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Using Noninvasive Genetic Sampling to Assess and Monitor Grizzly Bear Population Status in the Northern Continental Divide Ecosystem

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USING NONINVASIVE GENETIC SAMPLING TO ASSESS AND MONITOR GRIZZLY BEAR POPULATION STATUS IN THE NORTHERN CONTINENTAL DIVIDE ECOSYSTEM, MONTANA

By

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Wildlife managers need reliable estimates of population size, trend, and distribution to recover at–risk populations, yet obtaining these estimates is costly and often imprecise. The threatened grizzly bear (*Ursus arctos*) population in northwestern Montana has been managed for recovery since 1975, yet no rigorous data were available to evaluate the program's success. We assessed population status using data from a large noninvasive genetic sampling project and 33–years of physical captures. Our abundance estimate, $\hat{N}$ = 765 (CV = 3.8%), was more than double the working estimate. Based on our results, the total known, human–caused mortality rate was 4.6%, slightly above the 4% level considered sustainable. Genetic diversity approached levels seen in relatively undisturbed populations, with the only signal of population fragmentation that aligned with landscape features being across U.S. Highway 2.

I used these encounter data to parameterized a series of simulations to assess the ability of noninvasive genetic sampling, specifically surveys of naturally occurring bear rubs, to estimate population growth rates. I used data on 379 grizzly bears identified from bear rub surveys in a range of Pradel model simulations in program MARK. I evaluated model performance in terms of: (1) power to detect declines in population abundance, (2) precision and relative bias of estimates, and (3) sampling effort required to achieve 80% power to detect a decline within 10 years. Simulations suggest that annual bear rub surveys would exceed 80% power to detect a 3% annual decline within 6 years. Robust design models with 2 surveys per year provide precise and unbiased estimates of trend and abundance. Designs with 1 survey per year are less expensive but only yield trend and apparent survival estimates. I provide recommendations for designing a program to monitor population trends by sampling at bear rubs. Systematic bear rub surveys may provide a viable alternative to telemetry–based methods for monitoring trends in grizzly bear populations. This study illustrates the power of molecular techniques to rapidly assess population status and trends at landscape scales and provide detailed demographic and genetic data to guide and evaluate recovery efforts.

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Over 500 dedicated field technicians and volunteers assisted with field work, sample preparation, data entry, lure production, and a thousand other details over the life of these projects. By all accounts, these were massive efforts, and it would be difficult to express my gratitude to these people for being patient with our shortcomings. Anyone with experience running large projects knows that the complexity, labor demands, and opportunities for something to go wrong do not increase linearly with the size of the project. The success of these projects belongs in no small part to the hard work of many, many people working as a team.

My deepest gratitude goes to Kate Kendall and Dave Schirokauer, who opened the door to this wonderful work. When I got off the train in Glacier National Park for a three month volunteer position, I had no idea where it would lead over the next 10 years. During these years, there have been many truly special people who have inspired me and fostered my interests in noninvasive sampling techniques. Foremost among them are Wayne Simoneau, Mike Sawaya, Eric Penn, and Chris Kenyon. These are some of the hardest working people I've ever met, and their love of Glacier National Park, bears, and our projects has amazed and humbled me. I would be remiss if I didn't thank Montana Margo Coleman, who taught me many things about what data quality is all about. Of course I want to thank my family, who has supported my decisions throughout my life, no matter how divergent they were from the path I was expected to take. Finally, I want to thank Amy Macleod for innumerable and invaluable contributions to our projects and life in general. She has been a true unsung hero.

I thank committee members, Drs. L. Scott Mills, Fred Allendorf, and David Patterson. I realize my approach at graduate school was unorthodox, and I appreciate your patience, flexibility, and insights. In particular, I am truly grateful of the support and guidance of my advisor, Dr. Christopher Servheen. And, as every wildlife biology student at the University of Montana knows, Jeanne Franz is a true asset, and we all owe her a debt of gratitude. I thank Dr. Gary White and John Boulanger for assistance with concept and simulation development. Drs. David Paetkau and David Roon oversaw all genetic analyses. Drs. Bill Kendall and Trent McDonald assisted with model development used in Chapter II. Amy Macleod, Mike Sawaya, Julie Beston, and Drs. Paul Krausman and Sterling Miller provided helpful comments on earlier versions.

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Chapter I

INTRODUCTION

Noninvasive genetic sampling (NGS) was introduced to the world of bear research in 1996 (Woods et al. 1999). Since then, no fewer than three dozen studies have been conducted for North American grizzly/brown bears (*Ursus arctos*), European brown bears, and American black bears (*U. americanus*). The primary objective of nearly all of these studies was to estimate animal abundance. Consequently, the methods used to meet this objective have been refined with regards to field, laboratory, and statistical techniques. Laboratory methods were initially error–prone and had low success rates. An unknowable number of human errors such as scoring mistakes, contamination, transcription errors, and poor data management occurred, and samples that today would yield reliable genotypes were relegated as useless. Much of the early literature focused on these concerns and attempted to estimate error rates, yet focused almost exclusively on genotyping errors and ignoring the perhaps equally influential transcription/data handling errors (Roon et al. 2005, Creel et al. 2003, Mills et al. 2000) or accommodating genotyping errors with statistical methods incorporated into the mark–recapture analysis stage (Lukacs and Burnham 2005). During this same time period, lab methods were being improved, as was the recognition that data management was critical (Kendall et al. 2009, Paetkau 2003). Lab improvements also increased genotyping success rates, allowing lower quality samples to yield reliable genotypes.

As experience and the number of available datasets grew, so did researchers' ability to refine field methods. This facet of NGS projects has two primary components: study design and project execution. Analysis of a variety of study designs provided insights into what designs were most able to balance cost with sampling distribution and intensity. Issues such as optimal study area and grid–cell size, whether to move sites between sessions, and the number of required sessions were optimized to meet desired estimate precision. Project execution benefitted from most projects being initiated by a small number of researchers who determined what did and did not lead to a successful project. The ability to improve site locations for hair traps, for example, improved detection rates. Such improvements resulted in capture probabilities increasing from

≈0.1 in early studies in Alberta and Montana, to some recent projects that have seen capture probabilities approach 0.5, a dramatic improvement that has resulted in far more precise estimates.

The increased detection rates in conjunction with improved lab methods gave population modelers the data required to exploit advances in mark–recapture approaches beyond the classic closed population models of Otis et al. (1978) which have been the foundation for most studies for the past 30 years. For example, finite mixture models (Pledger 2000) use a mixture of two capture probability distributions to model heterogeneity of a single capture probability distribution. The Huggins (1991) models allow the use of individual (in addition to group and temporal) covariates to better model heterogeneity in capture probabilities. The abundance estimate ($\hat{N}$) is a derived parameter, therefore N is not in the likelihood, allowing use of covariates for only those individuals in the encounter history. Individual covariates such as history of previous live capture (which may reduce hair trap detection rates; Boulanger et al. 2008b) and distance of an animal to the edge of the sampled area (which helps to model effects of geographic closure violation; Boulanger and McLellan 2001) have improved the precision of estimates in a number of studies.

I have organized the research components of my thesis into two chapters and three appendices. Chapter II represents the final results of the Northern Divide Grizzly Bear Project (NDGBP), the largest application of NGS for bears undertaken to date. This paper was written by Katherine Kendall, myself, John Boulanger, Amy Macleod, David Paetkau, and Gary White (author order reflects that of the published, peer–reviewed paper). This project was initiated in 2002 at the bequest of the Manager's Subcommittee of the Northern Continental Divide Ecosystem (NCDE) to provide information on the status of this grizzly bear population. My roles in this project were diverse and built on my experiences in previous grizzly bear NGS research projects. I was actively involved in every aspect of project design, planning, execution, analysis, and publishing of results. Specifically, I was responsible for developing protocols and field tools, database design and maintenance, training field crews, developing budgets, coordination with our contracted genetics laboratory, blind sample testing of the lab, and various sections of our primary publications. I also participated in the hiring of field crews, coordination with

our 12 partner agencies, contract and agreement writing, purchasing, budget tracking, general information product development, professional and public presentations, and grant writing.

In 2004 we sampled 31,400 km^2 (7.8 million acres), representing the greater NCDE in northwestern Montana. The study area extended from the U.S.–Canada border to south of Highway 200; the western border followed Highways 93 and 35, extending east onto the plains of the Rocky Mountain Front. The study area included all lands thought to be occupied by grizzly bears at the time of sampling. While some very small number of bears may reside and/or pass through the area between the northwestern portion of the study area and the adjacent ecosystem to the west, our boundary was placed along Highway 93 for logistical and budgetary reasons.

As with most NGS projects, the primary objective of the NDGBP was to estimate abundance of the NCDE grizzly bear population. The study design, implementation, and analyses included many innovations. In addition to the unprecedented size (and subsequent degree of geographic closure), it was only the second study to incorporate a secondary, concurrent sampling method. Our previous work in this region successfully used hair from naturally occurring bear rubs to increase sample coverage and provide a backup method to estimating abundance. For example, collecting hairs from bear rubs during the NDGBP increased the minimum count of bears from 448 to 545 (22%), similar to the 24% increase in our previous work in the northern quarter of this ecosystem (Kendall et al. 2008). Also, we were able to use mixture models that allowed us to create a single encounter history for each bear that included detections from hair traps, bear rubs, and physical handling events (e.g., live captures) for estimating abundance. This approach had been shown effective in a simulation based experiment where we used data from our previous work to evaluate bias and precision of estimates with multiple data sources (Boulanger et al. 2008a). The resulting abundance estimate had a coefficient of variation (CV) = 3.8%, a level of precision not seen in hair trap–only projects, and far better than the CV≈20% that simulations predicted based on hair trap–only data. These improvements in precision give managers substantially greater confidence in making decisions based on abundance estimates.

Our estimate ($\hat{N}$ =765; 95% CI: 715–831), 2.5–fold larger than existing methods suggested, in conjunction with information on how bears are distributed across the NCDE will likely have major implications in the future management of this population. For example, despite the increase in estimated abundance, approximately one–half of the bears detected by the NDGBP were found in Glacier National Park, which represents only 13% of the study area. Using single numbers to reflect abundance or rates of population growth (λ) must be interpreted with caution in areas as diverse as the NCDE, as they are a cumulative function of very dynamic processes. However, benchmarks such as ecosystem–wide estimates of N or λ are critical components of effective management strategies. These estimates have been essentially unobtainable with such precision for populations like that of the NCDE until the advent of NGS methods and their complementary laboratory and mark–recapture techniques.

Chapter III addresses the second benchmark value, estimating population growth rates using NGS methods. Ongoing, yet at this time incomplete, efforts to estimate λ in the NCDE grizzly bear population require trapping, drugging, and collaring bears to obtain estimates of various vital rates such as survival and age of first reproduction. These rates can then be used in projection matrices to predict future asymptotic growth rates. As with any method, there are inherent limitations to such techniques. For example, it is increasing recognized that repeated handling and drugging events can have long–lasting impacts on bear behavior and survival, and, although rare, direct mortalities as a result of trapping do occur (Cattet et al. 2008). Further, it is not always logistically feasible to capture bears in all geographic regions, as is necessary for a density distributed design such as the one currently being used by the State of Montana. Budget limitations, collar malfunctions, and small sample sizes are other examples of the challenges of this traditional approach. Other methods to estimate population growth rates (e.g., the diffusion approximation; Dennis et al. 1991), exist; however, these methods have limitations, such as requiring precise abundance estimates from which trend is determined. While such methods may have potential for estimating λ in my study population, my purpose was not to review all of available methods, but to provide a relative comparison of the currently employed telemetry–based method and the method I present in Chapter III.

Noninvasive genetic sampling, in contrast, circumvents many of these limitations. Considerably less training and risk are involved with NGS and, as numerous projects have demonstrated, there are very few areas that remain inaccessible to sampling efforts. As evidenced by our work, bear rub surveys in particular represent an efficient means to obtain a large number of hair samples suitable for genetic analyses. Admittedly, bear rub surveys may never yield the quantity of samples that hair trap sampling does (assuming typical hair trapping efforts). However, bear rub sampling has several benefits over hair trapping. Rubbing is a natural behavior of bears, therefore, no lure is required and, subsequently, there is likely no behavioral response to sampling efforts either within or across years. Unlike hair trapping which requires substantial off–trail travel and/or helicopter use in the backcountry, bear rub surveys can be conducted entirely on recognized travel routes such as trails, forest roads, and powerpole lines. This is a tremendous advantage with regard to safety and efficiency. As with hair traps, bears of all sex–age classes have been detected at bear rubs, meaning that N and λ estimates include all bears in a population.

My objective in Chapter III was to evaluate the ability of grizzly bear detections at bear rubs, in conjunction with the mark–recapture–based models of Pradel (1996), to produce unbiased and precise estimates of λ. I used empirical data from the NDGBP as realistic parameter estimates of capture probabilities (p) and population abundance for simulations performed in program MARK (White and Burnham 1999). The primary simulations assumed a constant 3% annual decline (i.e., $\lambda = 0.97$) for both sexes, with sex–specific survival (φ), N, and p estimates. I considered three scenarios: (1) a single sampling event annually, (2) two sampling events (termed secondary occasions) annually, and (3) five secondary occasions. To derive $\hat{p}$ for each scenario, I assigned each bear detection from the NDGBP bear rub sampling effort into one, two, or five occasions, then divided that number by the total population estimate. As bear rub sampling was a secondary effort for the NDGBP, I consider these $\hat{p}$ values to be somewhat conservative. Increasing sampling effort (e.g., larger geographic distribution of rubs and/or longer season) would increase the number of detected individuals, resulting in improved precision of estimates and reducing the number of years required to detect a declining population.

The available data were too sparse to satisfy the five secondary occasion models, so this design was not considered in a full set of simulations. The one and two secondary occasion scenarios showed similar and expected patterns: pooled gender models showed greater power to detect a trend compared to gender–specific models, and both scenarios displayed rapidly improving power that began to asymptote around year 9. Precision of λ estimates improved as more years' data were available; however, as standard error estimates narrowed, confidence interval coverage (CIC) was reduced, but remained above acceptable levels ($\geq$ 80%) in all simulations through 10 years.

Estimates of apparent survival (φ) remained unbiased in all simulations other than those with five secondary occasions, and for the first two years of non–robust design models. The sparse data available for these simulation scenarios resulted in extreme underestimates of φ. Such results have not been documented in the literature, and require additional simulations to quantify the bounds on data requirements to achieve unbiased estimates (Gary White, Colorado State University, personal communication). Pradel model estimates of φ are a function of true survival and fidelity. Therefore, although the NCDE is not a truly geographically closed population, φ estimates based on bear rub surveys would provide an approximate estimate of true survival, as immigration and emigration are expected to be quite low (Kendall et al. 2009).

Although not initially a priority of my thesis, simulations suggest that robust design models (Kendall et al. 1997) with two secondary occasions may provide unbiased and reasonably precise gender–specific annual estimates of abundance. For populations where a reliable estimate of N is not available at the onset of a monitoring program, annual estimates would be a valuable component of assessing the overall status of the population. Annual abundance estimates performed in similar fashion to λ estimates. As more years' data were simulated, precision of estimates improved for both genders (pooled gender estimates were not evaluated), but confidence interval coverage declined as standard errors became smaller. Abundance estimates remained unbiased in all scenarios, as with λ estimates. It is an interesting condition of these models that, as more years' data are available, standard errors become so small that the 95% confidence intervals contain the actual N less frequently. Nonetheless, these models still perform

better than many methods (i.e., CIC ≥ 90%, CV< 9% for female estimates) and are a useful byproduct of monitoring population trajectory.

My second objective was to estimate the amount of annual sampling effort required to detect a population declining by 3% annually after 10 years. Based on the results of the initial simulations, I focused on designs of one and two secondary occasions, using the same parameter estimates for N and φ. I then iteratively manipulated values of $\hat{p}$ until adequate power at year 10 was achieved for both pooled gender and gender–specific models under one and two secondary occasion designs. I then translated the $\hat{p}$ values into a number of individuals of each gender that must be detected on bear rubs given our "known" population size from Kendall et al. (2009). Using data from four years of bear rub surveys, I used nonlinear regression to estimate the amount of survey effort needed to obtain the required number of detections under each scenario. Finally, based on our experience conducting large NGS projects, I estimated the personnel requirements to conduct the sampling. I estimate that 12 people would be required annually to detect a 3% annual decline in the NCDE grizzly bear population within 10 years. This assumes that personnel are dedicated to conducting surveys; however, existing personnel (e.g., park rangers), field courses, and volunteer groups could assist with surveys once a network of bear rubs has been established. This would reduce dedicated staff considerably.

In Appendix B I explored the effect that a fluctuating λ has on power to detect a net decline in abundance. As predicted, additional years' data (or increased capture probabilities) are required to achieve 80% power to detect a declining population when λ alternates between 0.94 and 1.01. These rates were selected simply to achieve an equal net reduction in abundance at year 10 as with a constant $\lambda = 0.97$. Estimates remained unbiased and showed the same patterns as a constant λ. However, it required eight and 10 years for the pooled and gender–specific scenarios, respectively, for both one and two secondary occasion designs, to achieve 80% power to detect a decline. Estimates of CV and CIC were very similar with either constant or fluctuating λ. Abundance estimates again followed similar patterns, with high and relatively stable CVs and reduced CIC as more years were simulated. Generally, performance of models declined with a

fluctuating λ, yet remained capable of detecting declining population abundance within a useful timeframe.

I also evaluated the ability of bear rub surveys to identify a modest increase in abundance. Surprisingly, models displayed less power to detect an increasing population ($\lambda = 1.03$) than to detect a decline of the same magnitude. Regardless, power to identify an increasing population (i.e., $\lambda > 1$) was achieved in nine years for the gender–specific one and two secondary occasion scenarios. Pooled gender simulations were not evaluated for $\lambda = 1.03$; however, based on other simulations, it would likely require one or two fewer years to detect an increasing population if pooled gender estimates were deemed adequate.

Another interesting aspect of the robust design Pradel model's annual estimates of abundance is that estimate precision improves retroactively as additional years' data are obtained. For example, with a single year's data, the female abundance estimate for the first year of sampling has a CV($\hat{N}$) = 16%; however, with 10 years' data, CV($\hat{N}$) = 8% for year one. So although estimates made early in a long–term program may be relatively imprecise, they rapidly improve across all years as more data become available.

In conclusion, simulations based on empirical data suggest that it is feasible to monitor population trajectory for the NCDE grizzly bear population by exploiting this unique behavior of bears. These methods have numerous advantages over traditional techniques including improved geographic coverage (which may be used in an occupancy modeling framework to monitor spatial trends), reduced cost, ability to monitor population genetic structure with relatively fine resolution, and essentially zero impact on bears. However, there are some disadvantages as bear rub–based methods only provide estimates of apparent survival and occupancy and do not provide information about other parameters that are useful in managing populations such as cause–specific mortality, mortalities of marked bears that would otherwise go undetected, movement in response to management actions, and habitat selection, which are all possible with telemetry–based monitoring. Nonetheless, either as a stand alone program or as a complement to telemetry–based methods, bear rub surveys offer a powerful tool to assist managers in assessing and monitoring this threatened population.

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Chapter II

DEMOGRAPHY AND GENETIC STRUCTURE OF A RECOVERING GRIZZLY BEAR POPULATION

ABSTRACT

Grizzly bears (brown bears; *Ursus arctos*) are imperiled in the southern extent of their range worldwide. The threatened population in northwestern Montana has been managed for recovery since 1975, yet no rigorous data were available to monitor program success. We used data from a large noninvasive genetic sampling effort conducted in 2004 and 33 years of physical captures to assess abundance, distribution, and genetic health of this population. We combined data from our 3 sampling methods (hair trap, bear rub, and physical capture) to construct individual bear encounter histories for use in Huggins–Pledger closed mark–recapture models. Our population estimate, $\hat{N}$ = 765 (CV = 3.8%) was more than double the existing estimate derived from sightings of females with young. Based on our results, the estimated known, human–caused mortality rate in 2004 was a 4.6% (95% CI: 4.2–4.9%), slightly above the 4% considered sustainable; however, the high proportion of female mortalities raises concern. We used location data from telemetry, confirmed sightings, and genetic sampling to estimate occupied habitat. We found that grizzly bears occupied 33,480 km^2 in the Northern Continental Divide Ecosystem (NCDE) during 1994–2007, including 10,340 km^2 outside the area thought to be occupied in 1993. We used factorial correspondence analysis to identify potential barriers to gene flow within this population. Our results suggested that genetic interchange recently increased in areas with low gene flow in the past; however, we also detected evidence of incipient fragmentation across the major transportation corridor in this ecosystem. Our results suggest that the NCDE population is faring better than previously thought, and highlight the need for a more rigorous monitoring program.

INTRODUCTION

World–wide, large carnivores are increasingly becoming endangered (Gittleman and Gompper 2001, Cardillo et al. 2005), but efforts to detect and reverse such declines are often hampered by limited data (Gibbons 1992, Andelman and Fagan 2000). Large

carnivores tend to be sparsely distributed over large areas and are difficult to observe (Schonewald-Cox et al. 1991). Grizzly bears (brown bears; *Ursus arctos*) exemplify these challenges and are threatened in many parts of their holarctic range.

The 5 remaining grizzly bear populations in the conterminous United States were listed as threatened in 1975 (U.S. Fish and Wildlife Service [USFWS] 1993; Fig. 2.1). Only 2 of these populations are currently thought to support more than approximately 50 individuals: the recently delisted population in the isolated Greater Yellowstone Ecosystem and our study population in the Northern Continental Divide Ecosystem (NCDE; Fig. 2.1) in northwestern Montana. The NCDE population is the only large population that remains connected to Canadian populations.

The Recovery Plan for the NCDE population identifies 6 recovery thresholds related to mortality rates and distribution of breeding females (Appendix A). The program is based on the best available science and relies on data acquired during routine agency activities rather than design–driven sampling (USFWS 1993, Vucetich et al. 2006). Multi–year counts of females with cubs are used to estimate population size and mortality rates because, in the absence of marked animals, individual females can be more easily identified than lone bears based on the number of cubs accompanying them. Despite strong public interest and costly management programs, there has been no rigorous, ecosystem–wide assessment of distribution and abundance in the NCDE, and the status of the population was unclear. Although sightings at the edge of the population's range have increased, suggesting population growth, allowable human–caused mortality thresholds have been exceeded every year for the last decade (USFWS 1993; Appendix A). To more rigorously assess the current status of this population, we conducted intensive noninvasive genetic sampling (NGS) across all lands occupied by grizzly bears in the NCDE and augmented these data with information collected during 33 years of research and management activities. We estimated abundance, distribution, and genetic population structure using individuals identified from multilocus genotypes of hair and tissue samples collected from bears that occupied our study area during our 2004 field season. We used our results to test assumptions about DNA–based mark–recapture analyses, estimate genetic error rates, and evaluate the USFWS program established to monitor this population.

