Closure violation in DNA-based mark–recapture estimation of grizzly bear populations

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Abstract: We use methods in the program MARK to explore the effects of closure violation when DNA-based mark–recapture methods are used to estimate grizzly bear (*Ursus arctos*) populations. Our approach involves the use of Pradel models in MARK to explore the relationship between recruitment, apparent survival rates, recapture rates, and distance between mean bear-capture locations and the edge of the sampling grid. If the population is demographically closed, it can be assumed that apparent survival estimates the fidelity of bears to the grid area and recruitment estimates rates of addition of bears to the grid area. A core bear population is defined from the Pradel analysis and is used to approximate the grid-based population size. The Huggins closed-population model in MARK is used to provide robust superpopulation estimates by explicitly modeling the relationship between capture probability and distance of bear-capture location from the grid edge. Data from a grizzly bear DNA-based mark–recapture inventory conducted in British Columbia is used to illustrate this method. The results of the Pradel analysis suggest that bears with mean capture locations within 10 km of the grid edge exhibit reduced fidelity rates and higher addition rates. Using the population of bears captured more than 10 km from the grid edge, a core-extrapolated estimate is derived, which is substantially lower than naïve CAPTURE superpopulation estimates. The Huggins model superpopulation estimate displays superior precision compared with CAPTURE model estimates. Our results illustrate the danger of naïve interpretation of closed-model estimates. This method allows further inferences to be made concerning the spatial causes of closure violation, and the degree of bias caused by closure violation to be explored.

Résumé : Nous avons utilisé des méthodes du programme MARK pour explorer les effets de l'absence des conditions préalables d'une population fermée lorsque les méthodes de capture–recapture basées sur l'ADN sont employées pour estimer les populations de Grizzlis (*Ursus arctos*). Notre approche suppose le recours aux modèles de Pradel du programme MARK pour étudier les relations entre le recrutement, la survie apparente, les taux de recapture et la distance entre le site moyen de capture et le bord de la grille d'étude. Lorsque la population est fermée démographiquement, on peut supposer que la survie apparente donne une estimation de la fidélité des ours à la grille et que le recrutement est un bon indicateur des taux d'addition dans la grille. Une population de base a été définie d'après l'analyse de Pradel et elle fournit une approximation de la taille de la population dans la grille. Le modèle de la population fermée de Higgins dans le programme MARK sert à obtenir des estimations robustes de la superpopulation par la modélisation explicite de la relation entre la probabilité de capture d'un Grizzli et la distance entre son point de capture et la bordure de la grille. Les données sur l'ADN obtenues au cours d'un inventaire par capture–recapture en Colombie-Britannique illustrent bien la méthode. L'analyse de Pradel indique que les ours dont les points moyens de capture sont en deça de 10 km de la bordure de la grille ont des taux de fidélité plus faibles et des taux d'addition plus élevés. À partir de la population d'ours capturés à une distance supérieure à 10 km de la bordure de la grille, on obtient par extrapolation une estimation de la population de base considérablement plus faible que les estimations simples de la superpopulation obtenues avec le programme CAPTURE. L'estimation de la superpopulation d'après le modèle de Higgins est plus précise que les estimations obtenues avec le programme CAPTURE. Nos résultats illustrent bien les risques d'une interprétation trop simpliste des estimations obtenues par des modèles applicables à des populations fermées. Cette méthode permet d'explorer davantage les causes spatiales de l'absence des conditions requises pour qu'une population soit fermée et de déterminer le pourcentage d'erreur relié à l'absence de ces conditions.

[Traduit par la Rédaction]

Introduction

Maintaining grizzly bears (*Ursus arctos*) is a major conservation challenge in North America (McLellan 1989). Besides being a high-profile species that is threatened in the U.S.A. (Serveen 1990) and vulnerable in Canada (Banci et al. 1994), grizzly bears are notoriously difficult to census in forested habitat (Mace et al. 1994), therefore monitoring their status is problematic. Until recently, the only methods available to estimate population size involved intrusive programs of capture and marking (McLellan 1989; Mace et al. 1994; Miller et al. 1997). Recent advances in genetics, how-

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ever, have made it possible to obtain mark–recapture population estimates using the genetic identities of grizzly bears as marks (Woods et al. 1999).

Although the use of DNA marks in a capture–recapture design shows promise, population closure is a major concern with animals whose home ranges are large relative to the sampling grid. Closure violation results in a positive bias in population estimates because animals move in and out of the mark–recapture grid, which inflates the number of marked animals and negatively biases capture-probability estimates. In some cases, closed-model estimates will describe the "superpopulation" of animals in the grid and surrounding area when closure is violated (Kendall 1999). While the superpopulation estimate may be a useful biological representation of the population, it cannot be used for density estimates because the sampling area is undefined. For this, a "grid-based estimate" of the average number of animals on the grid is needed, and this is most readily gained by using radio-collared bears to index movements in and out of the grid area (Kenward et al. 1981; Garshelis 1992; White 1996; Powell et al. 2000).

