An empirical test of DNA mark–recapture sampling strategies for grizzly bears

John Boulanger[1,7], Michael Proctor[2,8], Stefan Himmer[3,9], Gordon Stenhouse[4,10], David Paetkau[5,11], and Jerome Cranston[6,12]

[1]*Integrated Ecological Research, 924 Innes, Nelson, BC V1L 5T2, Canada*
[2]*Department of Biological Sciences, University of Alberta, Edmonton, AB T6G 2E9, Canada*
[3]*Arctos Wildlife Services Site 10, Comp. 7, R.R. 1, Crescent Valley, BC, V0G 1H0, Canada*
[4]*Sustainable Resource Development, Fish and Wildlife Division, Hinton, AB T7V 1X7, Canada*
[5]*Wildlife Genetics International, Box 274, Nelson BC V1L 5P9, Canada*
[6]*Foothills Model Forest, Box 6330, Hinton, AB, T7V 1X7, Canada*

Abstract: Despite the widespread use of DNA mark–recapture for estimation of grizzly bear (*Ursus arctos*) population size, there have been no designed experiments of DNA sampling strategies. We designed a large-scale study (8,820 km^2) in the foothills of Alberta, Canada, to test sampling strategies associated with the hair snag DNA method. The main sampling method for this project used a traditional design in which bait sites were moved within 180 7 x 7 km grid cells for 4 2-week sampling sessions in the spring of 2004. However, we also tested other strategies concurrently with the traditional design. We sampled fixed sites within each cell to test the utility of moving sites compared to the less-expensive method of not moving sites. We also placed a second, lower strand of barbed wire on bait sites to see if this could identify cubs, which are not typically sampled by the usual knee-height strand of barbed wire. We compared summary statistics, capture probability variation, population estimates, and the precision of population estimates for each design. The moved-sites designs captured more bears each session, captured more individual bears (especially females), and displayed population estimates that were 15–25% higher for females. Estimates for males were similar between designs. These results suggest that the moved-sites designs were more efficient in sampling the entire population at the 7 x 7 km grid cell size. These results highlight the need for all bears to have adequate trap encounter opportunities to ensure unbiased estimates. It also demonstrates the utility of collecting enhanced data sets to test and optimize DNA sampling strategies.

Key words: Alberta, DNA, grizzly bear, mark–recapture, population estimation, program MARK, *Ursus arctos*

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Despite the widespread use of DNA mark–recapture for estimation of grizzly bear (*Ursus arctos*) population size (Woods et al. 1999; Mowat and Strobeck 2000; Poole et al. 2001; Boulanger et al. 2002, 2004*b*; Mowat et al. 2005), there have been no designed experimental comparisons of field and analysis strategies. Because these surveys can be expensive, there is a need to minimize costs while not compromising accuracy and precision.

Woods et al. (1999) outlined the basic methodology for sampling grizzly bears for the majority of North American grizzly bear DNA projects. Since then, there has been experimentation with sampling intensity in the form of adjusting cell size and grid size and in moving and fixing sites (Boulanger et al. 2002) for individual projects. However, each project had its unique sampling conditions, making it difficult to compare results and determine optimal methods. For example, projects with fixed sites used a 25-km^2 cell, whereas projects that moved sites used larger grid cell (64 km^2). Thus, it was difficult to directly determine the utility and effects of moving sites on resulting population estimates. Fixed-site projects are significantly less expensive than moved-site projects when the cell size is the same. Thus it would be useful to know how the bias and precision of the abundance estimates of these strategies compare.

[7]boulange@ecological.bc.ca [8]mproctor@netidea.com [9]shimmer@netidea.com [10]Gordon.Stenhouse@gov.ab.ca [11]dpaetkau@wildlifegenetics.ca [12]Jerome.Cranston@gov.ab.ca

Another issue that has not been addressed directly is the effect of single-wire sampling on capture probabilities of younger bears. Boulanger et al. (2004*a*) used data from radiocollared bears to determine that cubs were sampled by single wire sampling; however, low sample sizes prevented direct determination of the effect of lower capture probabilities of younger bears on population estimates. Because cubs can represent up to 20% of the population (McLellan 1989), the issue of whether substantial numbers of younger bears were being missed is important (Boulanger et al. 2004*a*).

We designed a large-scale study (8,820 km^2) in the foothills of Alberta, Canada, to test sampling strategies associated with the hair-snag DNA sampling method. The main sampling effort for this project used a traditional design in which bait sites were moved within 180 7 x 7 km grid cells for 4 2-week sampling sessions. However, we also tested not moving sites and the use of a second wire concurrently within the same project.

Methods

Field methods

A DNA sampling area (8,820 km^2) was designated in the eastern foothills of Alberta's Rocky Mountains, bounded by Alberta Highway 16, Jasper National Park, and Highway 11. This area was overlaid with a systematic sampling grid of 180 49-km^2 grid cells. An extensive bear research project had occurred in the northern part of the sampling area from 1999 to 2004, including a DNA project (Boulanger et al. 2004*b*, Mowat et al. 2005) and resource selection function habitat modeling using bears that were GPS (global positioning system) collared (Nielsen et al. 2002, 2004).