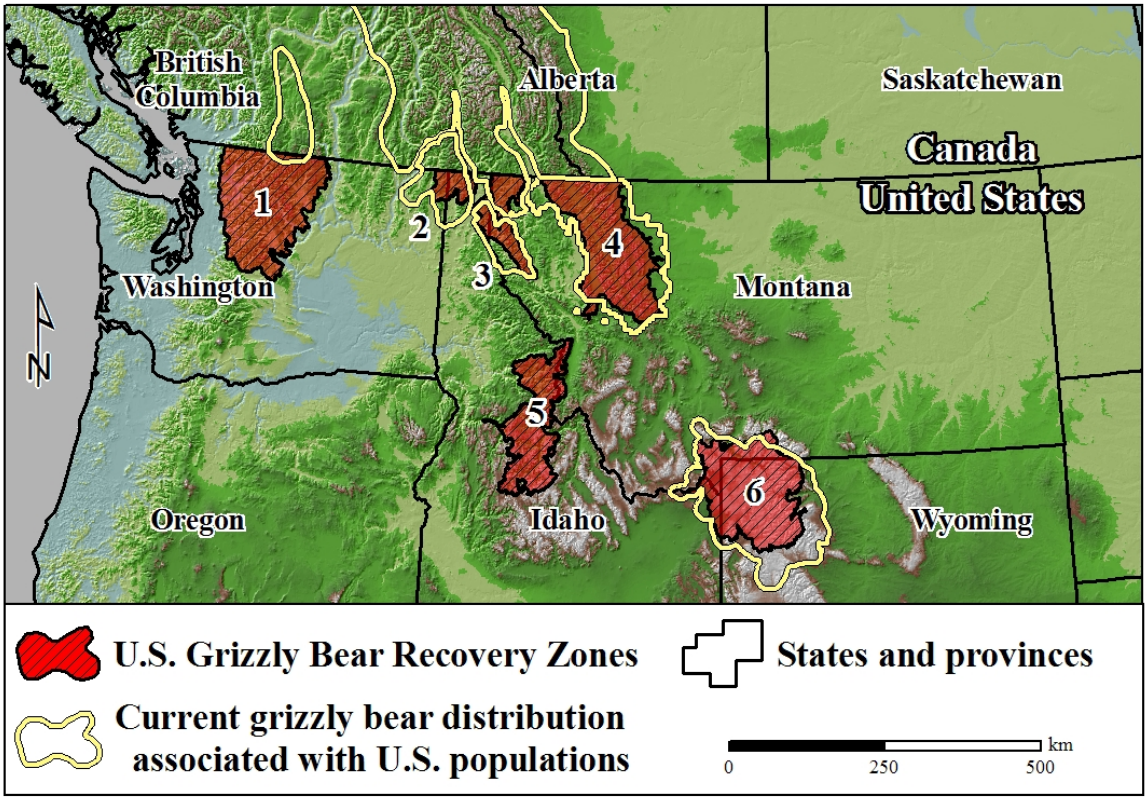


Figure 2.1. Location of remaining grizzly bear populations and Recovery Zones (established in the U.S. Fish and Wildlife Service Grizzly Bear Recovery Plan [1993]) south of Canada. Recovery zones: North Cascade (1), Selkirk (2), Cabinet–Yaak (3), Northern Continental Divide (4), Bitterroot (5), Yellowstone (6).

STUDY AREA

Our 31,410-km^2 study area in the northern Rocky Mountains of Montana, USA, encompassed the NCDE Grizzly Bear Recovery Zone (USFWS 1993) and extended to the edge of surrounding lands thought to have grizzly bears present during our study (Fig. 2.2a). The only exception was along the northern edge where the study area boundary was delineated by the U.S.–Canada border, which was open to bear movement. Black bears (*U. americanus*) occurred throughout the NCDE. The study area had a central core of rugged mountains managed as national park, wilderness, and multiple–use forest, surrounded by lower elevation tribal, state, and corporate timber lands, state game preserves, private ranch lands, and towns. Approximately 75% of the study area was mountainous and 35% was roadless. The study area included all of Glacier National Park, portions of 5 National Forests (Flathead, Kootenai, Lewis and Clark, Lolo, and

Helena), 5 Wilderness areas (Bob Marshall, Great Bear, Scapegoat, Mission Mountains, and Rattlesnake), parts of the Blackfeet Nation and Confederated Salish and Kootenai Indian Reservations, and hundreds of private land holdings. The east–west running United States Highway 2 and Burlington Northern – Santa Fe (BNSF) railroad form the largest and busiest transportation corridor in the NCDE (Fig. 2.2).

METHODS

Sampling Methods

To maximize coverage, we used 2 independent, concurrent NGS methods to sample the NCDE grizzly bear population. Our primary effort was based on systematically distributed hair traps using a grid of 641 7×7-km cells during 15 June–18 August, 2004. We placed 1 trap in a different location in each cell during 4 14–day sampling occasions. Hair traps consisted of 1 30-m length of 4–prong barbed wire encircling 3–6 trees or steel posts at a height of 50 cm (Woods et al. 1999). We poured 3 L of scent lure, a 2:1 mix of aged cattle blood and liquid from decomposed fish, on forest debris piled in the center of the wire corral. We hung a cloth saturated with lure in a tree 4–5 m above the center of the trap. We collected hair from barbs, the ground near the wire, and the lure pile. All hairs from 1 set of barbs constituted a sample; we used our best judgment to define samples from the ground and lure pile. We placed each hair sample in a paper envelope labeled with a uniquely numbered barcode.

We selected hair trap locations prior to the field season using consistent criteria throughout the study area based on Geographic Information System (GIS) layers and expert knowledge. We based selection on evidence of bear activity, presence of natural travel routes, seasonal vegetation characteristics, and indices of recent wildfire severity. Each trap was located ≥1 km from all other hair traps, ≥100 m from maintained trails, and ≥500 m from developed areas, including campsites. To help field personnel navigate to hair traps, we loaded all coordinates into Global Positioning System (GPS) units and made custom topographic and orthophoto maps for each site.

We also collected hair during repeated visits to bear rubs during 15 June–15 September, 2004. Bear rubbing was a result of natural behavior; we used no attractant. We surveyed rubs on approximately 80% of the study area; we omitted lands along the

eastern edge of study area due to insufficient personnel and a relative scarcity of rubs. We identified 4 primary types of bear rubs for hair collection: trees (85%), power poles (8%), wooden sign and fence posts (5%), and barbed wire fences (2%). We focused on bear rubs located along trails, forest roads, and power and fence lines to facilitate access and ensure that we could reliably find the rubs. Each rub received a uniquely numbered tag and short pieces of barbed wire nailed to the rubbed surface in a zig–zag pattern. We used barbless wire mounted vertically on bear rubs that had been bumped by horse packs. We found that the separated ends of double–stranded wire were effective at snaring hair but would not damage passing stock. During each rub visit, we collected all hair from each barb to ensure that we knew the hair deposition interval. We collected hair only from the barbed wire and passed a flame under each barb after collection to prevent contamination between sessions.

We compiled capture, telemetry, mortality, age, and past DNA detection data for 766 grizzly bears handled for research or management or identified during other hair sampling studies (Kendall et al. 2008) in the NCDE during 1975–2007. Of the bears for which tissue samples were available, 426 were successfully genotyped at ≥7 loci for individual identification. We used these data: 1) to identify bears that had been live–captured prior to 2004 for use as a covariate in mark–recapture modeling, 2) to investigate independence of capture probabilities among females and their dependent offspring, and 3) for our analysis of temporal trend in genetic structure. To determine the proportion of sex–age classes of bears detected with hair trap and bear rub sampling, we assumed that bears that met all of the following criteria were potentially available to be sampled: 1) ≥1 location on the NCDE study area during 15 June–15 September 1995–2006, 2) alive and ≤20 years old in 2004 (we included older bears if documented on the study area post–2003), and 3) not known to have died before 2004. We only included bears with reliable genotypes that were known to be present on our study area during our sampling period in our mark–recapture analysis.

Genetic Methods

We stored hair samples on silica desiccant at room temperature and blood and muscle samples either frozen or in lysis buffer. Samples were analyzed at a laboratory that specialized in low DNA quantity and quality samples, following standard protocols

(Woods et al. 1999, Paetkau 2003, Roon et al. 2005). We analyzed all samples with ≥1 guard hair follicle or 5 underfur hairs, and we used up to 10 guard hairs plus underfur when available.

The number and variability of the markers used to identify individuals determines the power of the multi–locus genotypes to differentiate individuals. We used 7 nuclear microsatellite loci to define individuals: G10J, G1A, G10B, G1D, G10H, G10M, and G10P (Paetkau et al. 1995). Preliminary data from this population suggested that randomly drawn, unrelated individuals would have identical genotypes (P_{ID}) with probability 1×10^{-7}, and full siblings would share identical genotypes with probability (P_{SIB}) 0.0018 for this marker set. These match probabilities assume a specified level of relationship, making it difficult to interpret them in the context of a study population in which the distribution of consanguinity is unknown. We obtained a more direct empirical estimate of match probability by extrapolating from observed mismatch distributions (Paetkau 2003). For each individual identified, we attempted to extend genotypes to 17 loci using the following markers: G10C, G10L, CXX110, CXX20, Mu50, Mu59, G10U, Mu23, G10X, and amelogenin (for gender; Ennis and Gallagher 1994).

For the first phase of the analysis we used 1 microsatellite marker (G10J), which has a high success rate and at which alleles with an odd number of base pairs are diagnostic of black bears. The only exception to this rule is a 94–base pair allele that exists in both species in our ecosystem. When this allele is present, species must be confirmed through additional analyses. We set aside samples that failed at this marker twice, as well as samples with 2 odd–numbered alleles. We analyzed all individuals with ≥1 94–base pair allele at G10J at all 7 markers that we used for individual identification, whether or not the second allele was even–numbered (presumed grizzly bears) or odd–numbered (presumed black bears).

During the next phase of lab analysis, we finished individual identifications by analyzing 6 additional markers on samples that passed through the G10J prescreen. We did not attempt to assign individual identity to any sample that failed to produce strong, typical, diploid (i.e., not mixed) genotype profiles for all 7 markers. We believe that this strict rejection of all samples whose genotypes contained weak, missing, or suspect data

(e.g., unbalanced peak heights) dramatically reduced genotyping error by eliminating the most error–prone samples.

Genotyping errors that result in the creation of false individuals, such as allelic dropout and amplification error, can bias mark–recapture population estimates (Mills et al. 2000, Roon et al. 2005). We used selective re–analysis of similar genotypes to detect and eliminate errors. We replicated genotypes for all: 1) individuals identified in a single sample, 2) pairs of individuals that differed at only 1 or 2 loci (1– and 2–mismatch pairs), 3) pairs of individuals that differed at 3 loci when those differences were consistent with allelic dropout (i.e., homozygous), and 4) individuals with samples geographically separated by large distances (Paetkau 2003, Roon et al. 2005, Kendall et al. 2008). We further minimized the risk of undetected genotyping error by replicating genetic data for all 17 markers (including gender) in ≥2 samples per individual or by repeating the analysis of all 17 markers in cases where just 1 sample was assigned to an individual. Whenever possible, we drew samples selected for reanalysis from a bear's 2 most distant capture points to potentially detect errors or true 0–mismatch pairs. We also made a photographic record of DNA liquid transfer steps to help determine the cause of handling errors when they occurred and to resolve them.

As part of our error–checking efforts, we submitted 748 blind control samples from 32 unique grizzly bears from throughout the NCDE to the laboratory. We constructed these samples to mimic the range of DNA quantity in hair samples collected in the field by varying the number of hairs with follicles per sample. Although lab personnel were aware that control samples would be randomly scattered among field samples, they were not aware of the number or identity of control samples. Genotyped bears for which sex was known from field data provided a similar opportunity to evaluate the accuracy of gender determinations. We also submitted 115 blind test samples that we created by mixing, in various proportions, hair from 2 individuals, mostly parent–offspring or full sibling pairs. As a final overall assessment of the reliability of our data, we contracted with Dr. Pierre Taberlet (Director of Research, National Centre for Scientific Research, Grenoble, France), an expert in issues of genotyping error in noninvasive samples (Taberlet et al. 1996, Abbott 2008), to conduct an independent assessment of our field, data entry, lab, and data exchange protocols. Among other tests,

P. Taberlet examined the results of 100 randomly drawn and 406 blind samples for errors and then checked if the data from the genetic analysis matched the database used for abundance estimates.

We replicated almost every genotype in the 17–locus dataset, either between samples, by repeated analysis as positive controls, or during error–checking, which provided an outstanding opportunity to detect genotyping errors. We recorded an error each time a genotype was changed after being entered into the database as a high–confidence score (i.e., not flagged as requiring reanalysis to confirm a weak initial result). The extra measures we employed to avoid the creation of spurious individuals, along with our large sample size, permitted us to evaluate the standard methods that formed the foundation of our genotyping protocol (Paetkau 2003). Before starting the analysis of supplemental markers (in duplicate, with emphasis on geographically distant samples), we generated a preliminary 7–locus results file using only the standard protocol of selective reanalysis of similar genotypes.

Estimating Abundance, Mortality, Distribution, and Genetic Population Structure

We developed an approach to abundance estimation that combined data from our 3 sampling methods (hair trap, bear rub, and physical capture) to construct individual bear encounter histories for use in Huggins–Pledger closed mark–recapture models (Huggins 1991, White and Burnham 1999, Pledger 2000, Boulanger et al. 2008a, Kendall et al. 2008). We performed all mark–recapture analyses in program MARK (White and Burnham 1999: Pledger model updated May 2007). The Huggins model allows the use of individual covariates, in addition to group and temporal covariates, to model capture probability heterogeneity. Pledger (2000) mixture models use ≥2 capture probabilities to model heterogeneity by partitioning animals into groups with relatively homogenous capture probabilities. Our candidate models included gender, bear rub sampling effort (RSE), history of previous live capture (PrevCap), and distance to edge (DTE) covariates. Rub sampling effort was the number of days since the last survey summed for all bear rubs surveyed in a session. We considered a bear to have a history of live capture if it had been captured or handled, regardless of method, at any time prior to or during hair trap sampling. Distance to edge was the distance of the average capture location of each bear from the open (northern) boundary.

We used a step–wise a priori approach to mark–recapture model development. To determine the best structure for each data type, we initially modeled hair trap and bear rub data separately. We pooled the other 2 data types and used them as the first sample occasion for each exercise. For example, in the hair trap models, we combined bear rub and physical capture detections as the first sample session followed by the 4 hair trap sessions. We then combined the most supported hair trap and bear rub models into a single analysis in which we constructed encounter histories for each of the 563 bears detected during 10 sampling occasions as follows: physical capture (1), detection during 4 hair trap sessions (2 – 5), detection during 5 bear rub survey sessions (6 – 10).

We evaluated relative support for candidate models with the sample size–adjusted Akaike Information Criterion (AIC_c). We obtained estimates of population size as a derived parameter of Huggins–Pledger closed mixture models in Program MARK (White and Burnham 1999, White et al. 2001). Calculation of 95% log–based confidence intervals (CI) about those estimates incorporated the minimum number of bears known to be alive on the study area (White et al. 2001). We averaged population estimates based on their support in the data, as indexed by AIC_c weights, to account for model selection uncertainty (Burnham and Anderson 2002).

We used our abundance estimate to calculate an estimate of the known, human–caused mortality rate in 2004 for comparison with mortality and abundance estimates generated using the Recovery Plan method (USFWS 1993). The Recovery Plan population estimate and the number of mortalities applied only to the Recovery Zone plus a 16.1–km buffer. Because our abundance estimate covered a larger area, we used the total number of mortalities for this area to calculate mortality rate.

To determine the current range of grizzly bears, we plotted confirmed records of grizzly bear presence from hair snaring, captures, telemetry, mortalities, and sightings from 1994–2007 on a 5-km grid. We defined the edge of current distribution as the outermost occupied cells adjacent to other occupied cells. We mapped an occupied cell as an outlier if it was separated from other cells with bears by >1 empty cell (Fig. 2.2a).

To investigate population genetic structure, we identified regional subpopulation boundaries using factorial correspondence analysis (FCA) conducted in GENETIX (Belkhir et al. 2004). We adjusted the number and location of geographic boundaries on

an ad hoc basis to minimize overlap of geographically defined genetic clusters (Fig. 2.2a). We used F_{ST} (Weir and Cockerham 1984, Barluenga et al. 2006) to estimate genetic differentiation between regions and visualized these values with Fitch trees (Fitch and Margoliash 1967). To determine gene flow across the United States Highway 2 and BNSF railroad corridor, we divided the corridor into 3 segments and used assignment tests (Paetkau et al. 1995) to compare the 50 individuals nearest to the highway on either side of the western and eastern sections (data not shown for the middle section).

To examine change in genetic structure over time we divided our dataset into 347 animals first captured prior to 1999 and 600 animals first captured more recently. We based the choice of 1998 as the cutoff for the earlier period on available sample size, which increased considerably after 1998. We conducted all population genetics analyses using ≥13–locus genotypes. We used 15 of the 16 microsatellite markers used in the NCDE in the datasets for bear populations in Canada and Alaska to which we made comparisons of genetic variability and population structure. Genetic distance calculations between the Prophet River and NCDE populations used 15–locus genotypes provided by G. Mowat (British Columbia Ministry of Environment, Nelson, B.C., Canada; Poole et al. 2001).

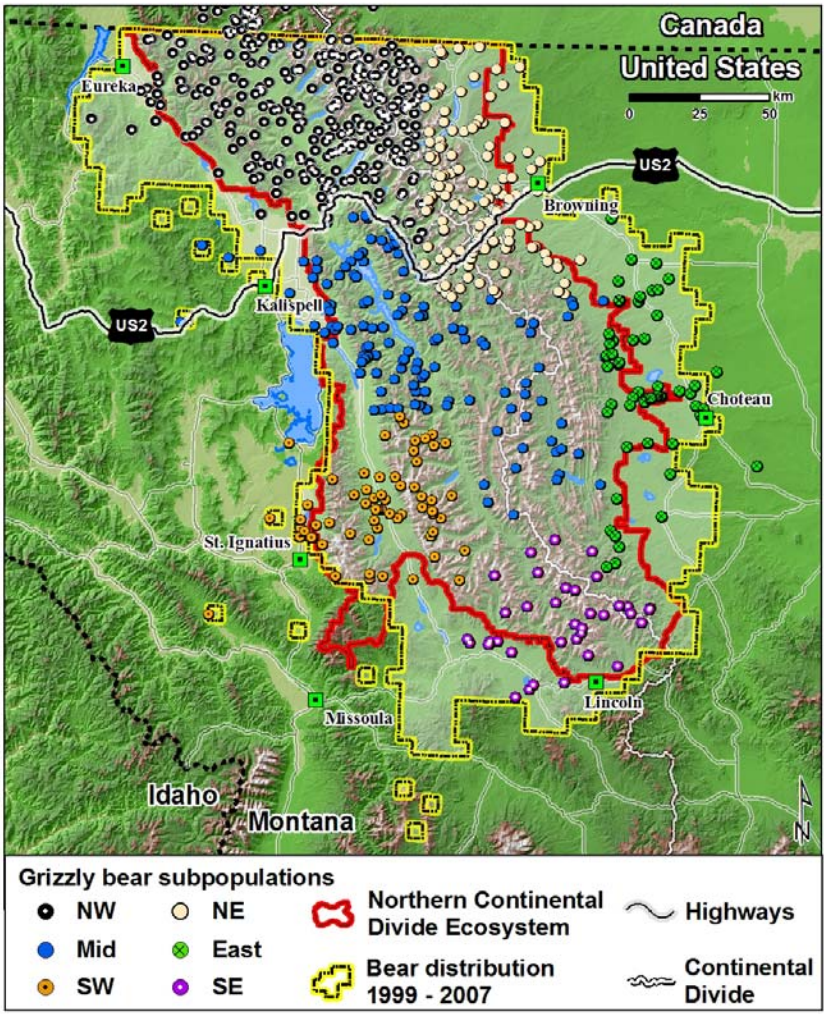
RESULTS

Sampling Effort

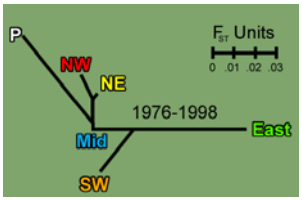
From 15 June–18 August 2004, we collected 20,785 bear hair samples from 2,558 scent–baited hair traps (Fig. 2.3a, Table 2.1). We also collected 12,956 hair samples from 4,795 bear rubs (Fig. 2.3b, Table 2.2). We conducted 18,021 rub visits during our 15 June–15 September, 2004, field season, for an average of 3.8 visits/rub (SD = 1.04; range 1–7; Table 2.2).

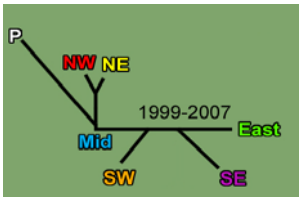
Genotyping Success, Marker Power, and Quality Control

We culled many of the 33,741 hair samples collected from hair traps and bear rubs before the first stage of analysis based on inadequate number of follicles (26.4%), obvious non–grizzly bear origin (2.3%), and subsampling criteria (2.1%). We attempted to genotype 23,325 (69.1%) samples. Genotyping success exceeded 70% with ≥3 guard hairs or ≥11 underfur follicles; success rates were similar for samples from hair traps and bear rubs.



A





B

Figure 2.2. Change in genetic differentiation between regions within the Northern Continental Divide Ecosystem (NCDE) brown bear population 1976–2006.
A) Map of region membership of brown bears within the NCDE as grouped by factorial correspondence analysis (8). Distribution of brown bears 1994–2007 in the Northern Continental Divide Ecosystem (NCDE) study area based on records of brown bear presence; total population range = 33,475 km^2; brown bear recovery zone = 23,130 km^2. B) Fitch tree of genetic distances (F_{ST}) (18) within the NCDE population for 1976–1998 (top) and 1999–2006 (bottom). The small number of genotypes available for the SE region for 1976–1998 (n = 2) precluded inclusion in that time period. Genetic distance to the Prophet River (P), British Columbia, brown bear population 1,150 km north of the NCDE was included for comparison to within–NCDE population distances.

Table 2.1. Grizzly bear hair trap results. We conducted hair trapping 15 June 2004 – 18 August 2004 in the Northern Continental Divide Ecosystem in northwestern Montana, USA, for 4 14–day sessions [a].

Session	No. sites	% traps with ≥1 grizzly bear sample	Grizzly bear samples/trap[b] $\bar{x}$	SD	Total no. grizzly bear samples	No. new bears F	No. new bears M	No. unique bears F	No. unique bears M
1	640	19.4	4.3	4.0	535	70	60	70	60
2	637	15.5	5.8	6.4	570	44	40	50	55
3	638	20.2	6.2	6.8	796	83	39	111	55
4	643	19.7	6.4	6.8	810	69	43	114	76
$\bar{x}$	640	18.7	5.7	6.0	678	67	46	86	62
Total	2,558				2,711			266	182

[a] $\bar{x}$ =13.98 days, SD=1.27

[b] Of those hair traps that had ≥1 grizzly bear hair sample

Table 2.2. Grizzly bear rub survey results. We conducted surveys 15 June 2004 – 15 September 2004 in the Northern Continental Divide Ecosystem in northwestern Montana, USA. We combined sessions with low sampling effort for mark–recapture analysis.

Session	No. bear rub visits	% bear rubs with grizzly bear hair	No. grizzly bear samples/rub[a] $\bar{x}$	SD	Rub tree effort[b]	Total no. samples	No. new bears F	No. new bears M	No. unique bears F	No. unique bears M
1–2	3,186	18.7	2.5	1.8	53,220	595	17	68	17	68
3	3,510	13.8	2.4	1.8	61,900	484	29	34	32	68
4	3,081	13.2	2.6	2.1	57,001	406	24	20	33	50
5	4,208	11.7	2.3	1.6	82,358	494	35	22	54	63
>6	4,036	10.4	2.2	1.5	63,999	380	15	11	39	50
$\bar{x}$	3,604	13.6	2.4	1.8	63,696	472	24	31	35	60
Total	18,021				318,478	2,359			120	155

[a]Of those bear rub visits that had at least one grizzly bear hair sample.

[b]Rub sampling effort (RSE) is the cumulative no. of days between successive hair collections for each rub sampled per session. For example, if we surveyed 3,000 rubs during session 3, each surveyed 20 days earlier, the RSE for session 3 would be: 3,000 × 20 = 60,000.