Unfortunately, methods that deal with closure violation are limited at this time. Tests for closure are available, but these are potentially biased by capture-probability variation and do not give any biological insight into causes of closure violation (Otis et al. 1978; Stanley and Burnham 1999). Methods to obtain density estimates from grid-based data, such as the nested-subgrid routine in the program CAPTURE (Otis et al. 1978), require much larger sample sizes than those collected in grizzly bear studies to correct estimates (Wilson and Anderson 1985). In addition, the alternative open models that can be used when closure is violated are not robust to heterogeneity bias (Pollock et al. 1990) and usually suffer from lower precision. Heterogeneity bias is especially relevant for mark–recapture studies based on grizzly bear DNA, owing to probable age- and sex-specific capture probabilities (Woods et al. 1999). Only sex, and not age, can be determined from DNA, therefore it is not possible to construct models that fully parameterize age- and sex-specific capture probabilities. Therefore, the use of the general closed heterogeneity models in CAPTURE is desirable with grizzly bear DNA mark–recapture data.

This paper presents a case study illustrating the problem of closure violation when DNA-based mark–recapture methods are used to estimate the size of grizzly bear populations. We extend the general closure test of Stanley and Burnham (1999) to explore closure violation based on apparent survival, recruitment, and recapture rates as a function of the distance of bear capture locations from the edge of the sampling grid. We use this relationship to estimate the superpopulation of bears, using the models of Huggins (1991), and explore the sensitivity of CAPTURE estimators to grid-edge-based capture-probability bias.

The case study involves the analysis of data from the Prophet River grizzly bear DNA mark–recapture study carried out in northern British Columbia, Canada. In this area it was not possible to topographically close the sampling grid in order to minimize closure violation. Since an unbiased population estimate was the main objective, this data set provides an excellent case study of closure-bias issues.

Methods

The Prophet River sampling grid consisted of 103 cells. Most were 9×9 km in size and the entire study area covered 8527 km^2 (Poole et al. 2001). One hair-collection site was installed in each cell for approximately 12 days before being moved to another site in the cell. Moving sites within each cell was done for 5 sampling periods, each new site being >1 km from all previous sites. At hair-collection sites, liquid bait was poured on a mound of logs, stumps, moss, and boughs. The mound was situated within a perimeter fence consisting of one strand of barbed wire running around >3 trees at about 50 cm from the ground (Mowat and Strobeck 2000; Woods et al. 1999). For the first 3 sampling periods, rancid fish oil and rotten-beef blood were used for bait. For the last 2 sampling periods, beaver (*Castor canadensis*) castor was hung at a height of 2 m in a tree in addition to the mounds containing fish oil and rotten-beef blood. Study-area boundaries followed the height of land between major drainages. None of these boundaries significantly hindered bear movement.

The hair samples from bait stations were analyzed by the Wildlife Genetics International Laboratory in Edmonton, Alberta. Standard procedures developed by Paetkau and Strobeck (1994), Paetkau et al. (1995), and Woods et al. (1999) were used to identify individual bears from hair samples. An equation given in Woods et al. (1999) was used to estimate the conditional probability that a given individual had the same genotype as a sibling (P_{sib}). New bears were accepted when P_{sib} was less than 0.05.

An assumption of this analysis is that genetic identification of captured and recaptured individuals is achieved without error. The most notable laboratory-based errors are scoring and amplification errors (such as allelic dropout), which cause false individuals to be introduced into the data set (Taberlet et al. 1999). To minimize laboratory-based genetic errors, D. Paetkau (personal communication) used populations of known individuals to confirm the ability of genetic markers to resolve individuals, and carefully checked and, if necessary, reproduced highly similar genotypes in order to search for genotyping errors. To minimize "shadow effect" errors caused by failing to separate closely related individuals, the conservative P_{sib} statistic was used to define individuals (Woods et al. 1999), and heterogeneity estimators that are relatively robust to shadow effects were considered for population estimates (Mills et al. 2000).

MARK methods for assessing closure violation

We developed a new method to assess closure violation based on the locations of bear captures on the sampling grid. The main premise of this test is that if closure violation was occurring, then the recapture rates for bears that were near the grid edge ("edge" bears) would have been lower, because their trap-encounter rate would be reduced while they were not on the grid, than recapture rates for bears farther from the edge ("core" bears). In addition, if bears moved from the grid for the entire sampling period, then edge bears would exhibit a lower relative estimated survival rate than core bears. Also, bears that immigrated into the grid area during sampling would be more prone to be captured near the grid edge. The "distance from edge" of the capture location was the shortest distance from the grid edge to the mean location of hair-collection sites where a bear was identified during the entire project.

The Pradel (1996) model as incorporated in the program MARK (White and Burnham 1999), which estimates apparent survival rate (ϕ), recruitment rate (f), and recapture probability (p), was used for this analysis. An example of the parameterization of the Pradel model for a bear captured during each of the 5 sampling periods is $\phi_1 f_1 p_2 \phi_2 f_2 p_3 \phi_3 f_3 p_4 \phi_4 f_4 p_5$. The estimates of the recapture rate are for the exact sampling period, whereas the estimates of the appar-

ent survival rate (φ) and recruitment rate (*f*) correspond to the interval before the given sampling period.

For this analysis we assumed that the bear population was demographically closed. The duration of sampling was ≈2 months and bear mortality rates are low (McLellan et al. 1999), so this assumption was reasonable. Apparent survival equals true survival (*S*) (because of mortality) times the fidelity of bears to the sampling grid (*F*) (φ = *SF*). Because the population was demographically closed, we assumed that true survival equaled 1 (*S* = 1), therefore relative changes in φ reflect bear fidelity to the sampling grid rather than actual mortalities, i.e., (φ = *F*). The Pradel recruitment rate is an estimate of the number of new individuals in the population at time *j* + 1 per individual at time *j*. We assumed that the number of births during sampling was minimal and therefore measures of recruitment reflected permanent immigration or "additions" of bears into the sampling grid. This assumption is also reasonable, since all bears would have given birth prior to the time of sampling. For the sake of simplicity, hereinafter we refer to φ as the rate of "fidelity" and *f* as the rate of "additions."