One challenge to this project was determining appropriate bait site density (cell size) given large differences in home range sizes of bears in our study area compared to areas in British Columbia where optimal cell sizes for DNA sampling had been determined. In British Columbia, cell size was based upon the mean size of female home ranges, which ranged from 25.0 km^2 (SD = 14.4, range 5.9–40.2, $n = 4$) for females with cubs in steep mountainous areas to 77.5 km^2 (SD = 36.0, range 5.9–155.2, $n = 31$) in the Flathead Valley (B.N. McLellan, British Columbia Ministry of Forests, Revelstoke, British Columbia, Canada, unpublished data). A cell size of 49 km^2 (with moved sites) was determined to be optimal based upon the approximate dimension of female home ranges and the results of DNA mark–recapture projects (Boulanger et al. 2002). A cell size of 25 km^2 with fixed sites was also considered optimal. In this case, cell size corresponded to the mean home range size of female bears in mountainous areas. In contrast, mean home ranges for females in our Alberta study area ranged from 208 km^2 (SD = 393.1, range 15.3–1,511.6, $n = 14$) for females with cubs to 336 km^2 for females without cubs (SD = 300.6, range 43.3–1,514.7, $n = 45$; G. Stenhouse, unpublished data). The large difference in mean home range sizes of bears further made us question whether moving sites was needed if cell size (relative to female home range size) was relatively small compared to the mean home range size for a female with cubs.

Within each cell, bait sites were sampled for a single 2-week session and then moved within the cell for each of 3 subsequent sessions. The bait site from the first session also was sampled for the subsequent 3 sessions to create a fixed-site data set. Site selection was done prior to fieldwork using a GIS (geographical information system) program and was based on expert opinion, grizzly bear habitat maps (Franklin et al. 2001), grizzly bear resource selection function models (Nielsen et al. 2002, Nielsen 2004), GPS collar locations, and orthophotos. The site sampled for the first session (and as a fixed site for the subsequent 3 sessions) had the highest quality grizzly bear habitat in the cell according to expert opinion. Bait sites were selected so that no locations within a cell were within 2 kilometers of each other. Two strands of barbed wire were used at all bait sites to test the utility of single versus double wire sampling and to maximize bear capture probabilities. The upper wire was at the traditional knee height (60 cm) and the lower wire was at approximately 30 cm. We then created a top-wire data set by only using data from the top wire and a both-wires data set by using data from both wires. Grizzly bear hair was collected from each bait site using methods documented in Woods et al. (1999), and applicable samples were then genotyped to 6 microsatellite markers (mean observed heterozygosity = 0.744) using methods and error checking protocols documented in Woods et al. (1999) and Paetkau (2003).

Genetic identification of parent–offspring pairs

We assessed genotypes of bears caught at the same site to determine if they were mother–offspring (cubs or yearlings) pairs. Fifteen-locus genotypes of individual were used for comparisons (Proctor et al. 2004). All pairs of bears that included at least one female and shared an allele at all loci were considered as potential mother–offspring pairs. To consider pairs that might be

mother–offspring where there had been a mutation at one loci or where a genotyping error may have obscured this allelic matching pattern, we used the parentage analysis software CERVUS (Marshall et al. 1998) to estimate probabilities of parent–offspring pairs from the genetic data. Pairs that shared an allele at all loci except one and had a confidence rating >80% were considered potential mother–offspring pairs along with pairs that shared an allele at all 15 loci.

We could not distinguish the age of potential offspring (cubs and yearlings) traveling with their mothers or independent offspring that may have been sampled at the same site as their mother by chance. Furthermore, Boulanger et al. (2004*a*) identified 1 cub-of-the-year that was sampled on a 50-cm high wire (confirmed with physical capture and radiotelemetry data). Consequently, our pool of 'cubs' traveling with their mothers may have been captured on the bottom or top wire in this study and may be indistinguishable from yearlings or independent offspring. Therefore, the only question we considered is the extent to which we would have missed this set of potential mother–offspring pairs by not using the bottom wire. Of particular interest was whether a greater proportion of potential cubs were captured only on the bottom wire. This would suggest that bottom wire sampling was effective in sampling cubs.

Sampling design comparison

We initially evaluated the efficiency of each method through comparison of summary statistics (Otis et al. 1978) from each effort. Each sampling strategy was compared in terms of bears captured per session (n_j), newly caught bears each session (u_j), and capture frequencies (f_j). We hypothesized that a more efficient design should capture more bears and more unique individuals.

One assumption of our experiment was that the fixed-sites and moved-sites sampling designs were independent. It could be argued that the presence of both moved and fixed sites after session 1 biased results toward moved sites since the moved sites were new and novel compared to the fixed sites. If bias occurred it would be expected that bears would be more likely to be captured at moved sites after previous capture at fixed sites. We tested this by tallying the sequence of the type of capture (fixed or moved site) from successive sessions for individual bears. In some cases bears were captured in both fixed or moved sites in a single session. In this case capture type was classified as fixed since the change in fixed site captures was of principal interest. We also tested the data with fixed and moved site captures set as "both" to further explore if this simplification changed test results. The resulting data were analyzed in a contingency table with previous session and subsequent session capture type frequencies as the row and columns. If fixed sites were being avoided in subsequent sessions, then capture frequencies in previous fixed site–subsequent moved-site cells would be higher than expected by chance, resulting in non-independence of contingency table cells. Independence of cells was tested using a Fisher exact test (Agresti 1990).