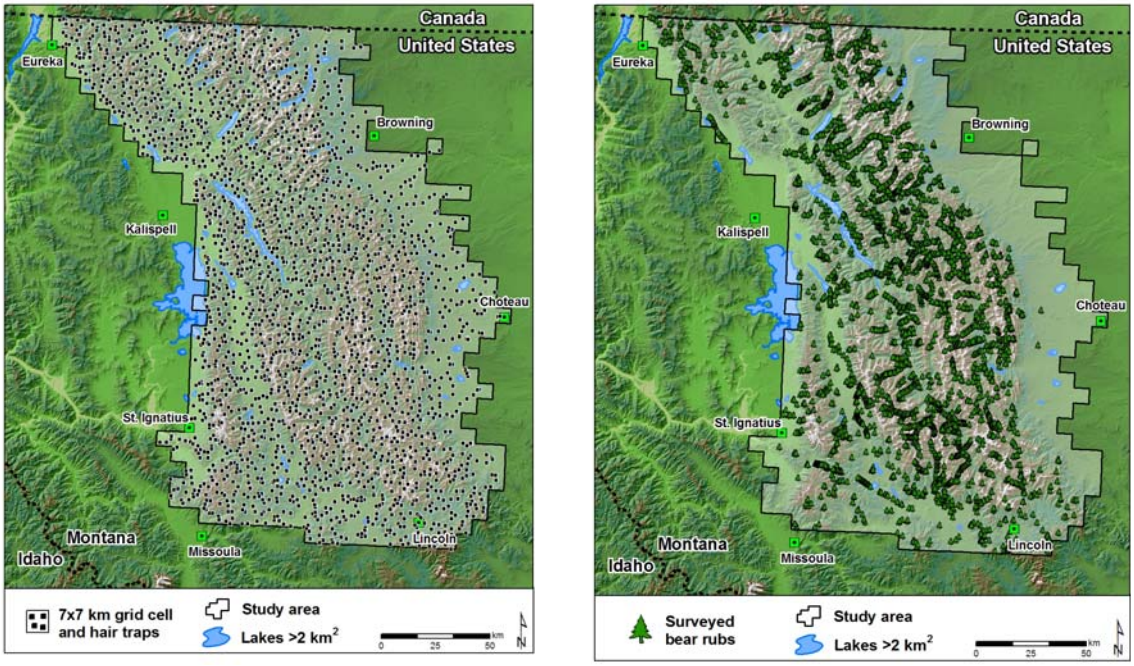


Figure 2.3. Location of grizzly bear hair snaring sites in the Northern Continental Divide Ecosystem, Montana, USA. A) Location of bear hair traps (n = 2,558). We conducted hair trap sampling 15 June–18August, 2004. B) Location of bear rubs (n = 4,795). We surveyed bear rubs on trails, forest roads, and power and fence lines during 15 June–15 September, 2004.

Of the samples we screened with the G10J marker, we set aside 17.3% after they failed twice and 51.2% identified as black bear (with 2 odd–numbered alleles). We obtained complete 7–locus genotypes for 74.2% (n = 4,218) of the samples that passed the G10J prescreen. We encountered samples with hair from >1 bear infrequently; we classified 0.4% of hair trap and 0.8% of bear rub samples as mixed based on the appearance of ≥3 alleles at ≥3 markers. Of the 563 individual grizzly bears we used in our analyses, 560 had complete genotypes at 17 microsatellite loci and 542 were fully replicated at all 17 markers with ≥2 independent, high–confidence genotypes.

Mean observed heterozygosity across the 7 markers used to identify individuals was 0.73 (Table 2.3). The probability that 2 randomly drawn, unrelated individuals would share the same genotype (P_{ID}) was 9×10^{-8} and the probability that full siblings

would have identical genotypes (P_{SIB}) was 0.0017. Extrapolation from the mismatch distribution in our dataset suggested approximately 1 pair of individuals with identical 7–locus genotypes. Expressed as a match probability, this equates to approximately 1/158,203, or 6×10^{-6}, midway between the estimates for siblings and unrelated bears (based on 563 × 562/2 = 158,203 pairs of individuals in the dataset, and a predicted 1 pair of individuals with the same 7–locus genotype).

Table 2.3. Variability of microsatellite markers used to determine individual identity of grizzly bears in the Northern Continental Divide Ecosystem in northwestern Montana, USA, in 2004.

Marker	H_E	H_O	A	P_{ID}	P_{SIB}
G10J	0.76	0.72	6	0.10	0.40
G1A	0.72	0.73	7	0.11	0.42
G10B	0.77	0.74	9	0.08	0.38
G1D	0.79	0.80	11	0.07	0.37
G10H	0.68	0.65	11	0.13	0.44
G10M	0.71	0.69	9	0.14	0.43
G10P	0.77	0.75	7	0.08	0.39
$\bar{x}$	0.74	0.73	8.6		
Overall probability of identity				9E–08	0.0017

H_E = expected heterozygosity; H_O = observed heterozygosity; A = no. of alleles; P_{ID} = probability of identity, P_{SIB} = probability of sibling identity.

When we considered all available markers, all individual bears differed at ≥ 3 loci. All 563 individuals identified by the original 7–locus analysis also had unique multilocus genotypes for the supplemental microsatellite markers. Given the low rate of genotyping error documented during data duplication (above) and by blind control samples (below) there was effectively zero probability that a pair of samples from a given individual would contain undetected genotyping errors in both the original 7–locus and

supplemental 9–locus genotype, so errors in the first 7 markers would be detected by discovery of matching genotypes at the supplemental markers.

As expected, some of the 748 blind control samples were of inadequate quality to obtain a reliable genotype. However, 100% of the 653 samples that we successfully genotyped were assigned to the correct individual, giving an estimated error rate for 7–locus genotypes of <1/653 (0.0015). As argued above, we believe that the actual number of false individuals is zero, but the blind controls provide an upper bound on the rate of error. Gender matched in all 514 cases for which we knew sex from field data. All of 115 deliberately mixed samples from 2 individuals were either assigned a genotype that matched 1 of the 2 source bears, failed to produce a clear genotype, or were correctly identified as mixed. In no case was a spurious individual recognized through mixing of alleles from 2 individual's genotypes, presumably because of the strict exclusion of samples with atypical genotype profiles at even one marker. The independent assessment of field and laboratory protocols concluded that: 1) all consistency checks strongly supported the reliability of the data, 2) no mechanism for systematic error was present, and 3) the error rate for the number of individual bears identified was ≤ 1%.

Factorial correspondence analysis (Kadwell et al. 2001, Belkhir et al. 2004) based on 6–locus genotypes (i.e., excluding G10J) provided unambiguous and independent species assignment for all individuals and confirmed that all individuals with ≥1 odd–numbered allele were black bears. The black bear genotypes that were closest to grizzly bears in the FCA had their genotypes extended to 16 microsatellite markers, as did genotypes that were homozygous for allele 94 at G10J. Subsequent 15–locus FCA analysis (excluding G10J) confirmed earlier 6–locus species assignments and identified 58 grizzly bears and 2 black bears that were homozygous for allele 94.

We estimated our rates of initial error (i.e., prior to error–checking) were 0.005 per locus per sample for the 7 microsatellites used on all samples, 0.002 for the 9 extra microsatellite markers, and 0.0007 for gender. Overall we classified 67% of the 234 detected errors as human errors (e.g., inaccurate scoring), 18% as allelic dropout, and 15% as false or irreproducible amplifications.

Population Abundance, Mortality, Distribution, and Genetic Structure

Our model-averaged abundance estimate for the NCDE population in 2004 was $\hat{N} = 765$ (95% CI: 715–831; Table 2.4). Although this represents a superpopulation estimate (Crosbie and Manly 1985), we estimated from radiotelemetry and DNA captures that only 0.5% of the bears we sampled moved outside of the study area to the west or east, and 1% of bears crossed the northern boundary of our study area (12% of the perimeter) during our 2004 sample period. Total known, human–caused mortality when calculated using our abundance estimate was 4.6% (95% CI: 4.2–4.9%); the female mortality rate was double the maximum allowed by the Recovery Plan (Appendix A; USFWS 1993).

Table 2.4. Total minimum counts and model–averaged estimates of grizzly bear population abundance in the Northern Continental Divide Ecosystem in northwestern Montana, USA, in 2004.

	Min.				95% log–based CI	
Parameter	count	Estimate	SE	CV (%)	Lower	Upper
M	242	294.58	12.01	4.1	276	324
F	321	470.60	26.16	5.6	427	531
Pooled	563	765.18	29.27	3.8	715	831

Our data supported 10 models as indicated by ΔAICc values ≤2 (Burnham and Anderson 2002; Table 2.5). However, our stepwise model development process resulted in very similar candidate models in the final stages of the analysis. In fact, the only parameters that varied were the sex–specific DTE threshold values. Our joint (physical capture–hair trap–bear rub) models suggested that hair trap capture probabilities mainly varied by sex, time, and PrevCap (Table 2.5). Average per–session capture probabilities were similar across genders for hair traps ($\hat{\bar{p}}_{\text{Male}} = 0.22$; $\hat{\bar{p}}_{\text{Female}} = 0.19$), with both genders having the lowest capture probabilities in session 2 and the highest by session 4 (Fig. 2.4). Bears with a history of previous live capture were 58.4% (95% CI: 42–79%) less likely to be captured in hair traps than were bears with no known record of capture. Bear rub capture probabilities varied by sex, sex–specific temporal trends, and RSE (Table 2.5). Males had approximately 3–fold higher average capture probabilities than

Table 2.5. Model selection results from mark–recapture analysis of the grizzly bear population in the Northern Continental Divide Ecosystem in northwestern Montana, USA, in 2004, sampled using physical capture (occasion 1), hair traps (occasions 2–5), and bear rubs (occasions 6–10). We present only models with ΔAICc < 2. Results from program MARK, 25 November 2007 build.

Model	AICc	ΔAICc	AICc wt	Model likelihood	No. parameters	Deviance
Base Model + $DTE_{Male15km}$, $DTE_{Female5km}$	5012.216	0	0.116	1	21	4970.051
Base Model + DTE_{5km}	5012.624	0.409	0.094	0.815	20	4972.474
Base Model + $DTE_{Male20km}$, $DTE_{Female5km}$	5012.894	0.678	0.082	0.712	21	4970.729
Base Model + DTE_{15km}	5012.947	0.731	0.080	0.694	20	4972.797
Base Model + $DTE_{Male25km}$, $DTE_{Female5km}$	5013.084	0.868	0.075	0.648	21	4970.919
Base Model + DTE_{10km}	5013.117	0.902	0.074	0.637	20	4972.968
Base Model + $DTE_{Male15km}$, $DTE_{Female10km}$	5013.132	0.917	0.073	0.632	21	4970.967
Base Model + $DTE_{Male30km}$, $DTE_{Female5km}$	5013.496	1.280	0.061	0.527	21	4971.331
Base Model + $DTE_{Male20km}$, $DTE_{Female10km}$	5013.806	1.590	0.052	0.452	21	4971.641
Base Model + $DTE_{Male10km}$, $DTE_{Female5km}$	5013.899	1.684	0.050	0.431	21	4971.735

Base Model Notation: PC (.) [HT: p(sex × t + PrevCap) RT: π (sex) $p_{1\&2}$ (× sex + sex × T + RSE)]

Base Model Description: Physical capture probability held constant. Hair trap: sex– and session–specific capture probabilities (p), with an effect of previous live capture (PrevCap), i.e., known to have a previous physical capture. Rub tree: sex–specific mixture probability (π). Capture probability is sex–specific with sex–specific linear trends (T), and an effect of rub sampling effort (RSE).

Parameter Definitions

PC = physical capture; HT = hair trap; RT = rub tree (includes all types of bear rubs). Mixture models only supported for RT data.

RSE = rub sampling effort: cumulative no. of days between successive hair collections across all sampled rubs/session. For example, if we surveyed 2,000 rubs during session 2, each surveyed 20 days earlier, the RSE for session 2 would be: 2,000 × 20 = 40,000.

DTE = individual covariate of distance to northern edge of study area. Effects of distance to edge are limited to the thresholds specified in model notation, e.g. $DTE_{Male15km}$ means that only male bears with an average capture location ≤15 km from the northern edge are modeled with this covariate.

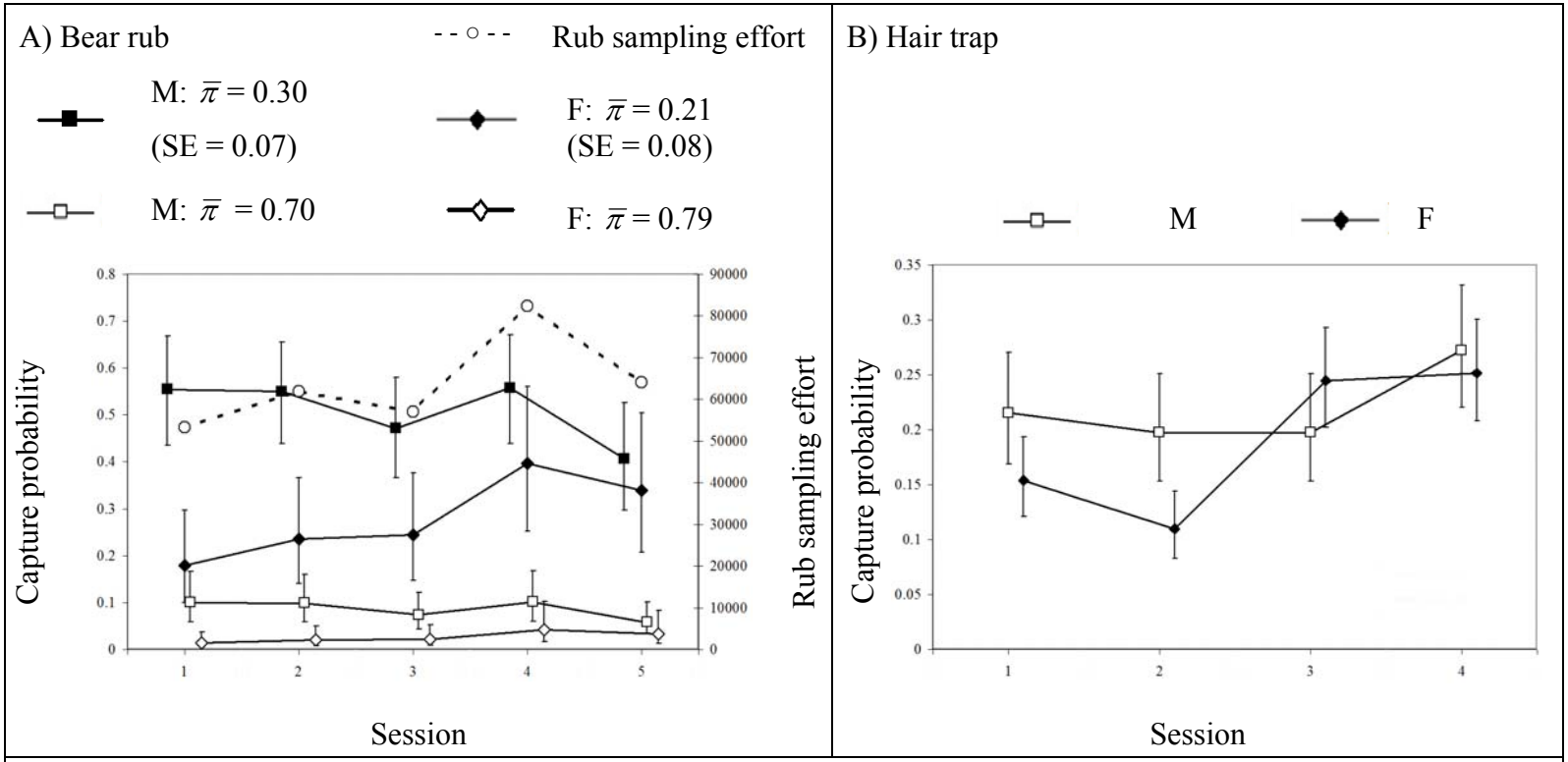


Figure 2.4. Gender–specific, per session grizzly bear capture probability estimates from (A) bear rub surveys and (B) hair traps in the Northern Continental Divide Ecosystem, Montana, USA. Sampling sessions were 2 weeks long, beginning 15 June, 2004. Pi (π) values represent the probability that an individual grizzly bear has 1 of 2 capture probabilities in the bear rub data. For example, in our data male bears had probability 0.30 of having the higher capture probabilities depicted in the top solid line. We derived estimates from the most selected models from Table 2.5. Rub sampling effort (RSE) was the cumulative number of days between successive hair collections summed over all bear rubs sampled per session; values are presented on the secondary Y–axis.

females, but males displayed slightly declining capture probabilities over time.

Conversely, females showed a slight increasing trend in capture probabilities over time and were nearly equal with males in session 4 (Fig. 2.4). In addition, there was undefined heterogeneity present in the bear rub data as indicated by the support for mixture models with this data type (Table 2.5). The DTE threshold values for the most supported model was ≤15 km and 5 km for males and females, respectively, which is consistent with bear biology as males are expected to move greater distances than females. Generally, as DTE increased above those levels, model support declined (Table 2.5).

Spring molting and behavioral differences between males and females could cause variation in hair deposition rates, sometimes in opposing directions. Because this may have influenced DNA capture probabilities, we examined our data for seasonal and gender–based differences in the number of hair samples deposited. Our data showed no seasonal trend in the number of hair samples left by females and a slight decrease in the number of samples deposited by males over the course of hair sampling. Although male and female hair deposition rates differed by sampling type (hair trap or bear rubs), this did not result in variable detection rates because we needed only one sample from each individual per hair sampling site to document presence.

In total, we detected 545 unique bears with our joint hair snaring methods, or 71% of the estimated population. By comparing hair snaring captures to genotypes from 276 handled bears of known sex and age class, we estimated hair snaring detected 44% of cubs, 80% of yearlings, and 89% of adult females known to be, or potentially present (Table 2.6). From our live–captured bear data, we knew of 6 family groups detected at hair traps. Of the 17 instances when we detected one member of a family group, we failed to detect other family members 53% of the time. Bear rub data also showed variable detection within families; we detected multiple members of the same group together in only 31% of 16 opportunities.

We detected 311 unique females and estimated there were 470 (95% CI: 427–531) in the NCDE population. We detected ≥1 (range: 1–55) female in each of the 23

Table 2.6. Number and proportion of grizzly bears that were present or potentially present that we detected with hair snaring in the Northern Continental Divide Ecosystem in northwestern Montana, USA, during the 2004 sampling period.

	Cub		Yearling		Subadult		Ad		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
F	11	36	7	100	11	55	118	89	147	83
M	5	60	8	63	20	75	96	94	129	88
Total	16	44	15	80	31	68	214	91	276	85

Bear Management Units defined in the Recovery Plan, as well as 12 females beyond the Recovery Zone boundary. Overall, population density declined along a north–south axis and toward the periphery of grizzly bear range (Fig. 2.5). Grizzly bears occupied 33,480 km^2 in the NCDE during 1994–2007, including 10,340 km^2 outside the Recovery Zone, which was thought to encompass most range occupied in 1993 [Fig. 2.2a]).

Factorial correspondence analysis identified 6 subpopulations in the NCDE (Fig. 2.2). In 4 of those subpopulations, genetic diversity approached levels found in undisturbed populations (15–locus mean H_E = 0.66–0.68). However, genetic variability was lower in the eastern (H_E = 0.61) and southeastern (H_E = 0.62) subpopulations.

Despite the general absence of geographically delimited genetic discontinuities, genetic differentiation between the northern NCDE and the southern and eastern periphery (F_{ST} = 0.05–0.09; 16–118 km apart) was similar to or greater than the value (F_{ST} = 0.06) observed between the northern NCDE and the Prophet River population in British Columbia, 1,150 km to the north (Fig. 2.2b, Table 2.7; Poole et al. 2001). When we compared population structure for animals first captured 1976–1998 with that of animals first captured 1999–2007, we found that the genetic distinctiveness of the eastern and southwestern periphery decreased over time (Fig. 2.2).

The only signal of population fragmentation that aligned with landscape features was across Highway 2 and the BNSF rail line (Fig. 2.2). There was little

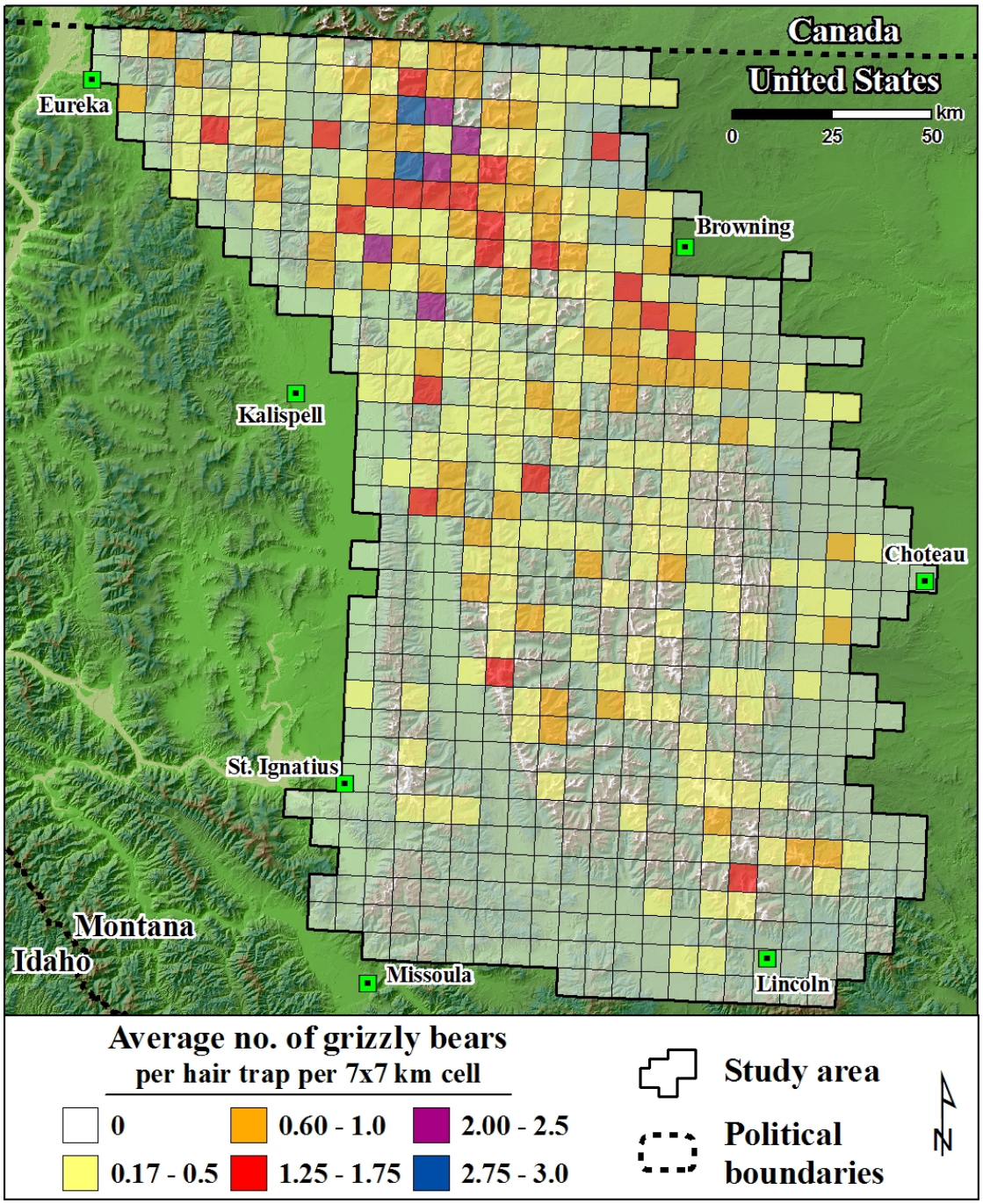


Figure 2.5. Relative density of grizzly bears in the 31,410 km^2 Northern Divide Grizzly Bear Project (NDGBP) study area in northwestern Montana, USA. We conducted sampling 15 June –18 August 2004 at 2,558 hair traps systematically distributed on a 7×7-km grid. Because equal sampling effort was required for this analysis, we used only hair trap data.