As an initial appraisal of population closure we evaluated the goodness of fit of Pradel models constrained to allow only certain forms of closure violation as first proposed by Stanley and Burnham (1999). The exact models used in Stanley and Burnham's (1999) test were the fully open Jolly–Seber model, a recruitment but not mortality model, a mortality but not recruitment model, and a closed model with no mortality or recruitment (*M*$_t$). Note that the closed model *M*$_t$ is analogous to the Darroch *M*$_t$ CAPTURE estimator. We emulated Stanley and Burnham's (1999) approach by fixing parameters to appropriately constrain the Pradel model.

We then used continuous covariates to model the relationship of distance from edge for φ, *f*, or *p* as a logistic function. The potential shapes that could be accommodated by the logistic curve that is used to model covariates in MARK was restrictive, and therefore logistic equations with log-transformed (+1) (Zar 1996) distance from edge and higher order polynomial (i.e., dfe^2 log (dfe)^2 + 1) distance from edge terms were also considered. Covariates were standardized in MARK by the mean and standard deviation of observed distances (White et al. 2001). A logit link was used for all analyses.

In addition to covariates, both sex- and time-specific model formulations were considered in the building of mark–recapture models. The fit of models was evaluated using the Akaike Information Criterion (AIC) index of model fit. The model with the lowest AIC$_c$ (sample-size-adjusted AIC) score was considered the most parsimonious, thus minimizing estimate bias and optimizing precision (Burnham and Anderson 1998). ΔAIC$_c$ values were also used to evaluate the fit of models when their AIC$_c$ scores were close. In general, any model with a ΔAIC$_c$ score of less than 2 was worthy of consideration.

There is no definitive method to assess the goodness of fit of Pradel continuous covariate models at this time. As suggested by White et al. (2001), we used a bootstrap goodness of fit for the Cormack–Jolly–Seber (Seber 1982) model to estimate overdispersion for the Pradel model under the assumption that lack of fit was due to recaptures of previously marked animals. If overdispersion was detected (as indicated by $\hat{c}$ > 1), we used the QAIC$_c$ instead of AIC$_c$ model-selection criterion to select optimal models (Burnham and Anderson 1998; White et al. 2001).

The CAPTURE closure test (Otis et al. 1978) was also used to allow comparison with results from the Pradel model analysis.

Population estimates

Superpopulation estimates

We used the Huggins (1991) closed model estimator as incorporated in MARK to estimate the superpopulation of bears in the sampling grid and surrounding area. The rationale for this approach is that differences in rates of bear fidelity or bear additions would be absorbed and reflected by estimates of closed-model capture probability (Kendall 1999). In addition, any change in capture probability as a function of distance from edge (due to closure violation) would appear as heterogeneity in closed models. By modeling capture probability as a function of distance from edge, this source of heterogeneity could be eliminated, leading to a more robust superpopulation estimate. As with the Pradel analysis, time-specific, sex-specific, and various forms of distance from edge covariates were considered in the model-building process, and AIC$_c$ methods were used to assess model fit.

Approximation of grid-based average N estimate

We now propose a method that uses the results of the Pradel analysis to produce a population estimate which approximates the grid-based population of bears. If estimates of φ and *f* stabilize near 1 and 0 (suggesting minimal dilution or additions of marks during sampling) as distance from edge increases, then it can be further assumed the capture probabilities for core bears that were captured in areas where φ = 1 and *f* = 0 are less affected by closure violation than edge bears captured closer to the grid edge. Using these core bears, a less biased population (and density) estimate of the grid-core area can be made. The population estimate for the grid core can then be extrapolated to the entire grid using the simple formula

$$[1] \qquad \hat{N}_{\text{extrapolate}} = \frac{\hat{N}_{\text{core}}}{A_{\text{core}}} A_{\text{total}}$$

In this formula, *A* is the total area of the grid and the area of the grid core. Because the measurements of the grid core and edge areas are a constant without error, the variance of the core-extrapolated estimate is found by simply multiplying the core variances by the square of the ratio of total-grid and grid-core areas. As discussed later, this estimator assumes that there is no error associated with estimating the cutoff distance from the edge between core and edge bears.

The extrapolated core estimate carries the assumption that bear densities in the core and edge areas were equal. The assumption of uniform density was tested by comparing observed and expected numbers of marked bears (based on uniform density) using a χ^2 test (Otis et al. 1978). Another potential problem with the core-based estimator is sensitivity to the definition of core and edge bears. An analysis was conducted to calculate extrapolated core estimates for 1-km distance from edge intervals. If the Pradel analysis properly defines core and edge areas, then the core-extrapolated population estimates should be biased high, then stabilize near the edge and core boundaries defined by the Pradel analysis.

Results

During the Prophet River project, 98 individual bears (46 females, 47 males, 5 of unknown sex) were identified in the genetic database (Table 1). The number of bears identified after the first round of genetic analysis was higher, but data scrutiny identified 5 scoring errors and 4 cases of allelic dropout, reducing the number from 107 to 98 (D. Paetkau, personal communication). All pairs of genotypes remaining in the data set that were identical at all but one locus were reproduced in a second round of analyses (including polymerase chain reaction (PCR)). Bears of unknown sex were excluded from the Pradel and Huggins analyses, which considered sex-specific model parameterizations, but were included (in a separate analysis) in Huggins model population estimates.