We used the Huggins (1991) closed-capture model in program MARK (White and Burnham 1999) to compare dominant factors influencing capture probabilities in data sets from each design. For this analysis, each sampling strategy (moved sites or fixed and top or bottom wire) and sex of bear was entered as a group in program MARK. Models were then built that constrained capture probabilities to vary as a function of the wire (top and bottom, or top wire) or sites (moved sites or fixed sites). Of particular interest was the degree of heterogeneity caused by each sampling design. To explore heterogeneity, we used the M_h Huggins (Huggins 1991) mixture models (Pledger 2000) in program MARK. M_h mixture models use a mixture of ≥ 2 capture probabilities to model heterogeneity of a single capture probability. This allows 2-point or multi-point distributions that may arise from heterogeneity of capture probabilities to be modeled. For example, the overall capture probability for an encounter history where a mixture of A distributions is used is $\Sigma_{i=1}^{A} \pi_i \theta_i^v (1 - \theta_i)^{t-v}$, where v equals the number of captures of the animal for t occasions, π_i is the probability the animal has capture probability θ_i, with the sum of the π_i forced to equal 1. Thus, for $A = 2$, $\pi_2 = 1 - \pi_1$. From Carothers (1973) the mean capture probability ($\bar{\theta}$) (based on 2 mixture distributions) and coefficient of variation for the mean capture probability (CV($\bar{\theta}$)) were estimated as $\bar{\theta} = \pi_1\theta_1 + (1 - \pi_1)\theta_2$ and $CV(\bar{\theta}) = \left[\sqrt{\pi_1(1 - \pi_1)} \bullet |\theta_1 - \theta_2|\right]/\bar{\theta}$. The coefficient of variation of $\bar{\theta}$ was used as an index of heterogeneity variation. A higher CV($\bar{\theta}$) would indicate a greater degree of dispersion in capture probabilities, suggesting a higher degree of heterogeneity.

The fit of models was evaluated using the sample-size adjusted Akaike information criterion (AIC_c) index of model fit. The model with the lowest AIC_c score was considered the model that best balanced bias and precision (Burnham and Anderson 1998). Change in AIC_c (ΔAIC_c) values were also used to evaluate the fit of models when AIC_c scores were close. In general, any model with a ΔAIC_c score of <2 was worthy of

consideration. AIC_c weights (abbreviated as w_i) were calculated to determine the proportional support for each of the candidate models. Parameter estimates were averaged (termed model averaging) based on their support by the data (as indexed by AIC_c weights) to further account for model selection uncertainty (Burnham and Anderson 1998). Model-averaged population estimates were compared for each strategy to determine the difference between the full data set (moved sites with both wires) and reduced data sets. In addition, model selection tests in program CAPTURE (Otis et al. 1978) were used to assess specific forms of capture probability variation in specific treatments that may have been missed by the meta-analysis in MARK.

We also compared costs between fixed-site and moved-site designs. Costs can vary widely depending on ease of access, methods of access, and density of bears. Rather than report actual costs for this project, we reported relative costs derived from average values of many projects using an example study area of 5,000 km^2. Results were standardized using the cost of the 7 x 7 moved-site design as a baseline. We used our experience from doing many ($n > 10$) DNA-based surveys to estimate the average costs associated with fieldwork effort to visit a cell for setup, sampling, and site removal. For field costs, we compared the numbers of site visits associated with each design. A four-session moved-site design requires that sites are checked and moved for three sessions, whereas the sites are only checked for a fixed-site design, thereby adding cost. For lab costs, we used average results from the number of hair samples collected/site. For a given study area size, a 5 x 5 (25 km^2) cell size design has almost twice as many sites as a 7 x 7 (49 km^2) design and therefore collects more hair samples that must be analyzed in the lab.