Table 2.7. Changes in genetic differentiation (F_{ST}) between regions within the Northern Continental Divide Ecosystem (NCDE) grizzly bear population in northwestern Montana, USA. F_{ST} values for 1976–1998 are below the diagonal; 1999–2007 values are above. The Prophet River, British Columbia, grizzly bear population 1,150 km north of the NCDE was included for comparison to within–NCDE population distances. Only 2 genotypes were available for the southeast region prior to 1999.

Region	Prophet	NW	NE	Mid	East	SW	SE
	99–07						
Prophet	76–98	0.07	0.07	0.05	0.10	0.09	0.10
NW	0.06		0.02	0.02	0.08	0.06	0.09
NE	0.06	0.02		0.02	0.07	0.05	0.07
Mid	0.05	0.02	0.01		0.05	0.03	0.05
East	0.12	0.10	0.08	0.06		0.05	0.04
SW	0.09	0.07	0.06	0.04	0.07		0.05
SE							

Table 2.8. Number and proportion of individual grizzly bears identified per sampling method during the Northern Divide Grizzly Bear Project, Montana, USA, 2004.

		M		F	
		No.	%	No.	%
Hair trap only		83	35	187	61
Bear rub only		56	24	41	13
Both NGS methods		99	42	79	26
Handled bears[a]		4	22	14	78
	Total	242	43	321	57

[a]Of those bears detected in ≥ 1 NGS methods, 31 (18 M, 13 F) also had a record of physical capture.

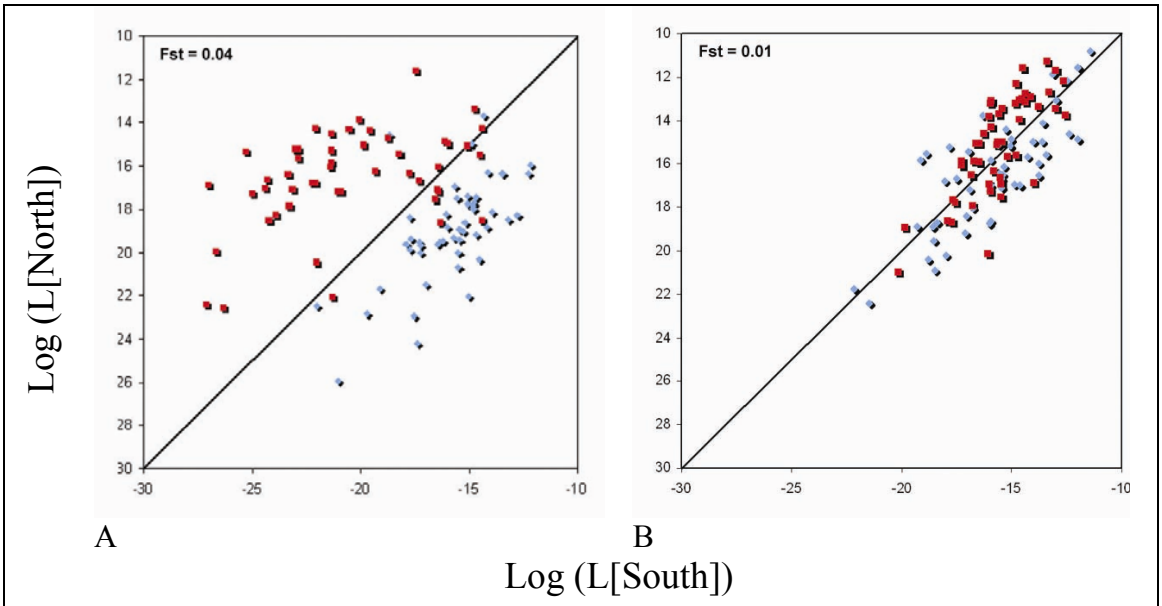


Figure 2.6. Genetic differentiation determined by assignment test between bears located on either side of the highway corridor for 2 segments of United States Highway 2; gray squares = bears north of highway; black squares = bears south of highway. A) Western segment with higher traffic volume and human density; B) Eastern segment with less traffic and development.

discernable genetic differentiation across the eastern portion of the corridor (F_{ST} = 0.01; Weir and Cockerham 1984), but at the western end, where human density and traffic volumes were higher, differentiation indicated reduced genetic interchange (F_{ST} = 0.04; Fig. 2.6).

DISCUSSION

Our study provides the first ecosystem–wide status assessment of the NCDE grizzly bear population. Our abundance estimate was 2.5 times larger than the recovery program estimate. However, density varied dramatically; we found the highest concentrations of grizzly bears in Glacier National Park but detected few were bears in the southern portion of the ecosystem. Our results suggested that the population was growing in terms of abundance, occupied habitat, and connectivity in areas of historically low genetic interchange. Our results also suggested that the population has generally remained genetically integrated and connected to Canadian populations. Conversely, we detected incipient fragmentation along the major transportation corridor in the NCDE and caution

that continued unmitigated development may lead to reduced gene flow within this population and reduced connectivity to adjacent populations. Our use of 3 data sources increased our sample coverage, resulting in improved estimate precision and greater resolution of genetic population structure. We demonstrated that our NGS methods detected bears of all sex–age classes and, therefore, our derived estimates reflect total population abundance. Our assessment suggests that grizzly bear recovery efforts have generally been successful; however, our results also highlight the need for improved monitoring techniques and reinforce the need to reduce the human–caused female mortality rate.

Grizzly Bear Demography and Population Structure

Abundance and mortality.– Our abundance estimate was more than double the existing estimate (Appendix A) and represents the first ecosystem–wide estimate of this population to include a measure of precision. Although our estimate reflects the superpopulation abundance, given the low rates of bear movement off our study area, we felt correcting for closure violation was unnecessary and would not impact inferences on population status. The known, human–caused mortality rate in 2004 when calculated with our abundance estimate was slightly above the 4% level considered sustainable (USFWS 1993). However, the number of mortalities in 2004 (n = 35) was the highest on record, and the female mortality rate was double the level allowed in the Recovery Plan. This is noteworthy because female survival is the most important driver of population trend (Schwartz et al. 2006). Although the Recovery Plan thresholds account for unreported mortality, this rate is difficult to measure and may vary over time (Cherry et al. 2002).

Knowing the sex–age classes included in population estimates is vital for monitoring population trend and making meaningful comparisons of density among populations. For example, dependent offspring can constitute 30% of grizzly bear populations (Knight and Eberhardt 1985). Because an animal's age cannot be determined from hair, it has been unclear if dependent offspring are sampled with hair snaring and included in abundance estimates derived from noninvasive sampling (Boulanger et al. 2004). Based on our large sample of bears (n = 276) for which sex and age was known, we found that hair snaring detected substantial proportions of the cubs and yearlings

known to be present (Table 2.6). This represents the most conclusive evidence to date that bear population estimates derived from hair snaring include all sex–age classes. Our estimate of the DNA detection rate was likely conservative because: 1) bears that have been previously live captured may be less likely to be sampled in hair traps (Boulanger et al. 2008a), 2) some known bears may have ranged beyond the study area boundary during our sampling season making them unavailable for DNA detection, and 3) unrecorded deaths could have occurred before DNA sampling.

Distribution.– Consistent with population expansion, we documented a substantial increase in habitat occupied by grizzlies in the NCDE since 1993. Female grizzlies were well distributed and found in all bear management units. Although not all were of breeding age, the number and wide distribution of females detected suggests good reproductive potential. However, density varied substantially from high levels in Glacier National Park in the north to low levels in the south (Fig. 2.5). Several areas in the NCDE had few or no detections, including some that contained high quality habitat, suggesting that there is still potential for population growth.

A single measure of bear density in a region as large and diverse as the NCDE would have little value and could be misleading when compared to other populations. Climate, topography, vegetation and land use were highly variable and likely influenced bear density patterns. Further complicating comparison to other populations, mammalian carnivore density estimates tend to vary inversely with study area size (Smallwood and Schonewald 1998). Typically, larger study areas include more habitat heterogeneity, which is often associated with variation in animal abundance. Smaller areas include proportionally more animals with home ranges overlapping the study area boundary, which, if not corrected for, can result in positively biased abundance estimates (Miller et al. 1997, Boulanger and McLellan 2001). At 31,410 km^2, our study area was much larger than those of most other terrestrial wildlife abundance estimation studies.

Population structure.– In general, the genetic health of this population was encouraging. Genetic diversity approached levels seen in relatively undisturbed populations in northern Canada and Alaska (Paetkau et al. 1998). Our results suggest that this population had not experienced a severe genetic bottleneck and that connectivity within the population and with the Canadian Rocky Mountain populations remained largely intact. The recent

increase in gene flow with the eastern periphery of the study area was consistent with population recovery. The historically low levels of genetic interchange and subsequently reduced diversity in the eastern and southeastern areas were similar to levels observed along the edges of the Canadian grizzly bear distribution and did not align with any landscape features (Proctor et al. 2005). However, our observation of reduced connectivity at the more developed western end of the dominant transportation corridor in the NCDE may signal the need for management intervention to ensure gene flow across this corridor in the future (Proctor et al. 2005).

Data Sources, Analytical Methods, and Data Quality

Supplemental data sources.– Having access to information such as mortality records, familial relationships, and animal movement data allowed us to investigate central assumptions of NGS studies. Some studies have assumed that juvenile bears are not sampled with hair snaring (e.g. Dreher et al. 2007). Our data showed that our abundance estimate based on hair snaring included all cohorts in the population. Noninvasive genetic sampling studies that assume juvenile bears are not vulnerable to sampling may overestimate total population abundance. In the absence of data on the detection rate of cubs and yearlings for individual study designs, our data argues for assuming that they are sampled. We also used management records to document partial independence of detection probabilities of family members traveling together, thus easing concern that a lack of independence among individuals creates bias in variance estimates.

The management and research records we gathered on grizzly bears in this ecosystem previously resided with individual researchers and wildlife managers from 8 agencies in dozens of locations in the United States and Canada. In addition to the assumptions investigated above, we used these data to: 1) increase sample coverage, extend encounter histories, and improve the precision of our abundance estimate, 2) produce a comprehensive map of grizzly bear occupied habitat in the NCDE, and 3) document the decrease in genetic differentiation among population segments over time. Management responsibility for most populations of wide–ranging species is shared by multiple agencies. Centralized databases with standardized data and tissue sample repositories can be extremely useful and will become more valuable with time as analytical techniques are refined.

Mark–recapture methods.– Noninvasive genetic sampling has been widely used for estimating abundance of grizzly and black bear populations (Boulanger et al. 2002, Boersen et al. 2003), but estimates have often been imprecise (CV > 20%; Boulanger et al. 2002) and thus, of limited use for detecting trends or guiding management policy, such as setting harvest rates. Factors that contributed to the precision of our estimate (CV = 3.8%) included the use of multiple sampling methods, the development of advanced mark–recapture modeling techniques (Boulanger et al. 2008a), and the large scale of our study. Combining detections from multiple data sources into single encounter histories yielded robust estimates with higher precision than a single–source approach (Boulanger et al. 2008a, Kendall et al. 2008). Mark–recapture models that can incorporate individual, group, and temporal covariates increase precision or reduce bias by more effectively modeling the heterogeneity in capture probabilities that is pervasive in wild populations (Huggins 1991, Pledger 2000, Boulanger et al. 2008a). Large study areas result in the larger sample sizes needed to model heterogeneity and reduce the effect of closure violation – a common source of capture probability variation. Our resulting population estimate was the most precise estimate obtained for a grizzly bear population using NGS.

Use of 3 sampling methods reduced estimate bias by increasing sample coverage; each method identified bears not sampled by the other methods (Table 2.8). Inclusion of physical capture data provided an opportunity to estimate capture probability for bears that were not detected using either hair snaring method and helped model heterogeneity in hair trap capture probabilities (Boulanger et al. 2008 *a, b*).

An important assumption in mark–recapture analyses is the independence of capture probabilities among individuals. Family groups (parent–offspring and siblings traveling together) are the largest source of non–independent movement in bear populations. Simulations suggested inclusion of dependent offspring causes minimal bias to population estimates but potentially a slight negative bias to variance estimates (Miller et al. 1997, Boulanger et al. 2004, Boulanger et al. 2008b). The magnitude of this phenomenon, however, has not been adequately explored with empirical data. Our evidence of partial independence of capture probabilities within family groups further

suggested that this source of heterogeneity was unlikely to be a significant source of bias in our estimates.

Heterogeneity caused by lack of geographic closure is also a major challenge for DNA–based abundance estimation projects using closed models (Boulanger and McLellan 2001, Boulanger et al. 2004). The most effective ways to decrease this source of bias are to sample the entire population or minimize the ratio of open edge to area sampled. We sampled essentially all occupied grizzly bear habitat associated with the NCDE in the United States and used telemetry data to assess movement rates across study area boundaries. We found extremely low levels of closure violation and, therefore, did not correct our estimate of abundance for lack of closure but used DTE to account for expected lower capture probabilities for bears along the northern edge of the study area.

Individual heterogeneity in capture probabilities is the most difficult problem facing the estimation of animal abundance (Link 2003, Lukacs and Burnham 2005b). The physical captures used in our encounter histories were not the result of even sampling effort across the study area. However, their inclusion may have reduced heterogeneity–induced bias resulting from unknown sources, such as behavioral traits or age, neither of which are known from DNA data and, therefore, cannot be modeled (Boulanger et al. 2008b). We included the PrevCap covariate in hair trap models because Boulanger et al. (2008b) found that detection probabilities at hair traps can be lower for bears that have been live–captured due to caution associated with similar lure and human scents. This effect was not expected at bear rubs as rubbing is a natural behavior with no association with human encounters; therefore, we did not consider the PrevCap covariate in bear rub models. We included terms to model the effects of gender–specific heterogeneity and gender–specific temporal trends in capture probabilities for both hair trap (Boulanger et al. 2004) and bear rubs (Kendall et al. 2008). Our results were similar to those of Kendall et al. (2008) who found increasing capture probabilities for females in both sampling methods in the northern portion of the NCDE. Males showed less consistency in temporal trends in capture probabilities across projects; however, males showed higher capture probabilities than females in bear rub data across all years of sampling. Our results suggest that sampling later in the season results in greater capture

probabilities, especially for females, and should result in more precise abundance estimates.

Data quality.–Some researchers advocate modeling genotyping error rates in mark–recapture analyses (Lukacs and Burnham 2005a). However, we not only employed a protocol than has been shown capable of reducing error–rates to a trivial level (Paetkau 2003), we also went beyond that protocol to duplicate all genotypes, whether or not they were similar to another genotype, and to confirm the authenticity of all 563 identified individuals using an independent set of microsatellite markers. This provided strong evidence that no spurious individuals were created through undetected genotyping error. This does not rule out the possibility that we sampled 2 individuals with the same 7–locus genotype, but it does demonstrate that such events were exceedingly uncommon, if they occurred at all. The estimated error rate for the number of individual bears identified through genotyping was ≤1%. Errors of this magnitude do not bias mark–recapture population estimates, whereas addition of a parameter (error rate) to the population estimation model would reduce the precision of the estimate.

We used bar–coded sample numbers and scanners to help ensure that genetic results were associated with the correct field data by eliminating transcription and data entry errors in the field, office, and lab. We employed data entry personnel with extensive experience in data quality control. Our database contained integrated error–checking queries that immediately identified questionable data and allowed us to resolve issues at the time of entry. We used GIS to verify the origin of samples, and we reviewed the detection history of each individual bear for inconsistencies. Further, field crews received 9 days of training in protocols, project background, laboratory methods, bear ecology, GPS use, and other topics that contributed to successful execution of field duties. Our use of such rigorous quality control measures contributed to our confidence in our results.

Monitoring Populations with Noninvasive Genetic Sampling

Monitoring and recovery programs for threatened and endangered species are usually a compromise between the quality of data desired and the cost of obtaining it (Doak and Mills 1994, Miller et al. 2002) and are often woefully inadequate (Vucetich et al. 2006). Abundance estimates are the most common quantitative criterion in recovery plans

(Gerber and Hatch 2002); however, they are often imprecise, error–ridden, or based on guesses (Holmes 2001, Campbell et al. 2002). In some cases, insufficient or erroneous data can directly influence how management efforts are prioritized and may result in misallocation of finite conservation resources (McKelvey et al. 2008). For example, inaccurate abundance estimates may result in misleading forecasts of population persistence because the magnitude of demographic stochasticity effects are a function of population size (Schwartz et al. 2006). Interpretation of per capita growth rate estimates may also be impacted by poor data, as growth rates can be affected by demographic stochasticity due to density–dependent factors (Drake 2005). For example, a monitoring program estimating trend would predict a flat or declining growth rate if the population was believed to be at or above carrying capacity (K). However, with inaccurate estimates of N or K, a declining growth rate could suggest that the population is experiencing a density–independent decline and elicit unnecessary management intervention.

To reliably monitor population trend, researchers must understand underlying patterns of variation in density and vital rates to guide stratified sampling, or sampling must be intensive enough to capture the variation. Measures of population trend such as those developed from projection matrices, commonly used for bears, may be insensitive to declines in some components of the population (Doak 1995). Using NGS methods for long–term monitoring therefore may be appealing when there is substantial heterogeneity in animal density and vital rates within a population, as with grizzly bears in the NCDE. Systematic NGS of the entire study area may be able to detect changes in local density (Fig. 2.5), patch occupancy, and genetic structure (Fig. 2.2), as well as ecosystem–wide abundance and apparent survival. Low intensity or periodic genetic sampling, such as with bear rub surveys, could be an efficient complement to, or more effective than, sightings– and telemetry–based methods for monitoring dispersal, distribution, genetic structure, and population trend.

MANAGEMENT IMPLICATIONS

Our results indicate that the NCDE grizzly bear population is faring better than the USFWS monitoring program had previously indicated. However, it is likely that continued unmitigated development along the Highway 2 corridor will result in genetic fragmentation of the grizzly bear population in the NCDE. Increased traffic volume and development along the other highways in the NCDE carries similar risks. Any long–term management strategy for this population should include ways to facilitate continued genetic interchange across transportation corridors and the associated development that tends to grow along them

The results of a 1-year study cannot measure population trend. Nonetheless, the recent decrease in genetic differentiation and apparent expanded distribution in the NCDE were consistent with population growth. In addition, the number and wide distribution of females we detected bodes well for the population. However, not all recovery criteria have been met. For example, even with our higher abundance estimate, the female mortality rate in 2004 was double the maximum allowed by the Recovery Plan. This suggests that, overall, management efforts have been effective in protecting this population but additional strategies are needed to reduce the female mortality rate, which is particularly important because the level of unreported mortality is difficult to assess. Clearly, a more intensive program should be considered to monitor population status and determine if mortality rates are sustainable. Based on our results, along with evidence of bear movement among populations and the recent initiation of a telemetry–based population trend study, the USFWS initiated a Status Review of threatened grizzly bear populations. This represents the first step in developing scientifically rigorous Recovery Plans for grizzly bears in the contiguous United States.

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Chapter III

EVALUATION OF BEAR RUB TREE SURVEYS TO MONITOR GRIZZLY BEAR POPULATION TRENDS

ABSTRACT

Wildlife managers need reliable estimates of population size, trend, and distribution to make informed decisions about how to recover at–risk populations, yet obtaining these estimates is costly and often imprecise. The grizzly bear population in northwestern Montana has been managed for recovery since being listed under the U.S. Endangered Species Act in 1975, yet no rigorous data were available to evaluate the program's success. I used encounter data from 379 grizzly bears identified through bear rub surveys to parameterize a series of Pradel model simulations in program MARK to assess the ability of noninvasive genetic sampling to estimate population growth rates. I evaluated model performance in terms of: (1) power to detect gender–specific and population–wide declines in population abundance, (2) precision and relative bias of growth rate estimates, and (3) sampling effort required to achieve 80% power to detect a decline within 10 years. Simulations indicate that ecosystem–wide, annual bear rub surveys would exceed 80% power to detect a 3% annual decline within 6 years. Robust design models with 2 surveys per year provide precise and unbiased annual estimates of trend, abundance, and apparent survival. Designs incorporating 1 survey per year require less sampling effort but only yield trend and apparent survival estimates. I provide recommendations for designing a program to monitor bear population trend by sampling at bear rubs. Systematic, annual bear rub surveys may provide a viable complement or alternative to telemetry–based methods for monitoring trends in grizzly bear populations.

INTRODUCTION

Without effective monitoring programs to identify declines in population abundance, distribution, and connectivity, managers are less able to take appropriate actions to ensure the persistence of populations (Nichols and Williams 2006, Joseph et al. 2006, Pollock 2006). This is especially true for small, isolated populations, and for species that reproduce as slowly as grizzly bears (*Ursus arctos*; Abrams 2002). Despite being listed

as a threatened species since 1975, rigorous estimates of population trajectory (λ) for the Northern Continental Divide Ecosystem (NCDE) grizzly bear population have never been obtained (Mace 2005). In 2006, Montana Department of Fish, Wildlife, and Parks (MTFWP) released a draft grizzly bear management plan for western Montana that explicitly stated that population trend will be MTFWP's guide to management decisions (Dood et al. 2006). The plan lists several potential methods for monitoring population trend, including periodic DNA–based sampling, radiotracking a sample of bears, and using indices based on unduplicated sightings of female bears with young (a method that has proven to be problematic in the NCDE [Servheen et al. 1996, Kendall et al. 2009]).

In 2004, MTFWP initiated a trend monitoring program for the NCDE grizzly bear population that uses live capture and collaring to track the fates of independent female bears. This program focuses on female bears because this cohort drives population trend (Eberhardt et al. 1994, Mace 2005, Garshelis et al. 2005). The program requires that ≥25 independent female bears be radio–collared, in perpetuity, to estimate population trajectory, reproductive rates, cause–specific mortality, and unreported mortality rates. To maintain a sample of 25 radio–collared females, far more bears will be captured and handled each year (i.e., males, dependent offspring, and black bears [*U. americanus*]). For example, during 2004 – 2006, the monitoring program captured 97 grizzly bears 111 times, yet only 45 of these were independent females (Mace and Chilton 2007).

In contrast to methods relying on live capture, noninvasive genetic sampling (NGS) protocols permit study of populations without the need to handle or even see the study animals. Systematic collection of bear hair samples for genetic analysis has been used to estimate population density at large geographic scales, with high levels of precision, in areas where live trapping would have been difficult and costly, and where methods based on visual sightings have proven problematic (Boulanger et al. 2002; Kendall et al. 2008, 2009). Although application of NGS methods to study bear populations have primarily focused on estimating abundance (Woods et al. 1999, Boulanger et al. 2002) or measuring population fragmentation (Proctor et al. 2002, Proctor et al. 2005), their potential for long–term monitoring of population trajectory and distribution has been recognized (Apps et al. 2005; Karamanlidis et al. 2007; Kendall et al. 2008, 2009).

Most NGS bear studies have used only baited hair traps that are systematically distributed on a grid of even–sized cells. Hair traps are placed in each cell in locations intended to maximize bear detections, and rely on a scent lure to attract bears. Limitations of this kind of sampling include accessibility of optimal locations for placing hair traps, variation in the attractiveness and persistence of lure, imperfect site construction, potential decline in individual recapture rates due to reduced attraction to the lure over time, and the logistical complexities inherent in satisfying mark–recapture sampling requirements (e.g., strict sampling intervals). Although hair trapping requires less training and is safer than live trapping, there are substantial study design and logistical challenges, especially in large, remote, and rugged areas typical of grizzly bear habitat (Boulanger et al. 2008a, Kendall et al. 2009).