Table 1. CAPTURE summary of capture–recapture statistics for the Prophet River Grizzly Bear DNA Mark–Recapture Project on 5 occasions in 1998.

	1	2	3	4	5	Total
No. of animals caught (n_i)	22	36	18	42	24	
No. marked in population (M_i)	0	22	52	61	86	98
No. newly caught (u_i)	22	30	9	25	12	
Frequency (f_j)	63	26	9	0	0	

Table 2. Results of Pradel analyses for the Prophet River Grizzly Bear DNA Mark–Recapture Project, 1998.

Model No.	Model terms and covariates[a]			Model-selection results			
	Fidelity rate (ϕ)		Recapture rate[b] (p)	Addition rate (f)	QAIC$_c$	ΔAIC$_c$	No. of parameters
Covariate models							
1	$(.)^c$	+ d, d2	t_{135}, t_{24}	t_{24}, t_{35} × d, d2	452.44	0.00	11
2	t_{24}, t_{35}	+ d, d2	t_{135}, t_{24}	t_{24}, t_{35} × d, d2	454.76	2.32	12
3	Sex	+ d, d2	t_{135}, t_{24}	Sex + d, d2	456.03	3.58	10
4	(.)	+ d, d2	t_{135}, t_{24}	(.) + d, d2	456.49	4.05	8
5	(.)	+ ld, ld2^d	t_{135}, t_{24}	(.) + ld, ld2	456.58	4.14	8
6	Sex	× d, d2	t_{135}, t_{24}	Sex × d, d2	459.36	6.92	14
7	(.)	+ d	t_{135}, t_{24}	(.) + d	460.70	8.26	6
8	(.)	+ d, d2	t_{135}, t_{24} + d, d2	(.) + d, d2	462.09	9.65	10
9	(.)	+ ld, ld2	t_{135}, t_{24}	t_{24}, t_{35} × ld, ld2	462.93	10.49	11
9	(.)		t_{135}, t_{24} + d, d2	(.) + d, d2	463.71	11.27	8
10	(.)	+ d	t_{135}, t_{24}	t_{24}, t_{35} × d	464.01	11.57	9
Initial models							
11	1^e		t_{135}, t_{24}	0^e	464.93	12.49	2
12	1		t_{135}, t_{24}	(.)	465.63	13.19	3
13	(.)		t_{135}, t_{24}	(.)	465.84	13.40	4
14	(.)		t_{135}, t_{24}	0	467.02	14.58	3
15	t_{24}, t_{35}		t_{135}, t_{24}	(.)	467.75	15.31	5
16	(.)		t_{135}, t_{24}	t_{24}, t_{35}	467.95	15.51	5
17	Sex		t_{135}, t_{24}	Sex	469.67	17.23	6
18	t_{24}, t_{35}		t_{135}, t_{24}	t_{24}, t_{35}	469.94	17.49	6
19	Sex × (t_{24}, t_{35})		Sex × (t_{135}, t_{24})	Sex × (t_{24}, t_{35})	480.59	28.15	12

[a]Covariates (symbolized by d or ld) were modeled as an interaction (×) (term-specific slope and intercept) or as additive (+) (term-specific intercept but common slope).
[b]Time-specific parameterization (sampling periods 2 and 4 and sampling periods 3 and 5 pooled).
[c]Term is constant (no time or sex variation).
[d]Log + 1 transformed distance from edge.
[e]Parameter fixed at 1 (ϕ) or 0 (f).

The mean distance from edge of capture locations was 12.07 km (SD = 8.7 km; n = 98). Comparison of observed and expected counts of bears at successive 4-km distance from edge intervals suggests that densities were reasonably uniform throughout the sampling grid (χ^2 = 6.65, df = 6, P = 0.35). Marginal closure violation was detected by the CAPTURE (Z = –1.634, P = 0.052) closure test.
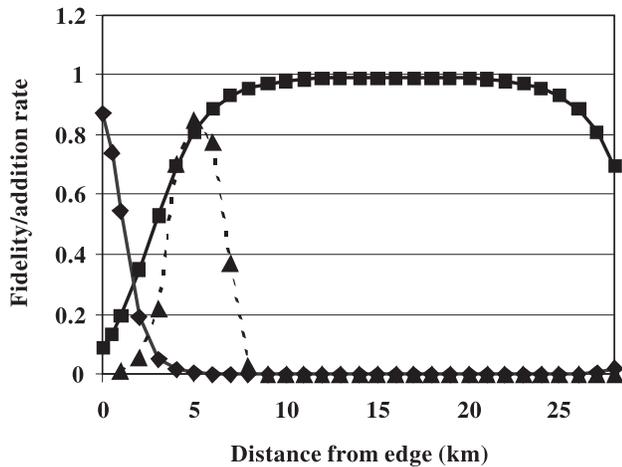
Pradel analysis

A bootstrap goodness of fit of the Cormack–Jolly–Seber model was used to estimate the degree of potential overdispersion caused by recaptures of bears (White et al. 2001). Sparseness of data precluded the use of a fully parameterized model ϕ(sex × time) p(sex × time) (as characterized by large standard errors of parameter estimates), therefore a slightly reduced model ϕ[sex × time($t_{j=24,35}$)] p[sex × time($t_{j=24,135}$)] (see below) was used to estimate a $\hat{c}$ value of 1.08, which suggests slight overdispersion. QAIC$_c$ values that incorporated $\hat{c}$ were used for model comparisons.