Results

Analysis of individual identity

We collected 3,363 hair samples, of which 24% were set aside because they were jet black along their entire length (presumed to be from black bears, *U. americanus*) and 27% were set aside because they lacked suitable material (no guard hairs and <5 underfurs with visible roots). Another 29% of samples were analyzed at microsatellite marker *G10J* and found to have genotypes diagnostic of black bears, and 9% of samples were analyzed but failed to produce genetic results. This left 366 grizzly bear samples with multilocus genotypes suitable for assigning individual identity, including 14 samples that produced consistently weak data for 1 of 6 markers and whose assignment to individual was therefore based on 5 markers. There were 46 multilocus genotypes (presumed individuals) among the 366 grizzly bear samples, so each genotype was replicated in an average of 8 independently scored samples. Five of the genotyped bears were caught in non-barb wire samples and were therefore only used for genetic analysis but not the mark–recapture analysis. Nineteen of these 46 genotypes also matched genotypes of bears that had been physically captured in other research efforts. All multilocus genotypes differed at >2 markers in the final 15-locus dataset, although 7 pairs of genotypes had matched at 5 of 6 markers before selective re-analysis (Paetkau 2003) had been used to detect and correct errors in preliminary 6-locus genotypes. Given the low rate of single-locus error that we detected, the absence of errors at 2 markers, and the highly distinct nature of the 46 multilocus genotypes in our dataset, we conclude that our method of selective data re-analysis was sufficient to prevent the identification of any false individuals through inconsistent genotyping of different samples from the same individual. Paetkau (2003) used data from captured bears to show that individuals with identical multilocus genotypes are ~100 times less common than individuals whose genotypes differ at 2 of 6 markers. We had 1 pair of genotypes that differed at 2 of 6 markers, and conclude therefore that our marker system was sufficiently variable to have a high probability of creating a unique genotype for every individual that we captured.

Genetic identification of parent–offspring pairs

Of 41 unique individuals identified using barbed wire sampling, four bears were captured only on the lower wire. Of these, 3 were captured only at 1 site and 1 was captured at 2 sites. Two of the bears were captured at sites where other bears were also captured. The other 2 were captured at sites where no other bears were captured. Therefore, we were able to explore potential parent–offspring relationships for only 2 of the 4 bears that were captured only on the bottom wire.

There were 55 pairs of bears captured at the same site during the same session that could be examined for potential mother–offspring relationships. Of these, CERVUS plus 15-locus matches identified 8 potential parent–offspring pairs. Of these pairs, 6 were both captured on the top only or the top and bottom wire. For the bottom-wire-only bears, one pair was two males, and thus clearly not a mother–offspring pair traveling

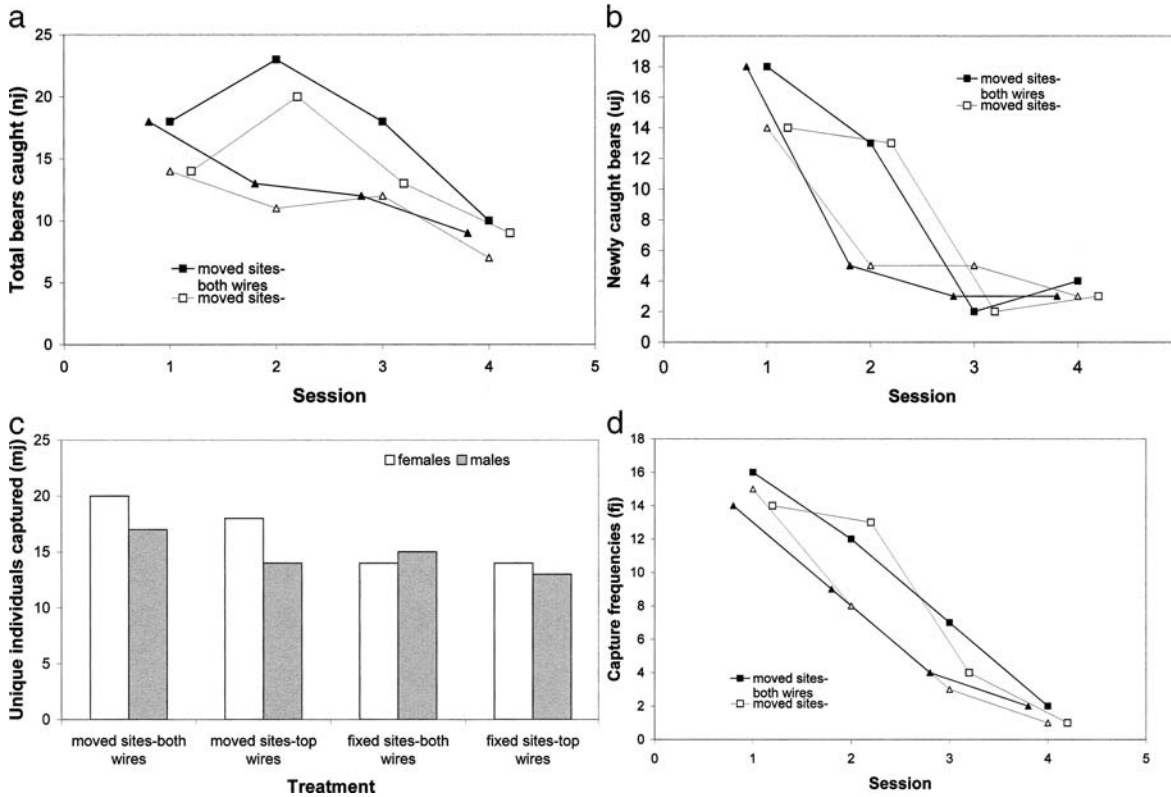

Fig. 1. Summary statistics as a function of sampling session for the 4 sampling scenarios, Alberta Foothills 2004 DNA mark–recapture project. Total bears caught (a) is the number of individual captured during each sampling session. Newly caught bears (b) is the number of newly identified bears in a sample for each session. Number of unique individuals captured (c) is the count of unique individual captured at the end of the project and is subdivided by sex. Capture frequencies (d) is a frequency distribution of the number of sessions each individual bear was captured.

together. For the other pair, one individual was captured on the top wire and the other was captured only on the bottom wire, making it more likely to be a cub. Therefore, of the 2-bottom-wire-only samples that were captured with other bears, one was potentially a cub.