Recently, two large–scale research projects in northwestern Montana, USA, have estimated grizzly bear abundance employing two concurrent noninvasive sampling methods (Kendall et al. 2008, 2009). In addition to the traditional grid of baited hair traps, hair samples were collected periodically from naturally–occurring bear rubs during the Greater Glacier Area Bear DNA Project (GGABDP) during 1998 – 2000 and the Northern Divide Grizzly Bear Project (NDGBP) in 2004. These projects were able to collect a large number of hair samples suitable for genetic analysis from both baited traps and unbaited bear rubs.

Typical bear rubs include trees, posts, power poles, or other objects that bears actively rub against. Hair is also passively left by bears as they cross wire fences or brush against gates, etc., that are common in non–forested areas such as the Rocky Mountain Front in Montana (Kendall et al. 2009). Bear rubs occur at varying densities along trails, forest roads, and power lines across a wide range of land management regimes throughout the NCDE and other populations (Karamanlidis et al. 2007, Kendall et al. 2009). This behavior is not well understood; however, rubbing likely includes some component of communication with other animals in the vicinity, despite grizzly bears not being considered territorial (Green and Mattson 2003).

Sampling bears by collecting hair left on naturally–occurring bear rubs is a promising complement, and potential alternative, to sampling with baited hair traps to meet a number of research and management objectives. Bears deposit hair through

rubbing in most, if not all, brown bear populations around the world (Green and Mattson 2003, Karamanlidis et al. 2007). The potential for using the frequency of bear sign such as rubbing activity, feces, and tracks to monitor trends has been suggested for black bear (Burst and Pelton 1983) and brown bears populations (Kendall et al. 1992, Clevenger and Purroy 1996, Karamanlidis et al. 2007). Based on annual surveys of bear sign, Kendall et al. (1992) reported that it may be possible to detect changes in abundance given adequate sampling effort. Because this work predated microsatellite genotyping of hair samples, it was limited to the abundance and distribution of bear sign encountered rather than identification of individual bears. In contrast to live capture and most other methods of obtaining information on grizzly bears, collecting hair from bear rubs can be performed by people with little specialized training, experience, or equipment, and with no more risk of injury (to bears or people) than would be expected on any hike in bear country.

In this paper I used simulations based on empirical data collected during two large–scale NGS projects in the NCDE to evaluate the potential for systematic, periodic bear rub surveys to detect a decline in population abundance. I provide recommendations on study design and the amount of sampling effort needed to detect a decline, and describe the relative benefits of different study designs to meet management objectives.

STUDY AREA

The 31,410 km^2 NDGBP study area represented essentially all lands occupied by grizzly bears in and around the NCDE in northwest Montana, USA (Fig. 3.1; Kendall et al. 2009). It extended approximately 240 km from the U.S. – Canada border to south of Montana Highway 200. The western boundary followed U.S. Highway 93 and the east shore of Flathead Lake. From the western boundary, the study area extended on average 125 km east onto the prairie beyond U.S. Highways 89 and 287. Lands within the NCDE were managed under numerous agencies, designations, and regimes. The study area included all of Glacier National Park (GNP), portions of five National Forests (Flathead, Kootenai, Lewis & Clark, Lolo, and Helena), five designated wilderness areas (Great Bear, Scapegoat, Bob Marshall, Mission Mountains, and Rattlesnake), the Blackfeet Nation and Confederated Salish & Kootenai reservations, Swan River and Coal Creek State Forests, large tracts of corporate timber land, hundreds of private land owners,

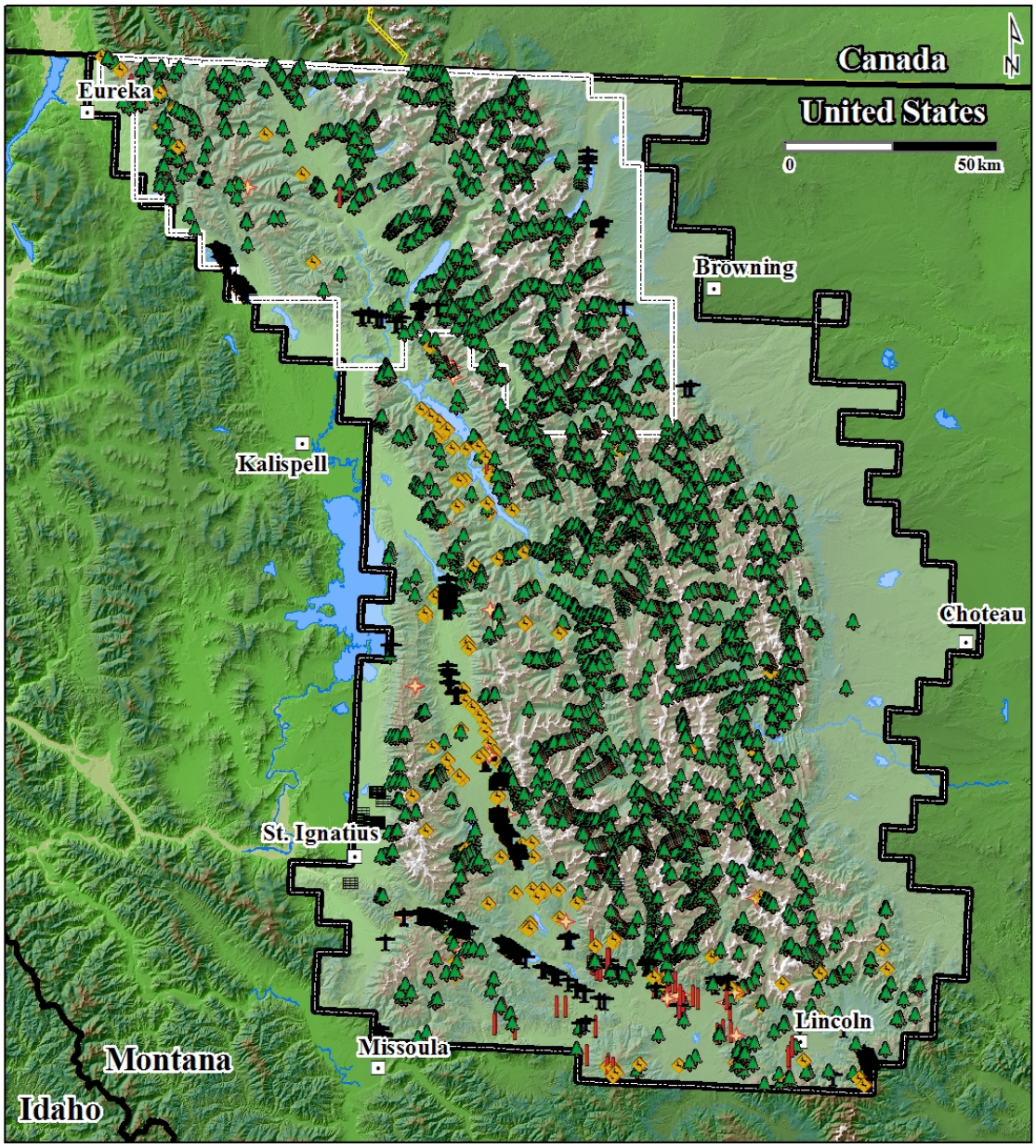

Figure 3.1. Map of the Greater Glacier Area Bear DNA Project and Northern Divide Grizzly Bear Project study areas, Montana, USA, and distribution of bear rubs surveyed by the NDGBP during 15 June–15 September, 2004.

and numerous other government and non–governmental organization landowners.

The study area was bisected longitudinally by the Continental Divide, which served as a geoclimatic boundary affecting weather patterns and, consequently, vegetation composition. Areas west of the Divide had a lower average elevation and, typical of a maritime climate, received considerably more precipitation than areas east of the Divide. Average annual total precipitation ranged from 102 cm along the west side of the Divide in GNP to 41 cm in the southeastern portion of the study area. Precipitation also varied with topographic features, with more precipitation falling at higher elevations. Roughly half of the annual precipitation comes during May through July. Elevation ranged from 780 – 3,190 m above sea level. The average maximum temperature for the hottest month (July) was 26.8° C; the average minimum temperature for the coldest month (January) was –11.5° C.

Primary tree species west of the Divide includes lodgepole pine (*Pinus contorta*), subalpine fir (*Abies lasiocarpa*), Douglas fir (*Pseudotsuga menziesii*), and Engelmann spruce (*Picea englemannii*), with stands of aspen and cottonwood (*Populus spp.*), ponderosa pine (*Pinus ponderosa*), and whitebark pine (*Pinus albicaulis*). Large, north–south oriented valleys exist along the three forks of the Flathead River and the Swan River. Potions of the Mission and Flathead Valleys were also included. To the south, the study area included the large, east–west running Blackfoot River valley.

Typical of a continental climate, lands east of the Divide receive more solar radiation and are known for frequent, high, sustained winds. Primary tree species east of the divide include aspen, subalpine fir, and cottonwood along river bottoms, with whitebark pine in upper subalpine areas. The majority of the eastern–most portion of the study area is in the prairie biome, dominated by open grassland. Shrub fields and alpine tundra exist in the mountains on both sides of the Divide.

The 8,000 km^2 GGABDP study area essentially coincides with the northern quarter of the NDGBP study area. Bear density is considerably higher in the Greater Glacier Area, primarily in GNP, than in the remainder of the NCDE (Kendall et al. 2008, 2009).

METHODS

Field Methods

Bear rub sampling occurred on approximately 50% and 80% of the GGABDP and NDGBP study areas, respectively. Surveys were essentially limited to GNP during the three years of the GGABDP. For the NDGBP, areas along the Rocky Mountain Front were omitted due to insufficient personnel and the relative scarcity of bear rubs (Fig. 3.1). The forested portion of the NCDE contained over 7,000 km of maintained trails plus many thousands of kilometers of forest roads and power pole lines. Bear rubs were found along these and other travel routes frequented by bears throughout the study area. Although bear rubs occur away from travel routes, search efforts were limited to such routes to ensure that surveys were repeatable and efficient.

For the both the GGABDP and NDGBP, each crew member was trained in the recognition of bear rubs, such as discoloration of tree bark and distinct animals paths leading to a rub (Fig. 3.2). Approximately 70% of identified rubs in the NDGBP had three 30 cm pieces of 15–gauge, 4–pronged barbed wire nailed to the rubbing surface to facilitate hair collection, obtain larger hair samples with more follicles, and reduce the rate of samples containing hair from multiple bears. I used double stranded wire (i.e., no barbs) on bear rubs that showed signs of being impacted by passing pack–stock to avoid damaging panniers. I found in field trials that the separated ends of the wire were nearly as effective as barbed wire at capturing hair samples. Each rub was tagged with a unique number, coordinates were obtained with a GPS unit, and information about the rub (e.g., type [tree, post, etc.]), tree species, distance from trail) was recorded. When searching for bear rubs, I attempted to have at least two crews survey each route because crews' ability to identify rubs improved with experience.

Each rub was surveyed periodically during the sampling period, with sampling effort varying across sessions and years. To account for this variation, I developed a measure of bear rub sampling effort (RSE), defined as the cumulative number of days between successive hair collections for all rubs sampled per time period. Upon each collection visit to designated bear rubs, each barb was inspected for hair. All hair from a given barb was placed into a uniquely numbered paper envelope and information, such as date, personnel, and the tag number of that rub, was recorded. To prevent contamination

between surveys, a flame was passed over the barbs to ensure that no stray hair fragments remained after the sample was collected. Only hair on barbs was collected (i.e., hair on tree bark were not collected) to make sampling effort comparable across rubs, to minimize the time required for collection, and to ensure that the period of time in which the sample was deposited could be determined. I included only those genotypes from hair samples where the period of deposition was known to ensure that no hairs left prior to my sampling were analyzed. All data were entered into a relational database with numerous integrated error–checking tools, as well as extensive post–analysis quality control measures (Kendall et al. 2008, 2009).

Lab Methods

Hair samples were stored on silica desiccant at room temperature until analyzed at a laboratory specializing in noninvasively collected samples following the protocols outlined in Woods et al. (1999), Paetkau (2003), and Roon et al. (2005). Species, individual identity, and gender of bears were determined through analysis of nuclear DNA extracted from hair follicles. Seven microsatellite loci were used to define unique individuals: G10J, G1A, G10B, G10C, G10L, G10M, and G10P (Paetkau et al. 1995). Gender was assigned using the amelogenin marker (Ennis and Gallagher 1994), the accuracy of which I verified through submission of samples from bears whose gender was known through management actions (Kendall et al. 2009). Exhaustive efforts to minimize errors were undertaken following the procedures of Paetkau (2003), Roon et al. (2005), McKelvey and Schwartz (2005), and Kendall et al. (2008, 2009). Details on marker power and genotyping success rates can be found in Kendall et al. (2009).

Simulation Methods

I conducted simulations based on empirically–derived capture probabilities to estimate the power to detect a declining population with bear rub detection data. For all simulations, I assumed $\lambda = 0.97$, where the population declines monotonically at 3% per year, resulting in a 26% decline after 10 years. This rate of decline would be considered rapid enough to warrant management intervention, but slight enough to demand a relatively powerful monitoring method to detect. Models with both gender–specific and gender–pooled λ estimates were evaluated.

Figure 3.2. Remote photograph of a free–ranging grizzly bear (*Ursus arctos*) using a rub tree in the backcountry of Glacier National Park, Montana, USA. Note the discoloration on the adjacent rub tree and the heavily used path between the rubs.

Parameter Values.– I obtained parameter values for use in mixture model simulations from the most supported (lowest AICc) model from the 2004 NDGBP abundance estimate model suite (Kendall et al. 2009). To estimate grizzly bear abundance in the NCDE, Kendall et al. (2009) used Huggins–Pledger closed mark–recapture models, which used a mixture of two capture probability distributions to model heterogeneity in a single capture probability distribution (Pledger 2000). These models provided estimates of gender–specific mixture probabilities, as well as gender– and session–specific capture probability estimates for bear rub data.

For non–mixture model simulations I used capture probabilities derived as simple ratios of the number of bears detected in bear rub sampling in the relevant sample period to total population abundance estimates (i.e., $\hat{p}_i = n_i / \hat{N}$; Table 3.1) from Kendall et al. (2009). For robust design models, where multiple sampling events are conducted each year (referred to as secondary occasions), capture probabilities were allowed to vary by gender and across secondary occasions to accommodate time variation in capture probabilities typical of bear rub data sets. I set recapture probabilities equal to capture probabilities as no behavioral response within or across years was expected because use

Table 3.1. Parameter values and simulation models used to evaluate the power of bear rub surveys to detect a 3% annual decline in the Northern Continental Divide Ecosystem grizzly bear population, Montana, USA. Five secondary occasion models are not shown due to unacceptable model performance.

Design type	Model notation	Capture probabilities used in simulations [a]			
1 Session (non–robust design)		M		F	
Gender–specific	φ(g) *p*(g) λ(g)	0.53		0.26	
Pooled genders	φ(g) *p*(g) λ(.)	0.53		0.26	
2 Session (robust–design)		Sess 1	Sess 2	Sess 1	Sess 2
Gender–specific	φ(g) *p*(g+t) λ(g)	0.37	0.36	0.13	0.19
Pooled genders	φ(g) *p*(g+t) λ(.)	0.37	0.36	0.13	0.19

[a] All models used $\varphi_{males} = 0.87$, $\varphi_{females} = 0.92$, $\lambda = 0.97$. Recapture probabilities were set equal to capture probabilities (c = p) in robust design models. True population sizes: males = 294, females = 470 (from Kendall et al. 2009).

of bear rubs is a natural behavior (Boulanger et al. 2008a; Kendall et al. 2008, 2009). Non–robust models simulated a single encounter each year; therefore, capture probabilities were held constant across years. I assumed capture probabilities to be independent for all animals based on my knowledge of variable detection rates for members of family groups in bear rub data (Kendall et al. 2009).

Values of apparent survival were approximations based on recent grizzly bear literature and considered appropriate for the NCDE population (Mace and Waller 1998, Garshelis et al. 2005). For all simulations, male apparent survival (φ) was set to 0.87 and female $\varphi = 0.92$ (Table 3.1).

Simulation Models.– To identify the most appropriate sampling design for monitoring trends in abundance with bear rub surveys, I evaluated scenarios for gender–pooled and gender–specific estimates based on 1, 2, and 5 annual (secondary) sampling occasions. I used three general formulations of the Pradel temporal symmetry model (Pradel 1996) in simulations performed in program MARK (v.5.1, build 2600, downloaded March 2008; White and Burnham 1999). The most complex simulations attempted to model heterogeneity of capture probabilities using robust–design Huggins–Pledger mixture models (Huggins 1991, Kendall et al. 1997, Pledger 2000). In addition to estimates of the realized rate of population change ($\lambda_i = [N_{i+1} / N_i]$), the robust design produces abundance estimates as a derived parameter. Robust design simulations also were used in a non–mixture framework, which provided the same categories of parameter estimates (i.e., λ, φ, and N), but considered only a single capture probability distribution for all individuals within a group for each secondary occasion. Finally, I evaluated non–robust, non–mixture formulations of the Pradel model. This approach collapsed all detections of each individual into a single event within each year. I evaluated all models in terms of power to detect declining abundance, percent relative bias, confidence interval coverage, and an index of precision based on the coefficient of variation (CV) of λ. Power to detect declining abundance was defined as the percentage of simulation runs where the upper 95% confidence interval on $\hat{\lambda}$ was < 1 (i.e., $\alpha = 0.025$). I evaluated robust design models with regard to bias, confidence interval coverage, and coefficient of variation (CV) of abundance estimates. Each simulation scenario was run 500 times, which results in power estimate uncertainty of ±3.5% at 80% power.

Monitoring Program Design

I explored several monitoring program designs from several perspectives. First, I estimated the number of years of annual sampling required to achieve 80% power to detect a declining population given capture probabilities achieved in the NDGBP bear rub sampling effort. Next, I estimated the amount of sampling effort required to detect a declining population within 10 years with ≥80% power for four basic sampling designs. For this, I again considered gender–specific and gender–pooled models with one and two sampling occasions per year (non–robust and robust Pradel formulations, respectively). Abundance estimates derived from the robust design models were also evaluated for precision, confidence interval coverage, and bias given the sampling effort required to meet the monitoring objective. I used data collected during four years of bear rub surveys in the NCDE in a nonlinear (logarithmic) regression to estimate the number of individual grizzly bears that could reasonably be expected to be identified given a specified amount of sampling effort. I iteratively manipulated capture probabilities in each of the four simulation model scenarios to the lowest possible values that still achieved ≥80% power to detect a declining population in 10 years. I then entered these capture probabilities into the regression to estimate the amount of sampling effort required to detect the desired number of bears. Regressions were performed for both robust and non–robust sampling designs to estimate the effort required to meet multiple management priorities. As with the previous simulations, I set male and female apparent survival to 0.87 and 0.92, respectively, with initial population sizes based on the results of Kendall et al. (2009).

RESULTS

Field Sampling

Rub tree sampling effort varied by year in the number of rubs surveyed, frequency of survey, and geographic distribution (Table 3.2, Fig. 3.1). From 15 June – 15 September, 2004, the period from which simulation parameter values were derived, I surveyed 4,795 unique bear rubs a total of 18,021 times. The average interval between visits was 17.8 (SD = 9.1) days. I collected 12,564 hair samples from bear rubs, for an average of 0.697 samples per visit. Results from the GGABDP (1998–2000; Table 3.2) were used only in

regression analyses to estimate the amount of effort required to obtain a desired capture probability. Details of GGABDP sampling effort and results can be found in Kendall et al. (2008).

Table 3.2. Bear rub sampling results in the Greater Glacier Area Bear DNA Project (1998 – 2000) and Northern Divide Grizzly Bear Project (2004), Montana, USA. Only data from surveys during 15 June – 15 September, and only those samples for which the time period of hair deposition was known, were included in simulations.

Year	No. rubs surveyed	Rub sampling effort (RSE [a])		No. samples genotyped		No. bears identified			
						M	F	M	F
		Session 1	Session 2	Session 1	Session 2	Session 1		Session 2	
1998	576	6,252	16,920	52	96	17	11	26	11
1999	740	28,710	28,297	309	148	59	26	40	25
2000	790	24,004	33,809	235	168	49	14	41	30
2004	4,795	172,121	146,357	1,026	865	110	60	106	88

[a] RSE = the cumulative number of days between successive hair collections summed over all bear rubs sampled per time period. For example: if 2,000 rubs were surveyed in a session and 30 days had elapsed since the previous survey, RSE would equal 60,000.

Genetic Analyses

Approximately 30% of the samples collected at bear rubs during the NDGBP contained too few follicles to be analyzed, 40% were from black bears, and 14% failed at various stages in the analysis. Individual genotypes could not be obtained from samples with hair from >1 individual; however, only 0.73% (n = 92) of the samples were mixed. A total of 1,891 (15.1%) grizzly bear hair samples were successfully genotyped at 7 microsatellite loci, from which I identified 155 unique male and 120 unique female genotypes. Individual genotypes were replicated, on average, in 9.1 (SD = 15.9) samples for males and 4.5 (SD = 3.4) samples for females. Details on marker power and blind tests of laboratory accuracy can be found in Kendall et al. (2009).

Simulations

Five Secondary Sampling Occasions Design.– I attempted simulations with Pradel–robust–mixture models based on five secondary occasions; however, sparse data, especially among female grizzly bears, resulted in unacceptable performance of the models. The Huggins–Pledger models used to estimate abundance (Kendall et al. 2009) estimated that 78% of females had capture probabilities of 0.02–0.05 for the five secondary occasions considered. This resulted in estimates of apparent survival (φ) for females to be biased by -14% after 10 years (i.e., with 'true' $\varphi = 0.92$, the simulation model estimated $\varphi = 0.78$). Further, confidence interval coverage on $\hat{N}$ was extremely poor for both genders, and approached 0 for females due to substantial negative bias in abundance estimates. Although estimates of power to detect a change in abundance appeared high, the inability to satisfy data requirements for other parameters in this model precluded further consideration.

Two Secondary Sampling Occasions Design.– Within this subset of simulations, I considered the relative performance of gender–specific versus gender–pooled λ estimates with a robust–design Pradel model based on two secondary occasions. As predicted, higher capture probabilities or more years of sampling were required to achieve adequate power to detect a change in gender–specific abundance than for gender–pooled models (Fig. 3.3). Based on my empirically–derived capture probabilities, power for gender–specific estimates improved nearly linearly with time, but did not exceeded 80% until year nine for both males and females. Conversely, gender–pooled models exceeded 80% in only six years, the least amount of time required for any model. Percent relative bias in $\hat{\lambda}$ rarely exceeded 0.6% in either formulation, and was <0.05% in year 10. Precision in $\hat{\lambda}$ improved rapidly for both gender–pooled and gender–specific models, converging at a coefficient of variation of <3% by year four, and continuing to decline asymptotically through year 10.