We investigated time variation in addition, fidelity, and recapture rates to determine if time-specific parameterizations should be considered in the analysis of distance from edge. The most noteworthy time variation was the increase in number of bears captured (n_j) and number of new bears captured in sampling periods 2 and 4 (Table 1). Models were built with unique rates of addition (i.e., model 16 in Table 2) and fidelity for the periods before sampling periods 2 and 4, and with recapture rates similar for sampling periods 2 and 4 and sampling periods 1, 3, and 5 (parameters that are symbolized by t_{135}, t_{24} in Table 2). Initially, a model that allowed time-

Fig. 1. Estimated fidelity rates (pooled for all sampling occasions) (■) and addition rates before sampling periods 2 and 4 (◆) and 3 and 5 (▲) as a function of distance of mean grizzly bear (*Ursus arctos*) capture location from the grid edge. Estimates are from the most supported model (model 1 in Table 2).


Fig. 2. Results of sex-based Pradel model analysis (model 3 in Table 2) of fidelity and addition rates by sex of bear as a function of distance of mean bear capture location from the grid edge. Solid lines and dotted lines show fidelity rates and addition rates for male bears (■) and females bears (▲), respectively.

specific variation in recapture rates but not in addition or fidelity rates was most supported by the data (model 13 in Table 2). This model was used as the basis for adding covariates; however, time variation in addition and fidelity rates was revisited during later stages of the analysis.

As an initial exploration of closure violation bias, the Pradel models were constrained to emulate Stanley and Burnham's (1999) "recruitment but no mortality" model (model 12 in Table 2), "mortality but no recruitment" model (model 14 in Table 2), and "no recruitment or mortality" model (closed model M_t; model 11 in Table 2) and the fully open Jolly–Seber model (model 18 in Table 2). Of the models considered, the most supported was model M_t (model 11 in Table 2), suggesting that the population closure violation was not severe. However, the difference in support between the models (as indicated by close QAIC$_c$ scores) was minimal, suggesting that closure violation was still likely with the data set.

Models that considered covariates as additive terms (indicated by + in Table 2) and interactive terms (indicated by × in Table 2) were introduced into the analysis. In additive models, each model term has its own intercept with similar distance from edge slope terms. In interactive models, each model term has its own intercept and slope term. Standard and natural log-transformed distance from edge covariates were used in the analysis. In general, log-transformed covariate distance from edge curves were gradual and did not asymptote as a function of distance from edge, whereas standard covariates did asymptote at 0 or 1.

The most supported Pradel model (model 1 in Table 2) assumed that rates of fidelity (ϕ) and additions (f) varied as second-order (quadratic) polynomial terms. Time-specific distance from edge curves were modeled for additions occurring before sampling periods 2 and 4 and sampling periods 3 and 5 (Fig. 1). From Fig. 1 it can be seen that the rate of fidelity is low and the rate of additions high at distances from edge of less than about 10 km. After this fidelity asymptotes at 1 and additions asymptotes at 0. The distance from edge curve for additions is notably different for periods before
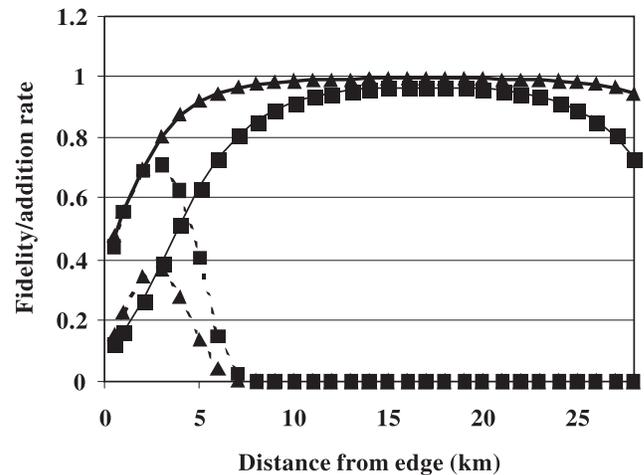
sampling periods 2 and 4 and sampling periods 3 and 5. This suggests that the majority of bears that potentially immigrated into the grid were captured a short distance (4–5 km) into the grid prior to sampling periods 3 and 5. In contrast, in sampling periods 2 and 4 potential immigrants were more likely to be captured at the very edge of the grid. A slight decrease in fidelity is noted at distances from edge greater than 23 km. This might be due to other factors influencing fidelity, or may be an artifact of the quadratic polynomials used to generate curves and the scarcity of marked bears ($M_{t+1} = 11$ bears) at distances greater than 23 km.

A model that considered additive sex-specific distance from edge curves (model 3 in Table 2) was marginally supported by the data but still worthy of biological consideration. The slope estimates from this model suggest that male bears are more prone to emigrate from or immigrate farther into the sampling grid than females (Fig. 2).

Population estimates

Huggins superpopulation estimates

Model-selection results from the Huggins analysis mirror the Pradel analysis, with the relationship between capture probability and distance from edge varying with sampling period. The most supported model modeled capture probability as a quadratic function (model 1 in Table 3), with separate slope and intercept terms for sampling periods 1, 3, and 5 and sampling periods 2 and 4, respectively. In addition, an additive quadratic model (model 2) and an additive cubic model (model 3) were also supported by the data. The slopes of the distance from edge curves from models 1, 2, and 3 suggest that less capture-probability bias due to distance from edge effects occurred in sampling periods 1, 3, and 5 than in sampling periods 2 and 4 (Fig. 3). A decrease in capture probability is noticeable at distances from edge greater than 23 km in Fig. 3. This might be due to other factors influencing capture probability or to an artifact of the quadratic model used to generate curves and the scarcity of bears ($M_{t+1} = 11$ bears) at distances greater than 23 km. A

Table 3. Huggins analysis model selection results for the Prophet River Grizzly Bear DNA Mark–Recapture Project.