Summary statistics

We compared summary statistics for sessions 2 thru 4, because each moved- or fixed-site design shared the same site during the first session. Summary statistics suggested that moving sites was a more effective method at capturing bears than fixed sites (Fig. 1). In contrast, a second lower wire did not influence summary statistics; most statistics for top-wire only paralleled the statistics for both wires. In terms of animals caught (n_j), the moved sites-both-wires design captured the most bears; however, it was closely paralleled by the moved sites-top-wire design (Fig. 1a). In contrast, the fixed-sites designs (single and both wires) captured fewer bears for the second and later sessions. In terms of newly caught bears (u_j) for each session, the moved-sites designs (single and both wires) captured more bears (Fig. 1b) and more individuals (m_j), especially females, than did the fixed-sites design (Fig. 1c). Sex ratios of captured individuals favored females with the moved-sites designs; whereas sex ratios were even or favored males with fixed-sites designs. The capture frequencies (f_j) were similar for all designs; however, the moved-sites designs had higher frequencies because more bears were captured (Fig. 1d).

Independence of treatments

Forty-five successive capture event pairs were used for the contingency test analysis. Of the 45 pairs, there were 29 pairs were a bear was captured previously at a fixed site. Of these cases, 19 were captured subsequently

Table 1. Akaike Information Criteria (AICc) model selection for design type meta-analysis from the Alberta Foothills 2004 DNA mark–recapture project. Main effects pertain to sex and design-specific model parameters. Time variation pertains to time variation in population capture probabilities that was additive to the main effects. AIC_c, the difference in AIC_c values between the *i*th model and the model with the lowest AIC_c value (Δ_i), Akaike weights (w_i), and number of parameters (K) are presented.

Main effects	Time variation	AICc	Δ_i	w_i	K	Deviance
M_{th2} π (.) θ_1&θ_2 (+sex)[a]	T(fixed)[b] + T^2(moved)[c]	640.9	0.00	0.204	6	587.0
M_{th} *p*(sex)	T(fixed) + T^2(moved)	641.1	0.16	0.188	4	591.2
M_{th} *p*(sex)	T	642.2	1.29	0.107	3	594.4
M_{th2} π (.) θ_1&θ_2 (.)	T(fixed) + T^2(moved)	642.3	1.37	0.103	5	590.4
M_{th2} π (.) θ_1&θ_2 (+sex+wire)	T(fixed) + T^2(moved)	642.8	1.87	0.080	7	586.8
M_{th2} π (.) θ_1&θ_2 (+sex+sites)	T(fixed) + T^2(moved)	642.9	2.01	0.075	7	587.0
M_{th2} π (.) θ_1&θ_2 (xsex)	T(fixed) + T^2(moved)	643.6	2.64	0.054	7	587.6
M_{th2} π (.) θ_1&θ_2 (xwire+sex)	T(fixed) + T^2(moved)	643.7	2.78	0.051	8	585.7
M_{th2} π (.) θ_1&θ_2 ((xsites)+sex)	T(fixed) + T^2(moved)	644.7	3.77	0.031	8	586.6
M_{th2} π (.) θ_1&θ_2 (+sex+wire+sites)	T(fixed) + T^2(moved)	644.8	3.87	0.029	8	586.7
M_{th2} π (sex) θ_1&θ_2 (xsex)	T(fixed) + T^2(moved)	645.0	4.06	0.027	8	586.9
M_{th2} π (wire) θ_1&θ_2 (+wire+sex)	T(fixed) + T^2(moved)	645.8	4.84	0.018	9	585.6
M_b *p*(sex+sites) *c*(sex+sites)		646.6	5.68	0.012	5	594.7
M_{th2} π (sites) θ_1&θ_2 (+sites+sex)	T(fixed) + T^2(moved)	646.7	5.74	0.012	9	586.5
M_{th2} π (wire) θ_1&θ_2 (wire)	T(fixed) + T^2(moved)	647.3	6.40	0.008	8	589.3
M_t *p*(t)		652.3	11.37	0.001	4	602.5
M_{tb} *p*(T) *c*(fixed only;(sex+T))		653.9	12.97	0.000	5	602.0
M_{h2} π (.) θ_1&θ_2 (.)		656.5	15.54	0.000	3	608.7
M_o *p*(sites+sex+wire)		657.2	16.23	0.000	4	607.3
M_o *P*(sites x wire)		661.0	20.11	0.000	4	611.2

[a]A + means that the term was additive to θ_1&θ_2 whereas an x means that the term was modeled individually for θ_1&θ_2.
[b]A linear trend in capture probabilities was denoted by T.
[c]A quadratic trend in capture probabilities was denoted by T^2.

in a fixed site and 10 were captured in a moved site. Results of the Fisher exact test suggested independence between previous and subsequent capture types ($P = 0.11$). Classification of capture type as both when bears were captured in fixed and moved sites did not affect test outcome ($P = 0.13$). We further stratified this test by sex, which also suggested independence of successive capture types for both sexes (males; $P = 0.59$, females; $P = 0.24$).