I also evaluated the robust design models for their ability to provide annual abundance estimates. As the underlying capture probabilities were the same regardless of how the model was parameterized for $\hat{\lambda}$, model performance with respect to $\hat{N}$ was essentially identical for all models (e.g., no paired annual estimates differed by >1.4%

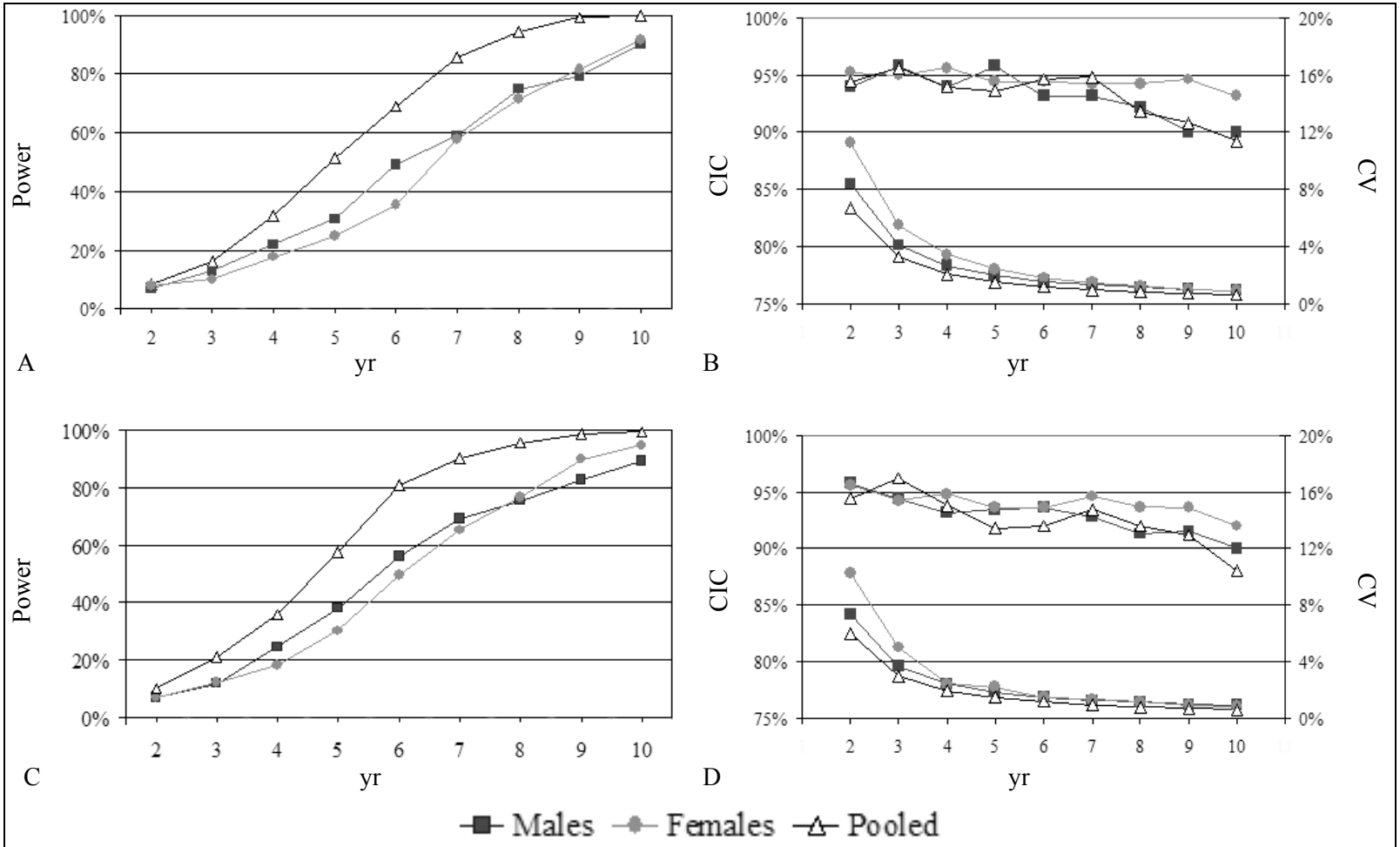

Figure 3.3. Power of bear rub surveys to detect a grizzly bear population declining at 3% annually, and coefficient of variation (CV) and confidence interval coverage (CIC) on point estimates of λ. A and B: 1 sampling occasion per year (non–robust design model). C and D: 2 sampling occasions per year (robust design model). Power estimates are ±3.5%. The bottom set of points reflect CV estimates with values given on the secondary y–axis. Parameter values used in simulations are provided in Table 3.1.

between models). By pooling detections into only two secondary occasions, I avoided the sparse data problems that were encountered with the five session mixture model approach. Pooling data also reduces heterogeneity in capture probabilities, which is difficult to model with sparse data (Boulanger et al. 2008a). Robust design Pradel models were consistently positively biased for $\hat{N}$; however, this bias remained between 1.5–4% for both genders for all 10 years. Standard errors of $\hat{N}$ declined by approximately 1.5–2% per year for both genders. However, as the simulated population was declining at 3% per year, the net result was a slightly increasing coefficient of variation on $\hat{N}$, although even at year 10, the CV for $\hat{N}$ remained <9% for females and <6% for males. Confidence interval coverage declined more dramatically for males than females, reaching 72.6% and 89.8%, respectively, at year 10 (Table 3.3).

Single Annual Survey Design.–I next evaluated non–robust models with one sampling occasion per year. I tested both gender–pooled and gender–specific models for their power to detect a 3% annual decline in abundance, and evaluated the bias and precision of λ estimates. For both approaches, percent relative bias of $\hat{\lambda}$ never exceeded 0.3%, and remained <0.1% by year seven. Power curves resembled those of robust design models (Fig. 3.3). Gender–pooled models exceeded 80% power in year seven, whereas gender–specific models required nine years. Precision of λ estimates was essentially identical to the robust design, converging in year five at CV ≈ 2%, and continuing to improve through year 10 (Fig. 3.3).

Predicting Sampling Effort Required to Detect λ=0.97 within 10 years.– My second objective was to estimate the amount of bear rub sampling effort required to detect a 3% annual decline in the NCDE grizzly bear population within 10 years with ≥80% power. As demonstrated by the simulations in the previous sections, each of the four general formulations (i.e., robust and non–robust for both gender–pooled and gender–specific estimates) had relative advantages. Gender–pooled approaches had greater power to detect declines in the overall population, but do not allow detection of different trajectories for the male and female portions of the population. Robust designs allowed estimation of abundance, but required higher capture probabilities to perform well. Capture probability, power, precision, and sampling effort estimates from the four

Table 3.3. Description of models and parameter values used to predict bear rub sampling effort required to exceed 80% power to detect λ=0.97 within 10 years for the grizzly bear population in the Northern Continental Divide Ecosystem, Montana, USA.[a] All model outputs are for year 10 of simulations.

Model type	Capture probabilities used in simulations[a]				Power[b] (%)		CV(λ) (%)		CV ($\hat{N}$)		% Relative bias		Estimated RSE[c]	
1 Session (non–robust design)	M		F		M	F	M	F						
Gender–specific	0.53		0.26		83.2	80.8	1.0	1.1			N/A		165	
Pooled genders	0.53		0.26		84.4		1.0				N/A		60	
2 Sessions (robust–design)	S 1	S 2	S 1	S 2	M	F	M	F	M	F	M	F	S 1	S2
Gender–specific	0.37	0.36	0.13	0.19	83.6	83.2	1.0	1.1	7.9	12.1	3.2	3.0	65	65
Pooled genders	0.37	0.36	0.13	0.19	83.2		0.8		9.7	15.4	2.9	4.0	40	50

λ = realized rate of population growth; φ = apparent survival

[a] All models used $\varphi_{males} = 0.87$, $\varphi_{females} = 0.92$, $\lambda = 0.97$, with recapture probabilities set equal to capture probabilities ($c = p$) for robust design models. True population sizes: males = 294, females = 470.

[b] Power estimates are ±3.5%.

[c] In thousands. Rub sampling effort (RSE) was defined as the cumulative number of days between successive hair collections summed over all bear rubs sampled per time period. RSE for non–robust designs was summed for the entire sampling period each year; robust–design effort was summed for each of 2 secondary occasions per year.

simulation scenarios are summarized in Table 3.3. The non–robust, gender–pooled model required the lowest annual sampling effort to detect a declining population, which is in contrast to previous simulations where the robust design showed greater power. This was likely due to low female capture probabilities in the first secondary occasion as simulated in this scenario. This hypothesis is supported with the gender–specific models, which required greater capture probabilities (and therefore effort) overall. This allowed the robust design model to perform better, and resulted in 21% less effort needed by the robust design than the non–robust design to attain adequate power with nearly identical precision.

DISCUSSION

Effective programs for monitoring wildlife populations should serve two primary purposes consistent with adaptive management: (1) provide periodic assessments of the status and trends of population metrics of concern, and (2) improve understanding of how populations respond to management actions (Pollock et al. 2002, Nichols and Williams 2006). As such, effective monitoring programs must focus on acquiring the information needed to make management decisions in a useful timeframe, as well as providing insight into the nature of the parameters being monitored and the factors impacting them (Nichols and Williams 2006). Predicting the response of an animal population to management actions is usually imprecise. In addition, even if a response is detectable, the time lag may be too long to change trajectory within an acceptable timeframe. Imprecise or irrelevant metrics often fail to identify problems until either it is too late to prevent precipitous population declines, or rescue would require extraordinary measures. Avoiding such scenarios through early detection of declines should be one of the primary objectives of any monitoring program.

Obtaining measures of population abundance continues to be an important focus of most wildlife management and conservation plans (Nichols and Hines 2002, Schwartz et al. 2007). Improved analytical techniques and specialized software applications have made it easier to produce robust and precise estimates of abundance for many closed and open populations (Pollock et al. 2002, Williams et al. 2002, White 2008). Advances in sampling methodologies, such as remote cameras and noninvasive genetic sampling, also

have led to improvements in our ability to sample wildlife populations at scales and intensities not possible just a few decades ago (Pollock et al. 2002, Kendall et al. 2009). However, despite these advances and the repeated warnings of statisticians, uncorrected counts continue to be used as indices of population abundance and trends (Slade and Blair 2000, Anderson 2001, Williams et al. 2002). For example, uncorrected indices usually assume that changes in raw counts reflect true changes in population abundance or density and not simply a change in detection rates, a condition that is difficult to assess (Williams et al. 2002). However, indices and various forms of convenience sampling may represent the only option for studying some systems and have been shown to be valid in some special circumstances (Sandercock and Beissinger 2002, Williams et al. 2002).

Grizzly bears have been a federally–listed threatened species in the contiguous United States since 1975. Despite this, monitoring of the NCDE grizzly bear population until recently consisted of opportunistic counts (sightings) of females with cubs, distribution of females with young, and known, human–caused mortalities. These measures were understood to be imprecise and, therefore, limited inferences about population status or trend based on them were possible (USFWS 1993, Mace 2005, Kendall et al. 2009). To improve our understanding of population processes, an ecosystem–wide monitoring program was initiated in 2004 that relied on maintaining a radio–collared sample of $\geq$25 independent females in the NCDE (Mace 2005). Live capture of grizzly bears is expensive, logistically difficult in remote areas, requires specialized training of field personnel, has inherent risk to both bears (Cattet et al. 2008) and trappers, requires aerial relocation of bears to monitor dependent offspring survival, and may be subject to intense scrutiny and potential moratoria on public lands. Although tracking the fates of individual bears is necessary to measure vital rates and may provide a better understanding of what drives population trend (e.g., cause–specific mortality), noninvasive genetic sampling represents a powerful complement, and potential alternative, to traditional methods of monitoring population trend.

Most NGS bear studies have been limited to baited hair traps to collect samples for genetic analysis and subsequent abundance estimation (Boulanger et al. 2002; Kendall et al. 2008, 2009). These studies have produced estimates of unprecedented precision for

a species that is difficult to detect, and have done so in expansive, remote areas. Recently, two of the largest DNA–based grizzly bear studies have used repeated surveys of naturally–occurring bear rubs in conjunction with baited hair traps to collect hair samples. In these studies, inclusion of samples collected from bear rubs increased the minimum number of bears detected by 22–24%, and resulted in more precise abundance estimates than would have been possible with hair trap data alone (Boulanger et al. 2008a; Kendall et al. 2008, 2009).

Collecting hair from bear rubs offers several significant advantages over grid–based methods, including more flexible sampling design and collection schedules which will ultimately reduce personnel needs and project costs. Surveys of bear rubs can be conducted entirely on maintained routes (e.g., hiking trails and power pole lines) eliminating the need for helicopters and off–trail travel often required in hair trapping studies. Further, bear rub sampling does not require the production, transportation, and application of putrid smelling lure because rubbing is a natural behavior of bears. Mace et al. (1994) noted that annual and seasonal variation in food availability had dramatic effects on success at baited, remotely triggered camera sets, resulting in wide confidence intervals in their population estimates. Harris (1984) reported extremely low bear visitation rates while using a number of scent baits in multiple study areas, including some areas with high bear density. There is also evidence that capture probabilities at hair traps are lower for bears that have been live captured (Boulanger et al. 2008b, Kendall et al. 2009). This results in greater heterogeneity of capture probabilities, which is difficult to model without knowledge of which bears have been live captured (Boulanger et al. 2008b). Another potential issue with hair sampling methods requiring scent lure is a waning attraction to the lure as bears learn that no food reward is present, analogous to the behavioral response of becoming "trap shy." Such a response is not expected with bear rubbing as it is a natural behavior that does not require artificial stimuli.

In what may be part of the reason bears evolved rubbing (marking) behavior, hair tufts and bear rubs in general are far more persistent and conspicuous evidence of a bear's presence than other kinds of sign such as scat and tracks (Karamanlidis et al. 2007). With regard to monitoring, scat and tracks may not persist long enough to be

detected under some environmental conditions, track surveys suffer from low power to discern unique individuals, and both methods rely on the assumption that counts share a direct relationship with animal abundance or density (Kendall et al. 1992, Hayward et al. 2002). Such assumptions are difficult to validate, and add uncertainty to perceived changes in population status.

Conversely, use of mark–recapture based methods to assess population rates of change have increased in recent years, and appear especially well–suited to noninvasively derived encounter data. For example, Boulanger et al. (2004) used DNA–based detections with the Pradel model to investigate the relationship between salmon availability and grizzly bear numbers in three sampling areas in British Columbia, Canada. Compared to using helicopters to count individual bears, they found that mark–recapture based methods yielded improved precision of demographic estimates and a better understanding of how changing environmental conditions affect population trends (Boulanger et al. 2004). Sandercock and Beissenger (2002) directly compared λ estimates derived from the Pradel model to those of asymptotic projection matrices and ratios of population counts. They found estimates to be in general agreement, but the Pradel model had greater precision and required less effort than the matrix–based method. However, Barker et al. (2002) advised that a clear distinction between the realized λ estimates of the Pradel model and asymptotic expectations of projection matrices must be made, and predictions based on retrospective mark–recapture data should be made only cautiously.

With proper model formulation, Pradel model estimates of λ have been found to be robust to moderately heterogeneous capture probabilities (Hines and Nichols 2002), such as those found in bear rub data. And although behavioral responses, especially permanent ones, can bias λ estimates (Hines and Nichols 2002), no such response is expected to exist with bear rub sampling. It should be emphasized, however, that λ estimates generated by the Pradel model are only applicable to the cohorts from which the encounter histories are obtained. Although NGS methods do not yield age information, Kendall et al. (2009) concluded that individuals of all sex–age classes were detected in bear rub samples. However, more research is warranted to better estimate detection rates of dependent offspring in this and other populations.

Other advantages of the Pradel (1996) temporal symmetry models include estimation of apparent survival, which incorporates both true survival and emigration. Therefore, in populations that are essentially geographically closed, the Pradel model provides approximate estimates of true survival. However, I found that estimates of φ with very low capture probabilities and/or few sampling occasions (e.g., $p \leq 0.05$; ≤ 5 occasions) appear to be substantially negatively biased with mixture model formulations. The only other instance of substantial bias (i.e., > 5%) of any parameters occurred with φ and p estimates with non–robust models with only two years' data. In all simulations, bias levels returned to <5% by year four. This indicates that estimates from the first few years of a monitoring program must be interpreted with caution for non–robust models. Robust–design models did not display this behavior, and bias levels remained <5% for all parameter estimates.

My simulations with the Pradel models in program MARK suggest that annual surveys of bear rubs, given sufficient sampling effort, have good power to detect even slight rates of population decline in the NCDE grizzly bear population. Based on my experience conducting bear rub surveys throughout this ecosystem, I estimated that a dedicated staff of approximately 12 crews could survey a sufficient number of bear rubs in this > 32,000 km^2 area to detect a declining population ($\lambda \leq 0.97$) with $\geq 80\%$ power within six years. With data collected over multiple years of a monitoring program, the ability to include group (i.e., gender), temporal, and individual covariates (e.g., distance of average capture location to open study area boundary; Kendall et al. 2008, 2009) should increase the precision of λ estimates further, and may result in increased power to detect population trend. Different monitoring objectives, such as gender–specific estimates, would require adjustments to the required sampling effort and labor needs.

Annually fluctuating population growth rates may mask overall net declining (or increasing) abundance. Simulations suggest that additional years' data or increased capture probabilities would be required to achieve the same degree of power to detect a declining population under such a scenario (Appendix B). For example, with λ alternating between 0.94 and 1.01 annually, which results in the same net reduction in abundance after 10 years, two additional years' sampling are required to exceed 80% power to detect a decline given the same capture probabilities as I used throughout this

paper. Also, the ability to detect positive growth rates is clearly important to managers. Simulations with $\lambda = 1.03$ required nine years to exceed 80% to detect an increasing population for both robust and non–robust models. Precision increased rapidly; however, confidence interval coverage on λ and abundance estimates was slightly poorer than scenarios of a declining population. The power of bear rub surveys to estimate trend under variable or positive growth rates adds confidence to their application for long–term monitoring in real–world conditions.

Although regressions of bear rub sampling effort against the number of bears detected had high R^2 values (only the first secondary occasion female regression ($R^2 = 0.87$) was < 0.96) and seemed reasonable given my knowledge of bear rub surveys, I regard these predictions as rough approximations intended for exploring general survey design in this population. I believe the RSE measure adequately reflects the ability of bear rubs to detect bears at large spatial and temporal scales, in part because it has been overwhelmingly supported as a temporal covariate in abundance estimation models in both the GGABDP and NDBDP (Boulanger et al. 2008a; Kendall et al. 2008, 2009). However, simply increasing the number of bear rubs surveyed without allowing adequate time for hair to accumulate (e.g., 15–30 days) will not result in increased detections. Another important design issue is that changes in sampling design or study area can confound Pradel model estimates. For example, increasing the spatial extent of sampling may appear as an increasing population trend as more animals become available for detection (Barker et al. 2002). To avoid these effects, I recommend that a greater investment be made in the initial year of a monitoring project to establish as many bear rubs as possible over the geographic area of interest, and that the sampling design remains stable over the course of any multi–year project.

Numerous other sampling methods have been developed that may prove to be useful for monitoring growth rates for other populations and species. For example, Beier et al. (2005) devised a single–use hair grabber to be deployed along bear trails leading to salmon feeding streams. This concept offers the advantage of minimizing the risk of mixed samples in areas of high bear density, but it had a tendency to collect samples from non–target species such as deer, and would not be suitable for areas of human use (Beier et al. 2005). Also, with recent improvements in fecal genotyping methods (e.g.,

Bellemain and Taberlet 2004, Luikart et al. 2008), scat sampling has reemerged as a viable sampling method for numerous species. Although sampling design issues will have to be addressed for other species and populations, noninvasive methods such as bear rub or scat sampling may offer many advantages over traditional baited or live–capture based methods.

MANAGEMENT IMPLICATIONS

Bear rub surveys present an efficient, safe, flexible, noninvasive, and relatively inexpensive means to collect data capable of estimating rates of population change and abundance. With the extensive geographic coverage of bear rubs and the large number of individuals detected, a bear rub–based monitoring program could also: (1) detect genetic population substructure, (2) document changes in relative density patterns and occupied habitat, (3) provide an extensive genetic archive that could be maintained to monitor other aspects of population status and genetic health, the value of which has been demonstrated in the Greater Yellowstone Ecosystem (Miller and Waits 2003), and (4) provide biological material that can be used to investigate other important facets about the grizzly bear population, such as diet and contaminant load through stable isotope and elemental analysis.

Genetic sampling will be most powerful in conjunction with collaring–based efforts that allow researchers to investigate cause–specific mortality, unreported mortalities, reproductive rates, response to human activities, and habitat use. The synergistic effects of multiple monitoring methods may result in more responsive and efficient management than either method alone can produce.

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Appendix A. GRIZZLY BEAR RECOVERY PLAN MONITORING PROGRAM METRICS (U.S. FISH AND WILDLIFE SERVICE 1993) AND MOLECULAR SAMPLING RESULTS IN 2004 IN THE NORTHERN CONTINENTAL DIVIDE ECOSYSTEM (NCDE) IN NORTHWESTERN MONTANA, USA.

Recovery criteria type	Recovery Plan targets: must be met for population to be considered recovered	Monitoring interval	2004 Recovery Plan monitoring results	2004 NCDE Hair Snare Project results: comparison with recovery criteria
Demographic and distribution: population size inside and outside GNP	≥10 FwC inside GNP and ≥ 12 outside GNP within 16 km of RZ excluding Canada. Using the Recovery Plan method to derive population estimate from counts of FwC, total population needed = 391.	Running 6–yr average of FwC counted for use in estimating population size.	13 FwC inside and 8 FwC outside GNP. Using Recovery Plan method to derive population estimate from counts of FwC, total population = 304.	Min. count: 131 F and 98 M bears inside and 190 F and 144 M bears outside GNP. Total population estimate = 765 (471 F and 294 M). Note: direct estimate of population size and min. counts of bears in– and outside GNP can identify no. of F but not age or reproductive status.
Distribution: FwY–total	21 of 23 BMUs occupied by FwY; no 2 adjacent BMUs unoccupied.	Running 6–yr sum of observations	All BMUs occupied; No. of FwC /BMU not available.	All BMUs occupied by F of unknown age. No. of F/BMU range 2–56. Total count of F, not just FwY.
Distribution: FwY–specific	Mission Mountains occupied by FwY.	Not stated.	Mission Mountains occupied. No. unique FwY not available.	Detected 12 unique F (reproductive status unknown).
Mortality: Total	Known, human–caused mortality ≤ 4% of population estimate (based on 3–year sum of FwC.	Cannot be exceeded for any 2 consecutive years.	Total mortality = 10.5%; exceeds threshold.	Total mortality = 4.6%; slightly above threshold.
Mortality: Female subquota	Of the above 4%, ≤ 30% shall be females.	Cannot be exceeded for any 2 consecutive years.	Allowable F morts ≤ 3. Recorded F morts = 18 (6X allowable level).	Allowable F morts ≤ 9 based on 2004 NCDE population estimate. Recorded F mortality = 20 (2.2 X allowable level).

GNP = Glacier National Park, M = male, F = female, FwC = females with cubs, FwY = females with young of any age, RZ = Recovery Zone, morts = mortalities, BMU = Bear Management.

Appendix B

Additional Simulations of Bear Rub Data with the Pradel Models

INTRODUCTION

Population processes are exceedingly complex and dynamic. There are too few reliable estimates of population growth rates over extended time periods for grizzly bears to make predictions about what is realistic or likely to happen in other populations. While it is possible that the grizzly bear population of the Northern Continental Divide Ecosystem (NCDE) could experience a consistent annual decline for an extended period such as simulated in Chapter III, it is perhaps more likely that annual growth rates (λ) will fluctuate from year to year, and may even vary between genders. The simulations presented in Chapter III assumed a uniform $\lambda = 0.97$ for both males and females. This value was selected because it equates to a 26% overall decline in abundance after 10 years, a decline that would certainly warrant management intervention. A 3% annual decline would be a subtle change from one year to the next, and extremely difficult to detect given the low and variable density and cryptic nature of bears in this population.

I performed additional simulations with the Pradel (1996) models in program MARK (White and Burnham 1999) to evaluate the ability of bear rub encounter data to determine that a population is experiencing a net decline when λ alternates between 0.94 and 1.01 annually. These λ values result in the same overall decline in abundance after 10 years as with λ held constant at 0.97 (Fig. B1). With the exception of λ values, parameter values used in the simulations were the same as presented in Chapter III. As in Chapter III, I evaluated scenarios of gender–pooled and gender–specific λ estimates for both robust and non–robust designs. I also evaluated model performance with regards to annual abundance estimates for robust–design models. Each simulation scenario was run 500 times, which results in power estimate uncertainty of ±3.5%. Details of simulations and derivation of parameter values can be found in Chapter III.