Model No.	Capture probability[b]		Recapture probability	AIC$_c$	ΔAIC$_c$	No. of parameters
	Model terms and covariates[a]			Model-selection results		
1	t_{135}, t_{24}	× d, d²		494.12	0	6
2	t_{135}, t_{24}	+ d, d²		494.48	0.36	4
3	t_{135}, t_{24}	+ d, d², d³		495.8	1.68	5
4	t_{135}, t_{24}	× ld, ld²		496.38	2.25	6
5	t_{135}, t_{24}	× d, d², d³		496.98	2.86	8
6	t_{135}, t_{24}	+ ld, ld²[c]		497.18	3.06	4
7	Sex × (t_{135}, t_{24}) (t_{24}, t_{135}) × d, d²			497.48	3.36	8
8	t_{135}, t_{24}	+ d, d², d³, d⁴		497.73	3.61	6
9	Sex × (t_{135}, t_{24}) + d, d²			498.28	4.16	6
10	t_1, t_2, t_3, t_4, t_5	+ d, d²		499.04	4.92	7
11	Sex × (t_{24}, t_{135}) (Sex) × d, d²			501.33	7.21	8
12	t_{135}, t_{24}	+ d, d²	t_{135}, t_{24} + d, d²	501.57	7.45	7
13	t_{135}, t_{24}	+ d		502.05	7.92	3
14	t_{135}, t_{24}			502.33	8.21	2
15	t_{135}, t_{24}		(.)[d] + d, d²	502.70	8.58	5
16	(t_1, t_2, t_3, t_4, t_5) × sex		(t_1, t_2, t_3, t_4, t_5) × sex	527.0	32.88	18

[a]Covariates (symbolized by d or ld) were modeled as an interaction (×) (term-specific slope and intercept) or as additive (+) (term-specific intercept but common slope).

[b]Time-specific (t_{135}, t_{24}) parameterization (sampling periods 2 and 4 and sampling periods 1, 3, and 5 pooled).

[c]Log + 1 transformed distance from edge.

[d]Term is constant (no time or sex variation).

Fig. 3. Capture-probability estimates from quadratic (model 1 in Table 3) and cubic (model 3 in Table 3) Huggins closed models as a function of distance from mean bear capture location from the grid edge. The quadratic and cubic models were tied in terms of support from the data, as indicated by ΔAIC$_c$ values of less than 2 (Table 3). Squares (quadratic (■), cubic (□)) represent capture probabilities for sampling periods 2 and 4. Triangles (quadratic (▲), cubic (△)) represent capture probabilities for sampling periods 1, 3, and 5.
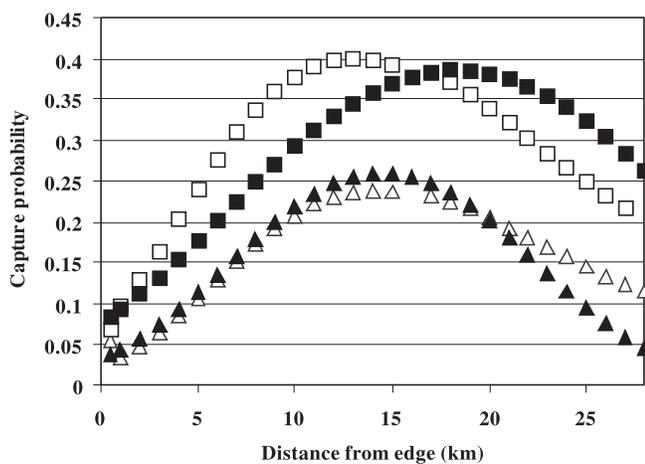
log-transformed quadratic model (model 4 in Table 3) that exhibited curves of similar shape to model 1 but with capture probabilities not decreasing at greater distances from edge was less supported (ΔAIC$_c$ = 2.25), suggesting that the standard quadratic model was an adequate representation of the data. Higher order (i.e., cubic) log-transformed poly-nomial models displayed poor fit, as characterized by high standard errors of parameter estimates, and were therefore not considered. A superpopulation estimate of 191 (confidence intervals (CI) = 163–229) was obtained from the most supported AIC$_c$ model (model 1 in Table 3) as a derived parameter (Table 4).

Grid-based estimates

The Pradel model results suggest that fidelity rates were close to 1 and addition rates were 0 at distances from edge greater than 10 km, therefore we chose this as the core bear – edge bear cutoff. The core population at distances from edge greater than 10 km consisted of 46 bears. CAPTURE core-extrapolated population estimates varied in terms of both distance from edge and the model used (Table 4 and Fig. 4). In Fig. 4 it can be seen that the estimates from all models steadily drop until 6 km from edge, after which they increase slightly and then stabilize after 8 km. In general, naïve estimates were 12–20% higher than core-extrapolated estimates for most estimation models (Table 4). The CVs of most core-extrapolated estimates were comparable to naïve estimates, presumably because the recapture rates for the core population were higher than those for the full (core bears + edge bears) population (Fig. 3).