MARK closed capture model analysis

AICc scores indicated that models with differences in time variation in capture probabilities between sites that were moved and fixed as well as differences in capture probabilities between sexes of bears were most strongly supported by the data (Table 1). Time variation was modeled as a linear term for fixed sites and quadratic term for moved sites. Models that also suggested differences in capture probabilities based upon the number of wires and sites were marginally supported. Models with undefined heterogeneity (M_{th2} models) and heterogeneity based upon sex alone (M_{th} *P*(sex)) were also supported. Inspection of CAPTURE model selection results suggested that males displayed a behavioral response to sampling when sites were fixed (moved sites, both wires, CAPTURE test 2 [$\chi^2 = 9.6$, 1 df, $P = 0.002$], CAPTURE test 5 [$\chi^2 = 0.67$, 2 df, $P = 0.715$]). This was not as readily detected by the MARK Huggins model analysis (model M_{tb} (*P*(T)), *c*(fixed only sex+T), $\Delta AIC_c = 12.97$); however, this may have been due to confounding of time variation and behavioral response in the data set.

The moved-sites both-wire treatment had slightly higher capture probabilities, especially for females (Fig. 2a). The degree of heterogeneity in capture probabilities (CV *p*) was higher for the fixed-sites designs than the moved-sites designs (Fig. 2b). Population estimates of females were 15 to 25% higher for the moved sites designs; estimates of males were similar between moved-sites and fixed-sites designs (Fig. 2c). For females, confidence intervals for the moved-sites designs did not overlap point estimates for the fixed-sites designs. In contrast, estimates for males were similar among designs with both wires or top wire only, regardless of whether sites were moved. Precision (CV $\hat{N}$) was highest for moved-sites designs (Fig. 2d).

Generally, field costs for fixed-site designs were lower than for moved-site designs when the total number of cells was constant. However, as cell size decreased, the total number of cells increased to cover

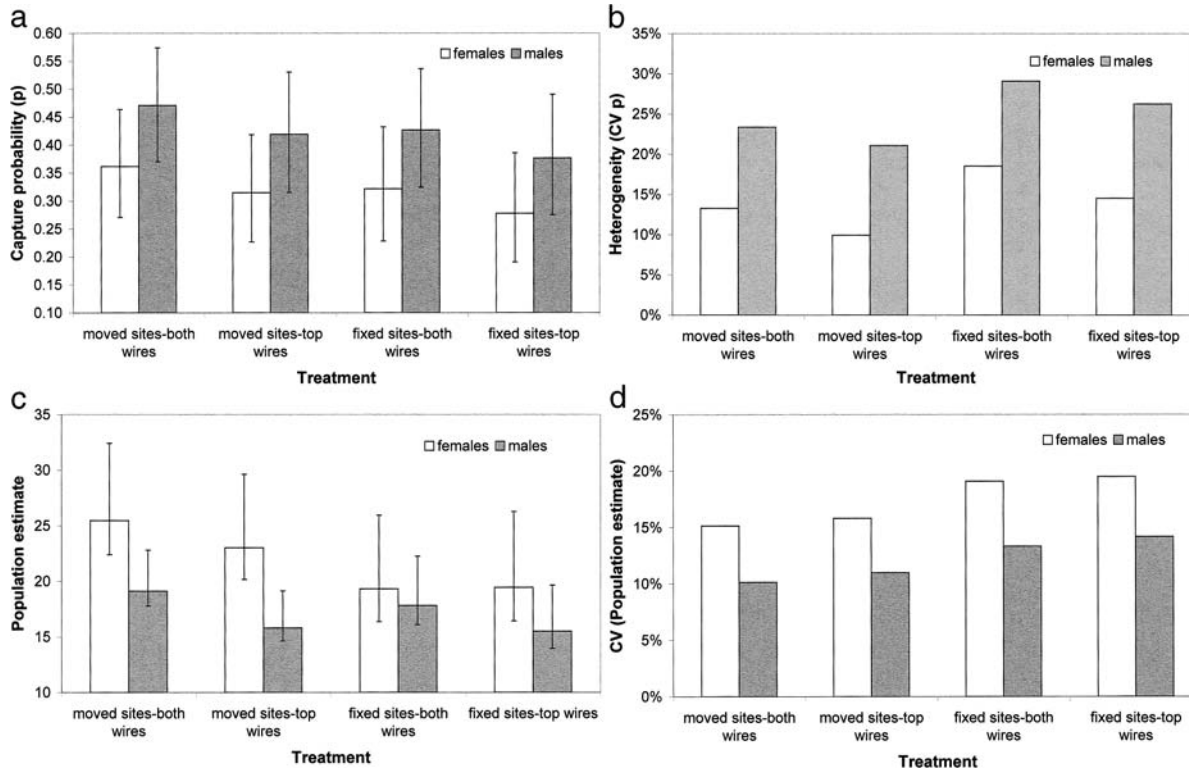

Fig. 2. Estimates of capture probability (a) and heterogeneity (b), and population estimates (c) and coefficient of variation (d) of population estimates from the 4 sampling designs for the Alberta Foothills 2004 DNA mark–recapture project. All estimates are model-averaged from the models in Table 1.