I also evaluated the ability to detect an increasing population trend ($\lambda = 1.03$) with the Pradel (1996) models using bear rub encounter data. I again used the same parameter values as given in Chapter III other than λ. For these simulations, I compared robust and non–robust, gender–specific models for power and precision of estimates.

RESULTS

Percent relative bias of λ estimates did not exceed 1.05% in any scenarios with λ fluctuating between 0.94 and 1.01. As predicted, it required more years' data to achieve 80% power to detect a declining population for both robust and non–robust models (Figs. B2, B4). Given that the underlying data, model properties, and output data types are analogous for estimating λ between robust and non–robust models, these estimates were effectively unchanged across model types. The pattern for estimates of coefficient of variation (CV) and confidence interval coverage (CIC) were also similar across robust and non–robust model types. In both cases, and for both gender–specific and gender–pooled estimates, CIC tended to decline as standard errors (SE) decreased, yet remained above 85% for all scenarios. The reduced SEs also resulted in improved CV estimates, which exceeded 5% only in the first two years' estimates.

Abundance estimates from the robust–design model showed similar patterns to scenarios with constant λ values, with estimated CIC declining for both genders, but more quickly for males. Also as with constant λ models, the CV on abundance estimates increased with additional years' data as standard errors improved over time, but not proportional to the decreasing abundance estimates themselves, resulting in an apparent, albeit very slight, decrease in estimate precision. All CVs on abundance estimates remained < 9%. Bias remained negligible, remaining below 1.5% for all simulations.

Scenarios with $\lambda = 1.03$ yielded power estimates surprisingly similar to scenarios of a constant declining population. In theory, identifying an increasing population trend should be easier, as more individuals are available to be detected each year. However, robust and non–robust gender–specific models required 9 years to achieve 80% power to detect an increasing population (Fig. B5). Estimates of precision also showed similar performance to other simulation scenarios. Confidence interval coverage maintained high levels (>90%) through all years for females; male CIC estimates remained >90% through year 8, then declined more dramatically yet remained >80% through year 10. Coefficient of variation estimates improved rapidly, staying below 4% by year 4 for all scenarios. Percent relative bias remained <1% for all scenarios.

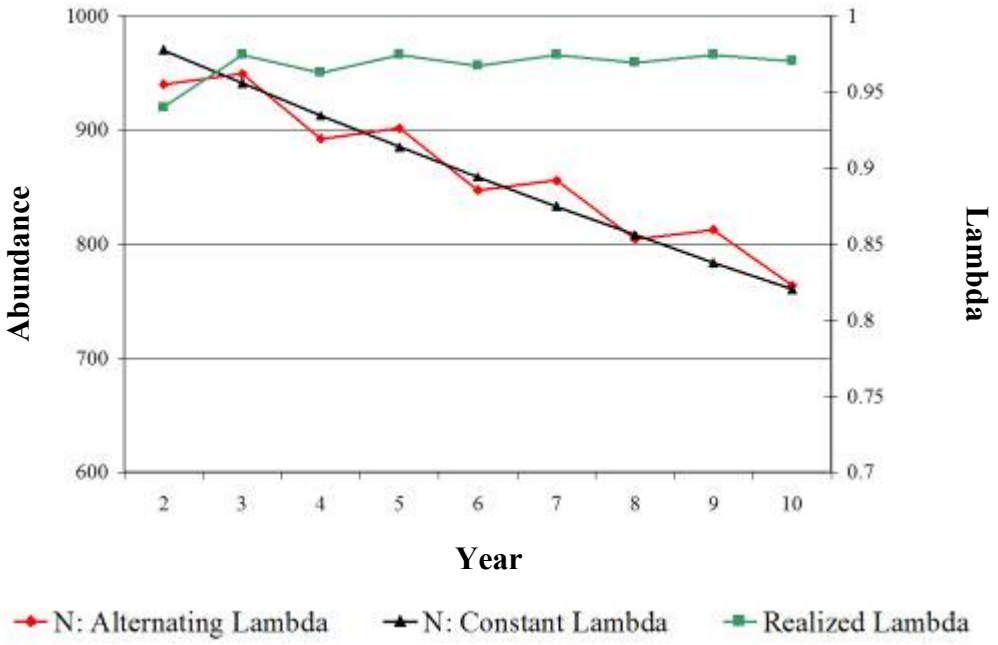

Figure B1. Relationship between abundance estimates under a constant $\lambda = 0.97$ and λ alternating between 0.94 and 1.01. Realized lambda refers to the geometric average of λ values alternating between 0.94 and 1.01; values given on secondary y–axis.

Abundance estimates under the scenario of $\lambda = 1.03$ were also somewhat surprising. Female CIC remained >87% through year 10; however, male CIC consistently declined over all years and approached 60% by year 10 (Fig. AB.6). Given the higher capture probabilities of males, this result is unexpected and warrants further exploration. It is possible, however, that more complex models with actual field data could yield better estimates. Coefficient of variation showed the opposite pattern to those of a declining population: SEs increased slightly, but less so than abundance estimates, resulting in nominally decreasing CVs. Bias remained <1.2% for all scenarios.

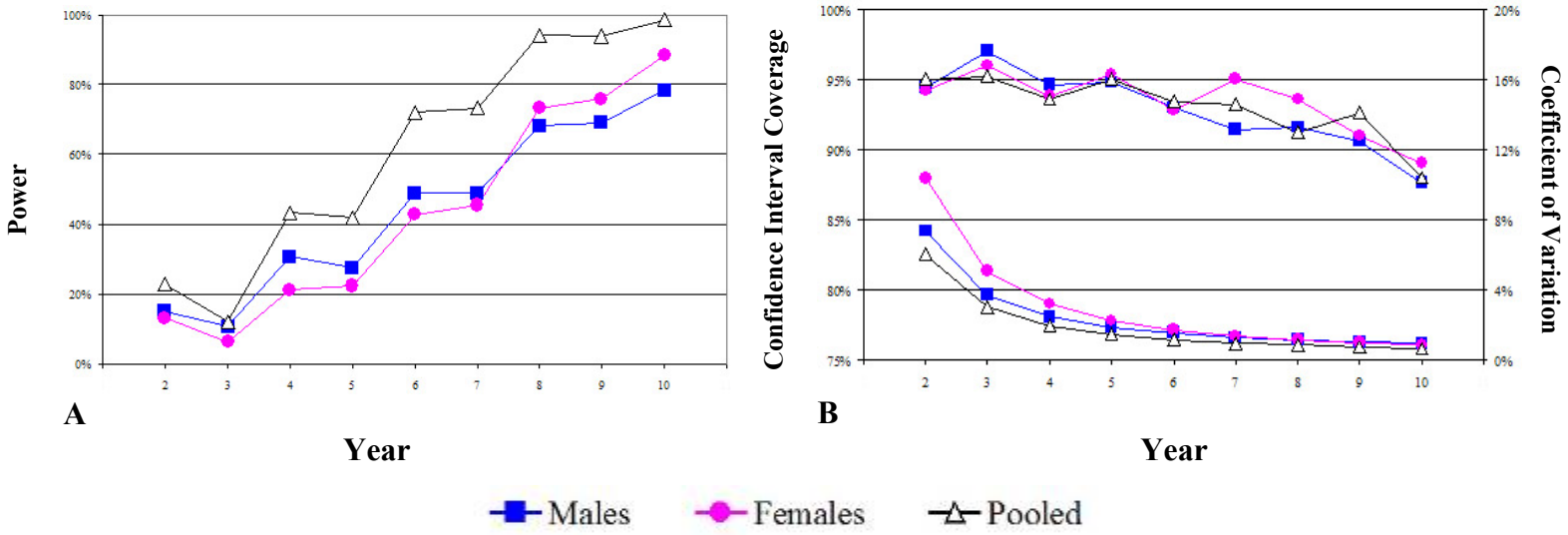

Figure B2. A) Robust–design model estimates of power to detect declining grizzly bear population abundance with λ alternating between 0.94 and 1.01 annually. B) Estimates of coefficient of variation and confidence interval coverage for gender–specific λ estimates. The bottom set of points reflect CV estimates with values given on the secondary y–axis. Based on two secondary occasions per year; parameter estimates as in Chapter III.

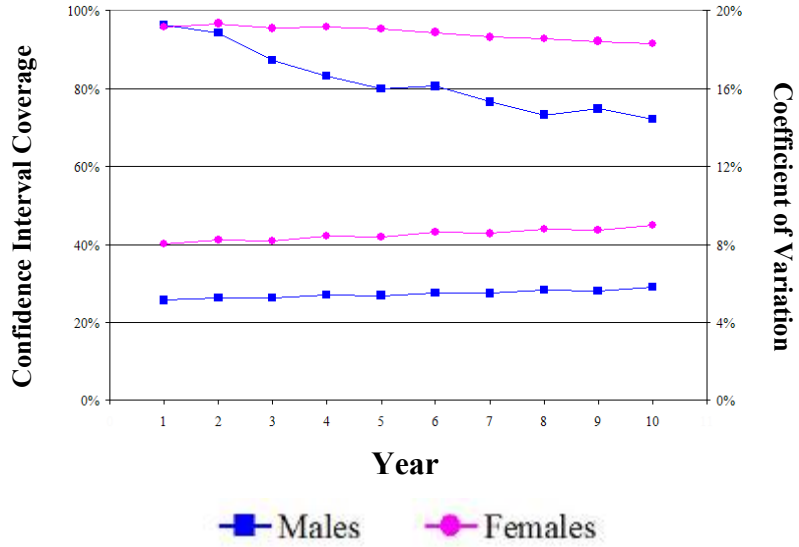

Figure B3. Robust–design model estimates of coefficient of variation and confidence interval coverage for gender–specific grizzly bear abundance estimates. The bottom set of points reflect CV estimates with values given on the secondary y–axis. Based on two secondary occasions per year; parameter estimates as in Chapter III.

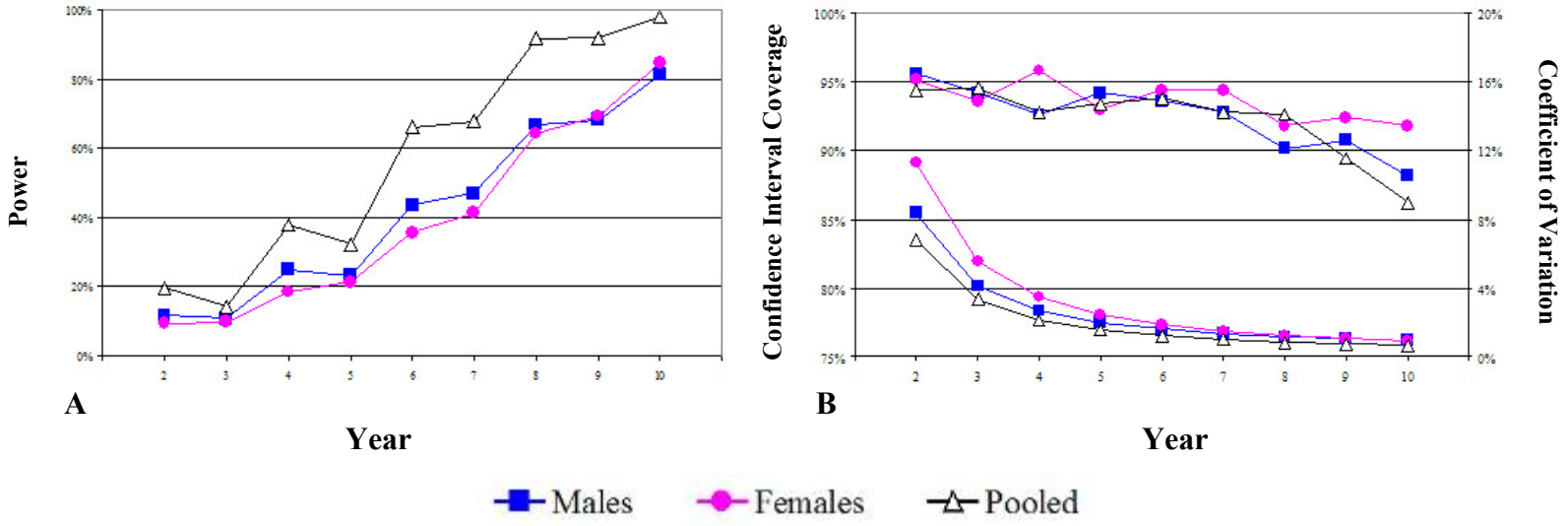

Figure B4. A) Non–robust design model estimates of power to detect declining grizzly bear population abundance with λ alternating between 0.94 and 1.01 annually. B) Estimates of coefficient of variation and confidence interval coverage for gender–specific λ estimates. The bottom set of points reflect CV estimates with values given on the secondary y–axis. Based on one sampling occasion per year; parameter estimates as in Chapter III.

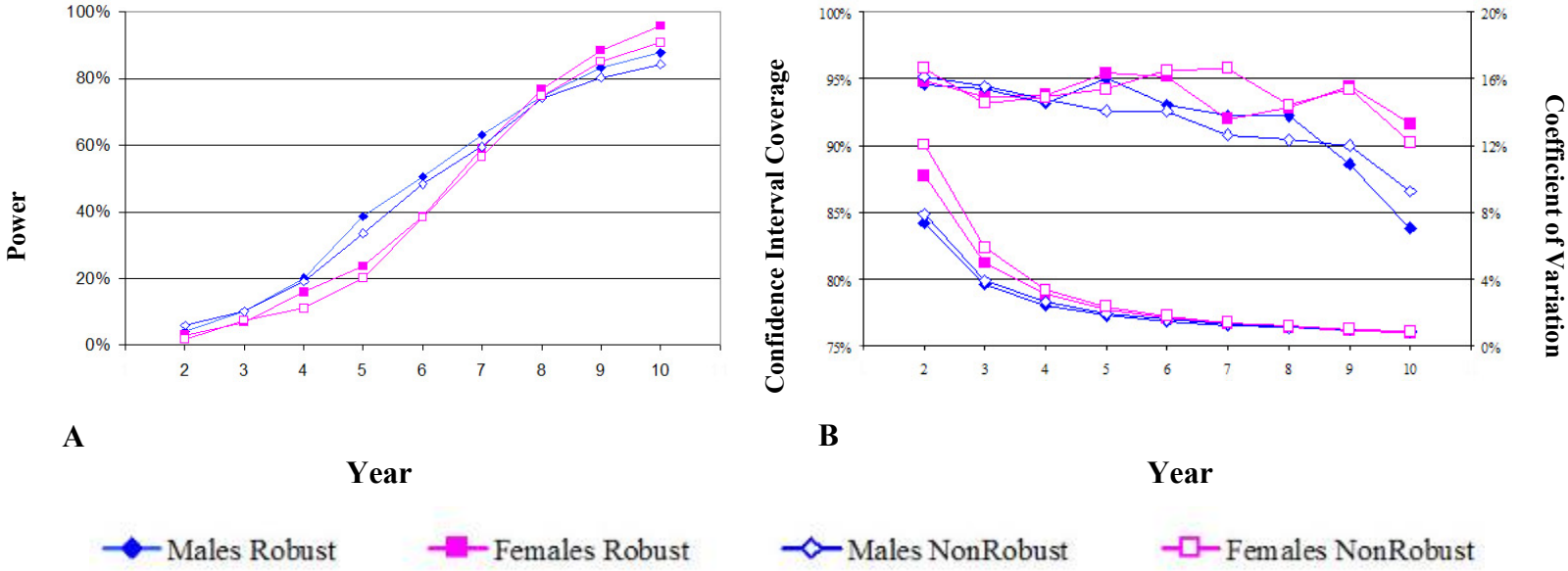

Figure B5. A) Robust and non–robust design model estimates of power to detect increasing grizzly bear population abundance with $\lambda = 1.03$. B) Estimates of coefficient of variation and confidence interval coverage for gender–specific λ estimates. The bottom set of points reflect CV estimates with values given on the secondary y–axis. Robust models based on two secondary occasions per year, non–robust models based on a single sampling occasion annually; parameter estimates as in Chapter III.

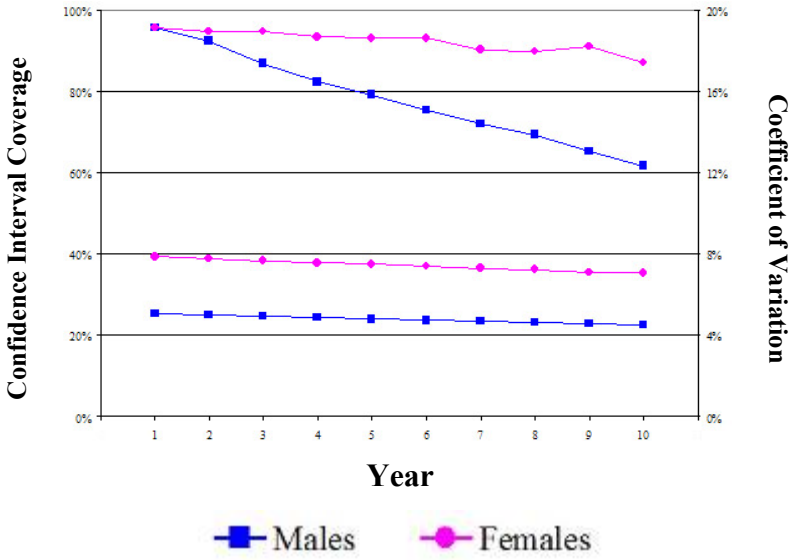

Figure B6. Robust–design model estimates of coefficient of variation and confidence interval coverage for gender–specific grizzly bear abundance estimates with λ = 1.03. The bottom set of points reflect CV estimates with values given on the secondary y–axis. Based on two secondary occasions per year; parameter estimates as in Chapter III.

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PRADEL, R. 1996. Utilization of capture–mark–recapture for the study of recruitment and population growth rate. Biometrics 52:703–709.

WHITE, G.C. and K.P. BURNHAM. 1999. Program MARK: survival estimation from populations of marked animals. Bird Study Supplement 46:120–138.

Appendix C

Working Example of Simulations with the Pradel Model in Program MARK

INTRODUCTION

This appendix is intended to provide sufficient information to allow someone new to program MARK to replicate the Pradel model simulations from Chapter III. Extensive, nearly overwhelming, documentation on MARK is available in a user's manual and in the Help menu included with the program. Further, a web–based forum is available to post questions and search the collective experience of users ranging from novice to expert. A word of caution, however, is that anyone posting a question covered in previous discussions, the Help menu, or in the manual will be quickly and publicly reprimanded. Perhaps the most common reply is "RTFM," which I'll leave the reader to determine the exact meaning of.

Program MARK is a powerful interactive software application that provides parameter estimates based on encounter data from marked or unmarked animals. The range of supported data types and analyses continue to be expanded. These currently include but are not limited to closed and open mark–recapture models for estimating abundance, nest survival, occupancy estimation, and Cormack–Jolly–Seber models. Users have the ability to incorporate a multitude of covariates into their models, select output data types, and perform model averaging based on Akaike's Information Criterion weightings. As is stated in the manual, MARK is the most comprehensive program available for analysis of encounter data and has become the standard for wildlife professionals. However, as the developer Dr. Gary White often says, MARK is like a sharp knife: it is a powerful tool, but can cut deeply if used improperly.

Program MARK is available for download from Gary White's webpage: http://welcome.warnercnr.colostate.edu/~gwhite/mark/mark.htm. This site also contains links to the online forum, a brief overview of MARK, a list of relevant articles and conference proceedings, information about upcoming courses, and a running list of updates to the program. The user's manual ("A Gentle Introduction"), currently in its 7th edition and nearly 800 pages long, is also available for download.

The parameter values and model design used in this example correspond to the robust–design, pooled gender, constant Lambda scenario with 10 years' data. Parameter values and definitions are provided in Table C.1.

Table C1. Description of simulation models and parameter values used to evaluate the power of bear rub surveys to detect a 3% annual decline in the Northern Continental Divide Ecosystem grizzly bear population.

Design Type	Model Notation	Capture Probabilities Used in Simulations [a]			
		Male		Female	
Two secondary occasion robust–design, pooled genders		Sess 1	Sess 2	Sess 1	Sess 2
	$\varphi(g)\ p(g+t)\ \lambda(.)$	0.37	0.36	0.13	0.19

[a] All models used $\varphi_{males} = 0.87$, $\varphi_{females} = 0.92$, $\lambda = 0.97$. Recapture probabilities were set equal to capture probabilities (c = p). True population sizes: males = 294, females = 470.

Figure C1. Opening splash–screen for program MARK. Select "Set up Simulation" from the File drop–down menu at top–left. Double–click in the open space of the dialog box that appears to override the warning.

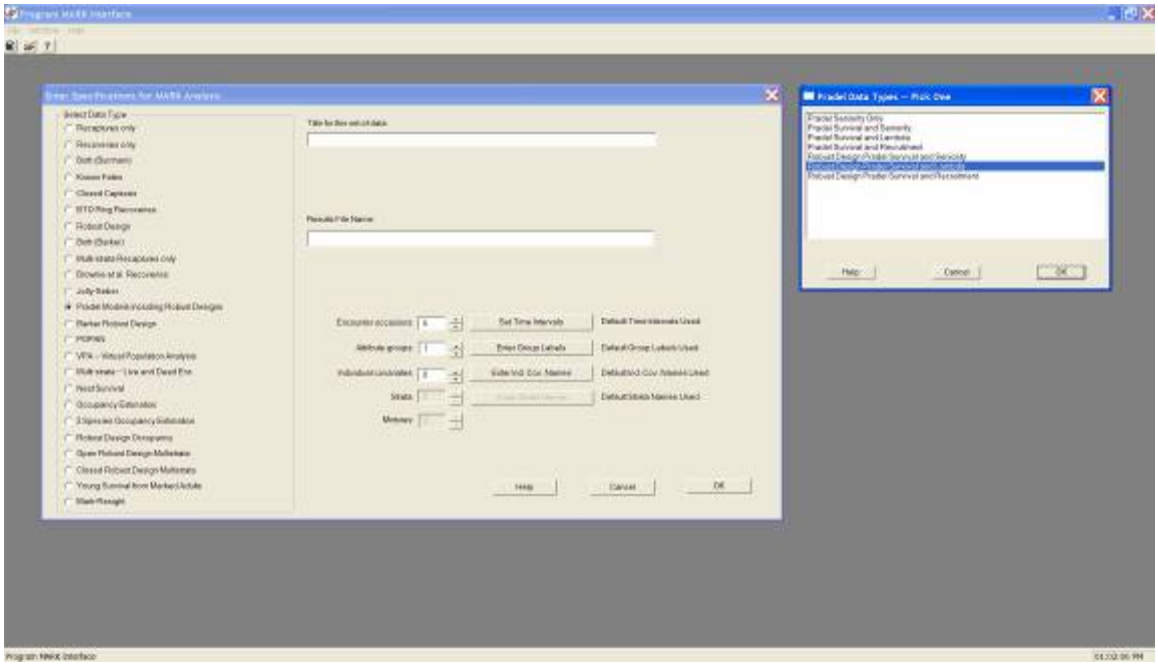

Figure C2. Initial dialog box where the simulation Data Type is selected. The Pradel models include seven options: seniority only, survival and seniority, and survival and recruitment. Robust–design models exist for the last three data types. Here I have selected the Robust Design Pradel Survival and Lambda model type.