In terms of closed-model selection, model M_t was selected as appropriate for the naïve superpopulation data set from the CAPTURE model selection routine. The tests for time variation were significant (CAPTURE test 3: χ^2 = 17.72, df = 4, P = 0.0017) but the tests for heterogeneity were not. For the naïve data set, model M_t was selected by the CAPTURE model selection routine.

The distance from edge at which bears were vulnerable to

Table 4. Naïve (superpopulation) and core-extrapolated grid-based population estimates for the Prophet River Grizzly Bear DNA Mark–Recapture Project.

Model	$\hat{N}$	SE	CI	CV
Naïve estimate				
M_h (Chao)	174 (21)	25.9	138–243	14.9
M_h (jackknife)	191 (24)	19.6	160–237	10.3
M_t (Chao)	155 (17)	19.6	128–207	12.6
M_{th}	166 (25)	26.2	132–239	15.8
Huggins covariate[a]	191	16.5	164–229	8.6
Core-extrapolated at 10 km				
M_h (Chao)	137	19.1	114–193	14.0
M_h (jackknife)	144	17.4	122–191	12.0
M_t (Chao)	124	13.7	109–165	11.1
M_{th}	131	16.0	112–178	12.2

Note: Numbers in parentheses show the percent difference from the corresponding core-extrapolated estimate.

[a]Model 1 in Table 3.

Fig. 4. Extrapolated-core CAPTURE estimates as a function of distance of the core area from the grid edge. CAPTURE models shown are as follows: ◆, M_h (Chao); ■, M_h (jackknife); □, M_t (Chao); and *, M_{th}.
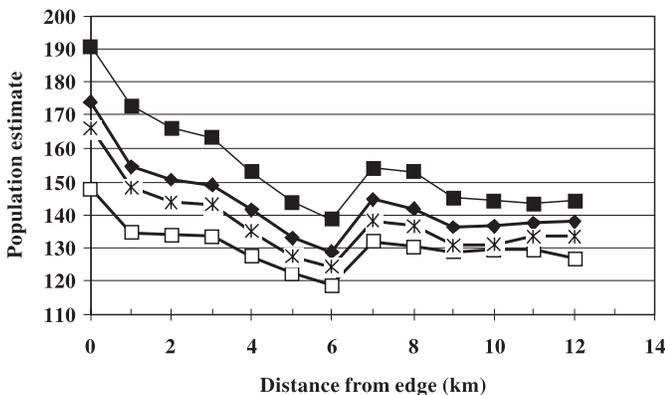
closure violation, approximately 10 km, is displayed graphically in Fig. 5. The mean capture locations of individual bears are also displayed. Many bears that were in the edge area had mean capture locations that fell very close to the grid boundary, therefore it is highly probable that they moved in or out of the grid area.

Discussion

In this analysis we attempted to fit plausible function shapes to relationships between distance from edge and mark–recapture parameters by using an approach similar to the curve-fitting techniques used with distance methods for line-transect data (Buckland et al. 1993). The analysis of the Prophet River data indicated a definite trend in addition rates, capture probabilities, and fidelity rates of bears as a function of distance from edge. The approximate area that this includes (10 km) can be conceptualized as an inner strip where addition rates, fidelity rates, and capture probabilities were most affected by closure violation. This does not suggest that edge bears did not penetrate more than 10 km into the grid. Rather, it means that bears which were found more than 10 km into the grid potentially encountered enough bait

stations during enough sampling periods that changes in fidelity and addition rates are not detectable when compared with rates for bears near the grid edge. The results of this analysis demonstrate that male bears are more prone to cause closure violation as exemplified by differences in distance from edge curves for male and female bears (Fig. 2), and this is verified by studies of sex-specific bear movements (Mace and Waller 1997).

An assumption of this analysis is that the prime factor affecting addition rates, capture probabilities, and fidelity rates is permanent or temporary emigration from or immigration into the sampling grid. Alternative factors such as habitat-specific capture probabilities or other forms of spatial variation in capture probabilities and associated rates potentially affect the relationship with distance from edge. For example, results from the Huggins analysis suggest that bears captured at greater distances from edge exhibited reduced capture probabilities compared with bears captured at moderate distance from edge (Fig. 3). The decrease in capture probabilities may be due to age- or sex-specific distributions of bears, or habitat-specific capture probabilities. However, in all cases the lowest capture probabilities, fidelity rates, or survival rates are exhibited by bears captured close to the grid edge, and in this study it is biologically reasonable to assume that this is most likely due to movement across grid boundaries. We suggest that factors other than distance from grid edge which account for spatial changes in capture probability, addition rate, or fidelity rate be considered in analyses if this method is applied in other studies.