the same study size area, and field costs increased (Table 2). Fieldwork costs from a 5 x 5 fixed-site design were only marginally more expensive than a 7 x 7 moved-site design. Lab costs increased as the number of cells increased. The lab costs of the 5 x 5 fixed-site design were approximately double those of a 7 x 7 moved-site design, given a constant study area size.

Discussion

This project represents the first designed experiment to determine optimal sampling methods for grizzly bears using hair snag approach. By conducting different sampling strategies within the same study, we avoided confounding factors that have compromised comparisons of study designs from different projects. For example, it would have been impossible to determine if moved-sites designs capture more bears and exhibited higher capture probabilities by comparing 2 studies because study-specific factors such as closure violation and habitat type sampled influence these parameters. However, our approach assumed that the results of the designs were reasonably independent. For example, for the last 3 sessions of sampling, 2 sites were present in each grid cell (1 fixed site and 1 moved site). We assumed that a bear captured in one of the sites did not

Table 2. Relative costs associated with cell size in a mark–recapture population estimation effort. These cost estimates assume the study area is of equal size (5,000 km^2 used as an example) and the number of cells varies depending on cell size. Results are standardized using the cost of the 7 x 7 moved-site design as a baseline. The cost of a second wire would cause lab costs to increase by a factor of 1.96 for any of the designs. These estimates are a rough guide and vary depending on field techniques used (helicopter vs. vehicle access) and lab prices.

Cell size	Design	Sites	Field cost	Lab cost
7 x 7	moved	102	1.00	1.00
7 x 7	fixed	102	0.73	1.00
6 x 6	fixed	139	0.89	1.36
5 x 5	fixed	200	1.14	1.96

affect its capture in the other site. Fisher exact tests suggested that bears were equally probable of being captured in fixed or moved sites after previous capture at fixed sites, suggesting that this assumption was reasonable. In addition, we assumed that presence of a bottom wire did not affect captures on the top wire only. Kernel 95% bear home ranges for the period of sampling in the study area ranged from 65 km^2 (a female with cubs) to 938 km^2 (an adult male; G. Stenhouse, unpublished data); the size of grid cells was well within the area traversed by a bear. In addition, the capture probabilities for both sexes of bears were among the highest observed in any DNA study for both fixed- and moved-site treatments (see below). This suggests that bears had ample opportunity to be recaptured, and the effect of 4 simultaneous treatments was minimal.

Our results suggest that moving sites sample bear populations more thoroughly than fixing sites. Moved-sites designs captured more bears on each sampling occasion and sampled more individuals overall (Fig. 1a and 1c) than fixed-site designs. Capture probabilities of bears were also higher (Fig. 2a). The fact that more bears were captured with the moved-sites designs is noteworthy given that the overall bait site densities for the fixed- and moved-site designs were equal (one bait site per cell). One potential reason for this is that the moved-sites designs allowed field workers to access the best seasonal habitats as vegetation greened up in the spring. Moving sites may have also mitigated habituation of bears to bait sites. In addition, it potentially allowed placement of bait sites in core home range areas of more individuals.

Male population estimates were similar for moved-sites and fixed-sites designs, but female estimates were lower for the fixed-sites designs (Fig. 2d). Both the number of female bears captured and female capture probabilities were higher with moved-site designs than fixed-site designs (Fig. 1c and 2a). One potential cause for reduced numbers of female bears captured from fixed-sites designs is that the cell size (49 km^2) was too large, reducing the probability of trap encounter for females with smaller core home ranges. In this case a segment of the population (females with cubs) may have been 'invisible' to mark–recapture sampling. This general result is further supported by the higher levels of heterogeneity estimated for the fixed-sites design, suggesting that some females had lower capture probabilities than others. Heterogeneity models can account for differences in capture probabilities between bears; however, an inherent assumption is that all bears have a non-zero probability of being sampled. If some females had no probability of being sampled with fixed sites, then our estimates would be biased low. In contrast, moving sites between sessions sampled more unique areas, better assuring us that all bears had a non-zero probability of capture. Simulation results suggest that the degree of heterogeneity of capture probabilities caused by unequal trap encounter will be minimized if cell size is reduced, to the point that estimates from fixed- and moved-site designs will be similar (J. Boulanger, unpublished data). Therefore, reducing cell size (to, for example, 6 x 6 km) could offset the issue of low trap encounter of females with the 7 x 7 km fixed-site design.