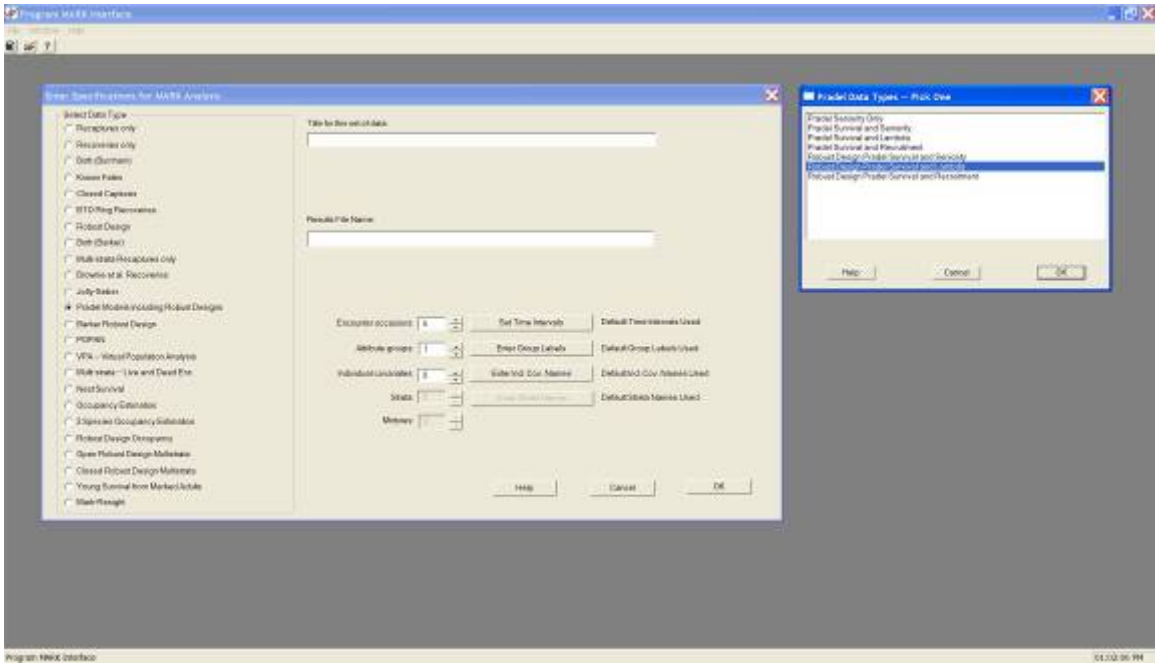

Figure C3. Within the Robust Design Pradel Survival and Lambda model type there are 12 closed captures data types to choose from: closed captures, Huggins closed captures, closed captures with heterogeneity, full closed captures with heterogeneity, Huggins heterogeneity, and Huggins full heterogeneity. Each of these has a corresponding version that allows misidentification of individuals (e.g., from genotyping errors). Heterogeneity cannot be modeled with only two secondary occasions, and I assumed no misidentifications (see Chapter II for justification). The Huggins closed captures was used for robust–design simulations. For non–robust models, there is no selection of data type beyond those options listed in Figure C2.

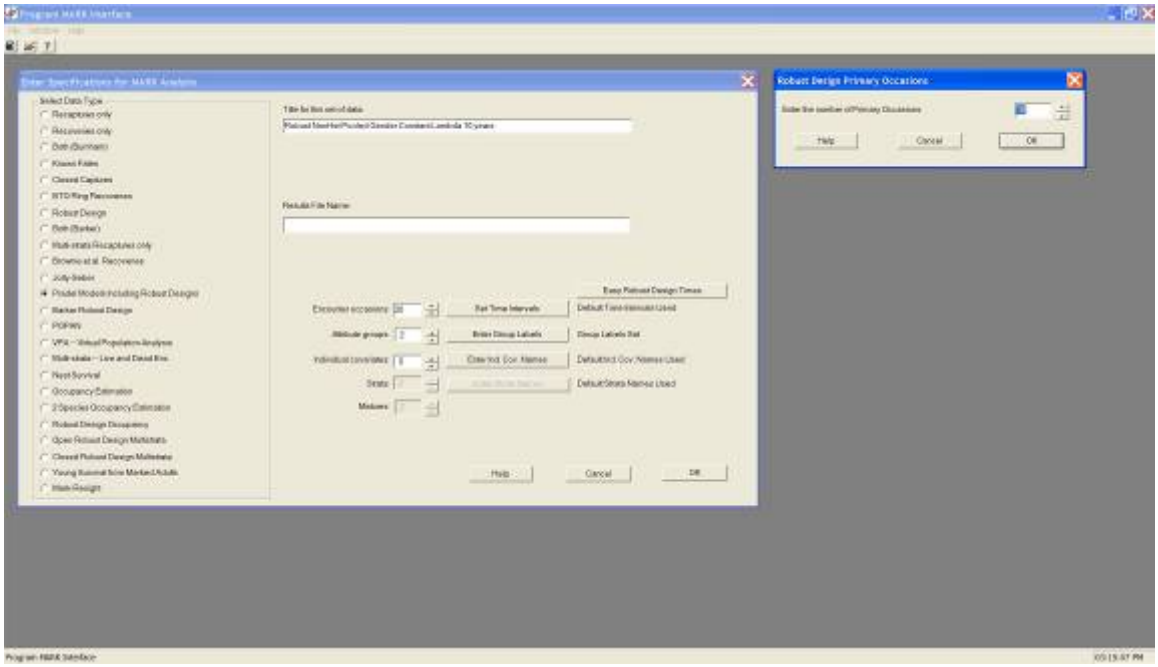

Figure C4. The total number of encounter occasions is the product of secondary and primary occasions. Therefore, for 10 years with two secondary occasions annually, the total number of occasions is 20. By clicking on the Easy Robust Times button, the user enters the number of primary occasions (10 in this case). MARK then verifies that the user desires to equally allocate secondary occasions across primary occasions. The number of attribute groups is also set at this stage. I simulated gender–specific values of survival and capture probabilities, so there are two attribute groups. Group labels can be entered here as well. Click OK.

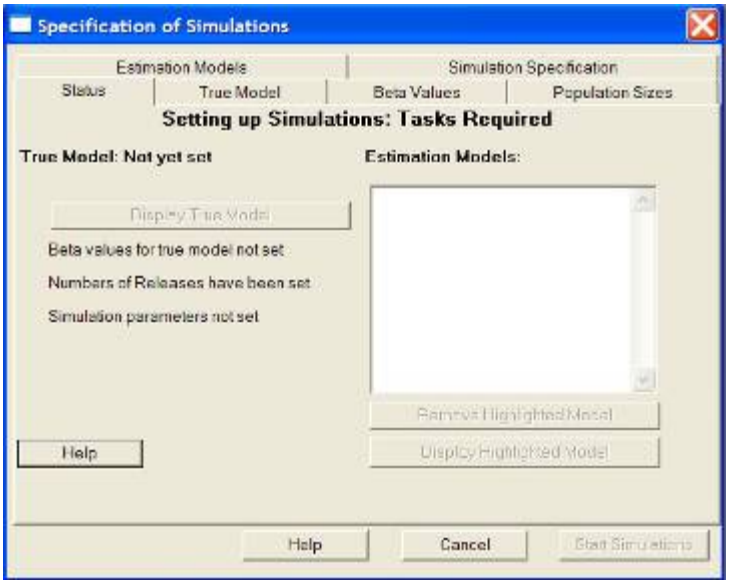

Figure C5. The next window shows a series of tabs that set the specifications of the simulation. Each must be completed before MARK allows the simulations to be run. MARK displays the status as each component is completed.

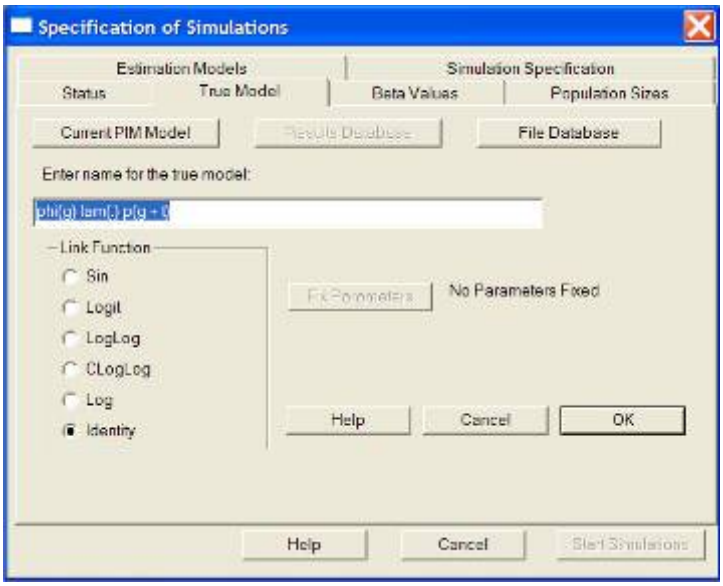

Figure C6. The True Model generates the simulated dataset from which the Estimation Model samples from. MARK provides a graphical interface to design models; the Parameter Index Chart (select under the PIM drop–down menu) initially displays the full model based on the number of encounter occasions, data type selected, and number of groups.

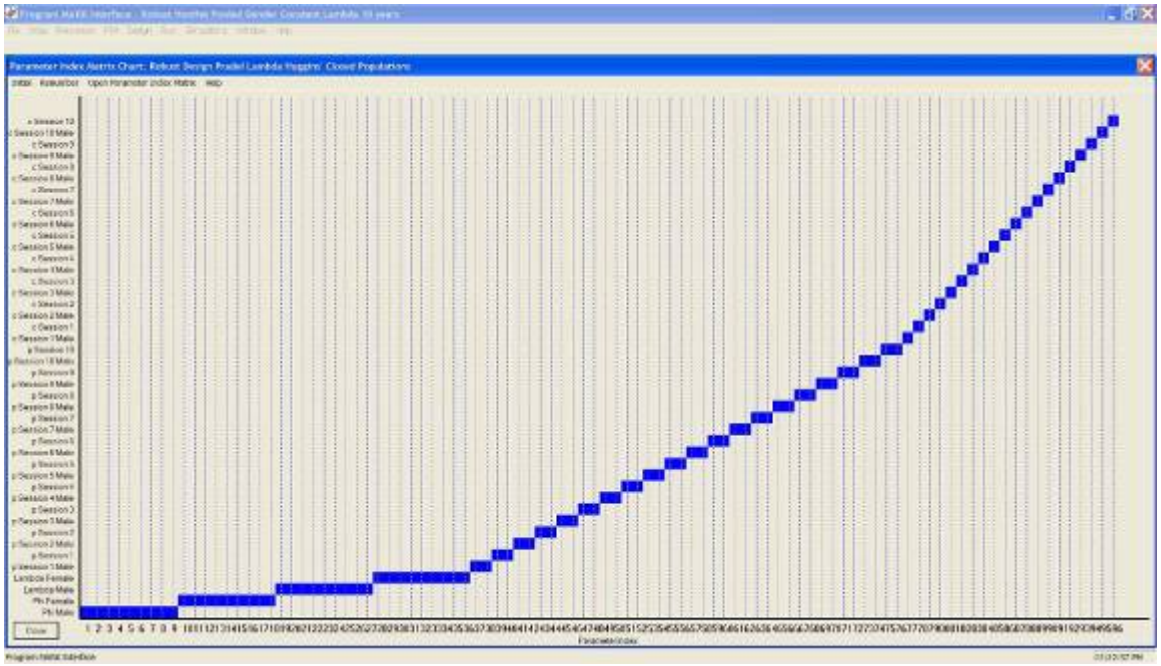

Figure C7. Example of a full model Parameter Index Chart. This model would allow unique survival, lambda, and capture probabilities for each group for each occasion. I assumed constant lambda and apparent survival (phi), with capture probabilities being unique only by group and secondary occasion (i.e., not different across primary occasions).

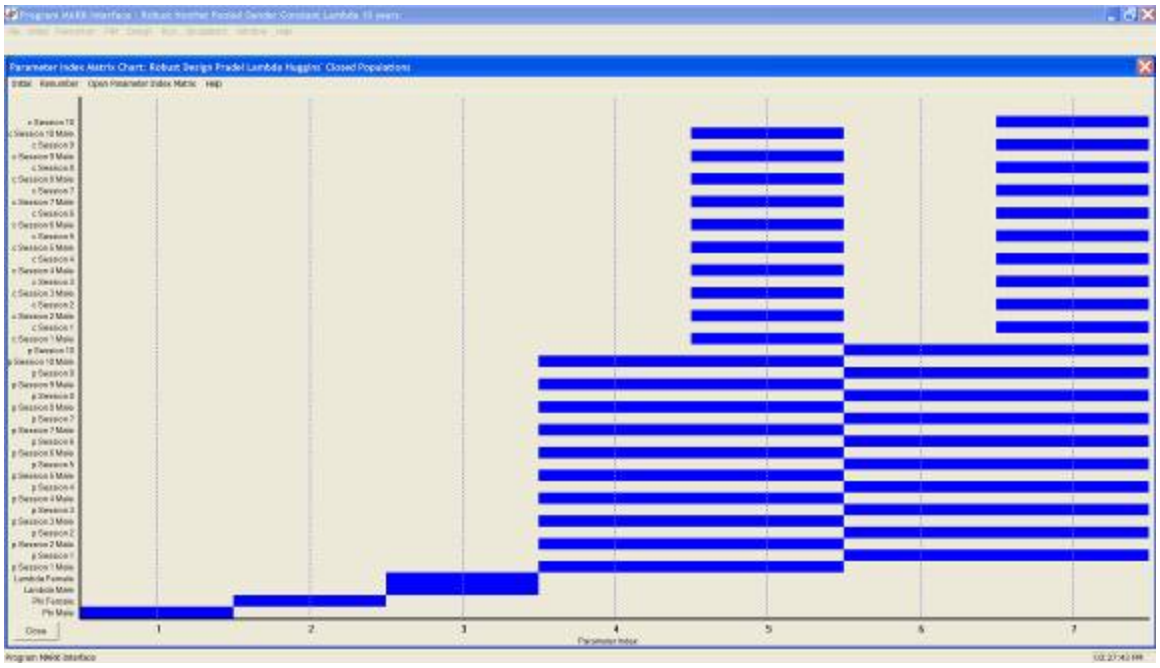

Figure C8. The Parameter Index Chart displaying the true model as described in the previous figure. Phi is gender specific but does not change across years, lambda is the same for both genders, and each gender has a unique capture probability for each secondary occasion (based on empirical estimates from Chapter III) that does not change across primary occasions. Click the "Current PIM Model" button, enter an appropriate name for the True Model, and click OK.

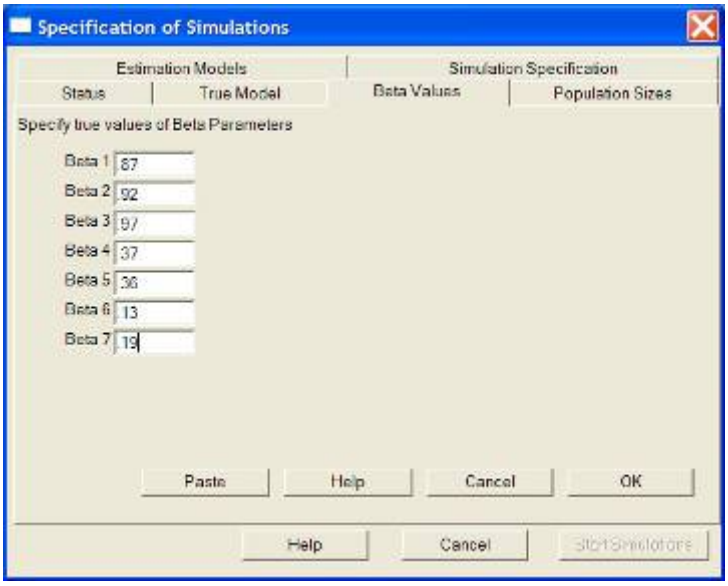

Figure C9. When the true model has been defined, the user enters the beta values to be simulated. Each beta corresponds to one of the blue bars in the Parameter Index Chart. For example, Beta 1 above represents male apparent survival (phi) and beta 6 represents female capture probability for the first secondary occasion. Click OK.

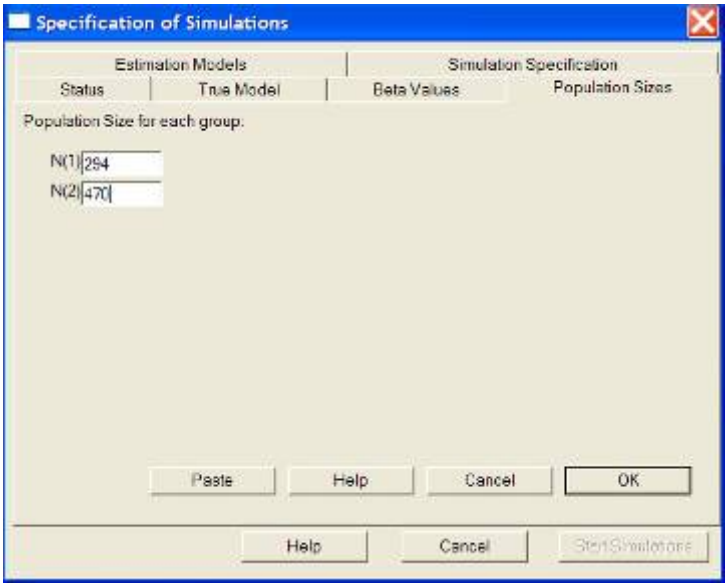

Figure C10. Enter the beginning abundance value for each group. N(1) represents the initial population size for males, N(2) for females. Click OK.

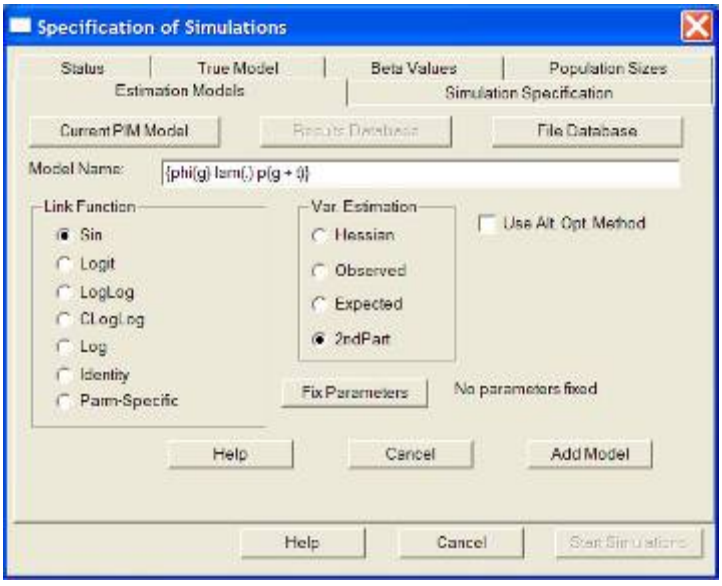

Figure C11. The estimation model is defined following the same basic steps as used for the true model using the Parameter Index Chart. The estimation model should be viewed as an *a priori* hypothesis based on your expectations of the population. In this scenario, I assumed the true and estimation model were the same, i.e., that males and females have different survival rates, different capture probabilities across secondary occasions, but a common lambda. Therefore, I did not change the Parameter Index Chart; click Current PIM Model, enter an appropriate model name, and click Add Model. As I was not interested in comparing or averaging models, each simulation used a single estimation model.

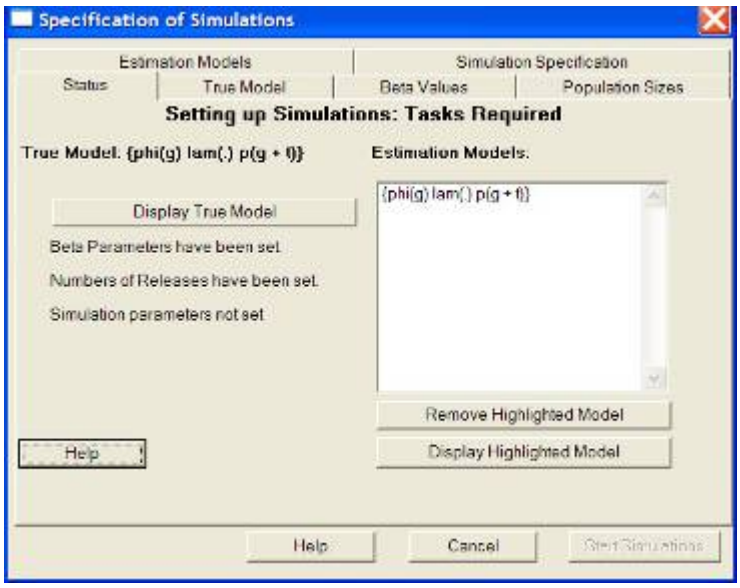

Figure C12. The specification window displays the estimation model that was just added as well as the current status of the simulation set up.

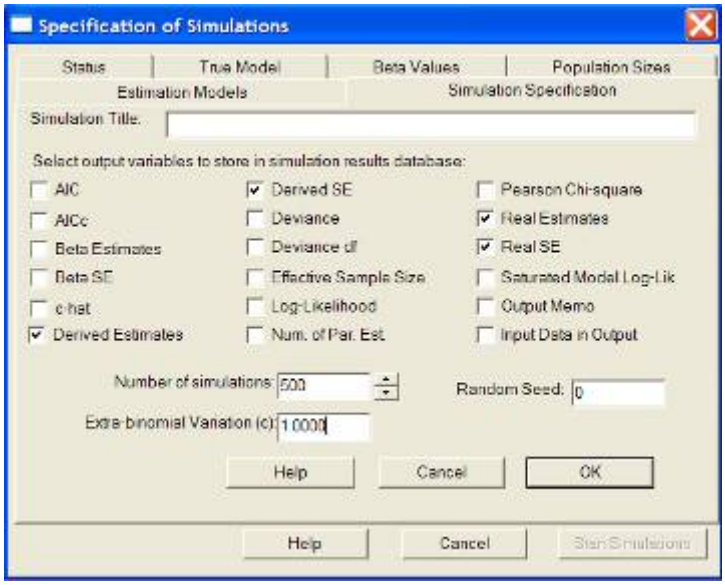

Figure C13. The final tab allows the user to select the output variables, number of simulation runs, and amount of extra-binomial variation. Abundance is a derived estimates for robust–design models; real estimates include capture probability, apparent survival, and lambda. Corresponding estimates of standard error are needed to calculate confidence interval coverage, coefficient of variation, and power.

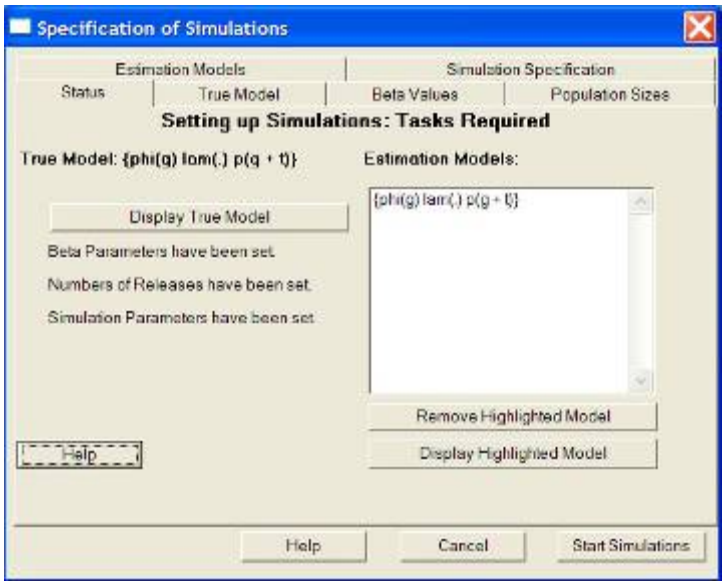

Figure C14. Once all specifications have been defined, the Start Simulations button becomes active. Upon clicking this, MARK prompts the user to name and select a location to store the output files. Output data are stored in a .DBF file, which can be analyzed and manipulated in any spreadsheet application such as Microsoft Excel. Additional simulation runs can be appended to an existing run within program MARK.

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