One consideration in comparison of designs is relative field and genetic costs (Table 2). Moving sites costs more because sites have to be taken down and setup between sessions. Although moving sites adds cost, this addition must be weighed against the added lab costs incurred by the alternative bias-reduction strategy of using smaller cell sizes (because given an equal sampling area, small cells will accumulate more samples for the lab). The actual cost of designs depends greatly on the degree of helicopter versus road access in a given study area, so the ratio of field to laboratory costs may vary between designs. For example the ratio of field to genetic cost for this project was 7 to 1, whereas the range of field to genetic costs for historic projects in British Columbia has ranged from 1 to 1 to 7 to 1.

Another issue with fixing sites is habituation of male bears to bait sites. CAPTURE test results suggested that fixing sites created a behavioral response, especially for the males. This would explain why recapture rates were lower with the fixed sites design for males. In this case the optimal model for the data would involve time, heterogeneity, and behavioral variation. Although modeling all 3 sources of variation simultaneously is possible within program MARK, estimates will tend to be imprecise because of the large number of parameters used for estimation.

One assumption in the double wire experiment was that the proportion of cubs in the population was similar in 2004 to other years. Reproductive rates of grizzly bears often vary substantially on a yearly basis. In 2004, 18 female bears were captured as part of GPS collaring efforts in foothills area of Alberta. Of these, 9 had no offspring, 8 had older dependent offspring (yearlings, 2-year olds, or 3-year olds), and only 1 had cubs born that year (Gordon Stenhouse, unpublished data). These data suggest the proportion of cubs in the population was unusually low during the study, but there were many female bears that had older dependent offspring.

Attempts to genetically distinguish cubs on lower wire samples were compromised by low sample sizes of bears captured only on the bottom wire. In addition, this procedure relied on both mother bears and cubs being simultaneously captured at sites. Scenarios in which only mothers or only cubs were captured at sites were not detectable. Also, because age cannot be determined from genotype, it was not possible to determine if captured offspring in potential mother–offspring pairings were cubs, yearlings, or older independent offspring. In addition, Proctor et al. (2004) documented that a small percent (11%) of sibling–sibling matches could be misidentified as parent–offspring pairs in CERVUS. Despite these limitations, potential captures of family groups, including the capture of a cub on the bottom wire, was suggested. This further backs the general claim that cubs and family groups cannot be excluded from estimates using single-wire or double-wire sampling.

The addition of a second wire did not substantially influence estimates. Summary statistics for double-wire and top-wire only treatments were closely associated. Estimates for males were slightly higher for double wire treatments; however, this difference was minimal compared to the relative uncertainty in population estimates. This general result follows the results of the genetic analysis as top wire samples identified the majority of bears, including potential family groups. These results suggest that double wire sampling may not be worth the additional expense. The double wire design also increases the number of hair samples analyzed. Of the 337 wire samples, 243 were top wire and 94 were bottom wire samples. The exact cost difference will vary depending on the sub-sampling rules one applies, but the double-wire design will add more genetic samples, increasing genetic costs.

We had higher capture probabilities in this project than similar projects in British Columbia. Of the projects reviewed by Boulanger et al. (2002), the highest capture probability (0.26) was from the Jumbo project, which used a 5 x 5 km cell size with fixed sites. In contrast, capture probabilities from this project were 0.27 (females) and 0.37 (males) for the comparable top wire–fixed sites design, and were even higher for other designs. There are many potential reasons for higher capture probabilities. Grizzly bear home ranges are larger on the eastern side of the Rockies, where this project took place, than on the western side, where many of the British Columbia projects occurred. This could result in higher trap encounter rates and higher capture probabilities. In addition, this project used *a priori* site selection based on GPS collar data, resource selection function modeling (Nielsen et al. 2002, Nielsen 2004), and high-resolution orthophotos. In contrast, many of the British Columbia projects used ground-based or helicopter-based reconnaissance. As a result, it is likely that site placement was more optimal for this project, leading to higher capture probabilities.

Management implications

Our results support the idea that moving sites between sessions at a given cell size is an advantageous sampling strategy for many reasons. First, it captures more bears per session, increasing the overall sample size. Second, it recaptures more bears, leading to higher capture probabilities. Third, it reduces capture heterogeneity, by increasing the likelihood that all bears have access to traps. Fourth, it potentially avoids behavioral responses to sampling due to trap habituation. Moving sites is more costly; however, we argue the cost is offset by these advantages. In contrast, the addition of a second wire for sampling increases costs substantially but does not seem to change estimates or improve estimate precision, leading us to conclude that single wire sampling suitably targets all bears in the population.

In terms of overall cost, one option is to use a smaller cell size (e.g., 6 x 6 or 5 x 5 km) and not move the sites. The main risk with this design is behavioral response caused by habituation to bait sites. In addition, care must be taken to ensure that bait site density is adequate when sites are fixed. The results of this study suggest that fixed-site designs result in lower estimates for females even when site density is reasonably high compared to female home range size.

This study demonstrated how designed experiments and program MARK can be used to infer optimal sampling patterns. The initial cost of this experiment was higher than if traditional approaches had been used. However, we believe these results will help optimize and ensure the robustness of future DNA-based mark–recapture estimation.

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