One limitation of covariate analysis is the restrictive shape of covariate curves that define the relationship between distance from edge and parameters of interest. Using higher order polynomials and log-transformed distance from edge values allowed the testing of a variety of potential curve shapes that represented plausible hypotheses concerning forms of spatial-closure violation. For example, log-transformed curves that were non-asymptotic modeled a situation in which parameters of interest (i.e., fidelity) changed constantly as a function of distance from edge, which suggests a higher degree of closure violation. In contrast, standard covariates displayed asymptotic behavior, suggesting more finite boundaries to closure violation. However, these curves will still not

Fig. 5. The 10-km distance from edge area (shaded) and core area (white) for the Prophet River grizzly bear capture–recapture grids. Data points indicate the mean capture locations for bears. Squares represent 9 × 9 km grid cells in which individual bait sites were placed during each sampling period for the duration of the project.
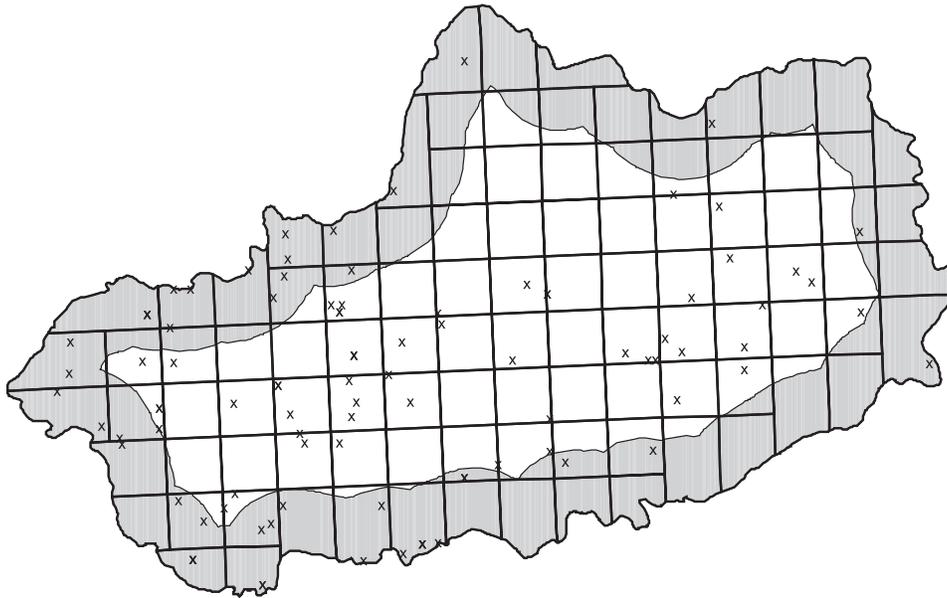
be sensitive to fine-scale changes in parameters as a function of distance from edge because sample size limits model complexity. The number of estimable parameters for a mark–recapture model is approximately $n/(10\hat{c})$, where n is the number of marked animals and $\hat{c}$ is the overdispersion parameter (K. Burnham, personal communication). Using this equation the optimal number of parameters for models would be approximately 9. The number of parameters in the most supported Pradel model is 11 (model 1 in Table 2), which is probably near the upper limit of model complexity. Other curve-fitting methods, such as the use of generalized additive modeling techniques, or more advanced transformation strategies may be useful for furthering the robustness of this analysis, given the limited sample sizes.

Huggins superpopulation estimates

This study illustrates the potential value of the Huggins closed model estimators. Unlike CAPTURE estimators, the number of parameters used with the Huggins estimator can be optimized through the use of AIC_c model selection procedures. In addition, the Huggins model can accommodate exact forms of capture-probability bias through the use of covariates, sex classes, and time-specific formulations. As a result, the precision of the Huggins estimator is much higher than that of the corresponding CAPTURE estimators (Table 4). However, a strong assumption of the Huggins estimator is that the most substantial form of capture-probability bias is caused by distance from edge effects and therefore its results will be biased if other non-identifiable (i.e., age-specific) biases are present in the data. Therefore, use of the more general CAPTURE heterogeneity estimators is still warranted. Future implementation of the likelihood-based heterogeneity estimators of Pledger and Efford (1998) and Norris and Pollock (1996) into MARK will allow more flexibility in modeling non-identifiable heterogeneity variation (G.C. White, personal communication).

CAPTURE closed model selection

Inspection of Table 4 shows a range of 36 bears between CAPTURE models in terms of population estimates. Heterogeneity variation was not decisively detected by the specific CAPTURE statistical tests; however, we suspect that there were age-specific capture probabilities due to cubs being minimally sampled because of the height of the barbed wire at bait sites. In addition, heterogeneity variation caused by increasing capture probabilities as a function of distance from edge was documented by the Huggins analysis (Fig. 3). Finally, it has been concluded from the results of simulation studies that heterogeneity estimators are most robust to hypothetical "shadow effects" caused by closely related individuals appearing to be identical in the genetic data (Mills et al. 2000). The minimal power of CAPTURE to detect capture-probability variation when capture probabilities are low is well documented (Otis et al. 1978; Boulanger and Krebs 1996; Stanley and Burnham 1998). In conclusion, model M_t was chosen by the CAPTURE model selection routine; however, model M_{th} should also be considered because heterogeneity is probable and the time (M_t) and heterogeneity estimators (M_h) are minimally robust to simultaneous time and heterogeneity variation (Otis et al. 1978; Chao and Jeng 1992).

Core-extrapolated population estimates

The Pradel analysis suggests that the majority of bears suspected of closure violation were captured at distances from edge that were less than approximately 10 km (Fig. 1). CAPTURE estimates are relatively stable at distances from edge greater than 8 km, which suggests reduced estimator sensitivity to the core bear – edge bear cutoff (Fig. 4). Therefore, the estimates should provide a better representation of grid-based population estimates than naïve estimates do. In addition, core bears exhibited higher capture probabilities (Fig. 3), therefore the precision of the core-extrapolated and naïve estimates is comparable despite the fact that the

core-extrapolated estimate is based upon fewer marked bears than the naïve estimates. However, caution should be exercised when interpreting core-extrapolated population estimates. First, determination of the core bear – edge bear cutoff (at 10 km in this case) is not estimated, therefore there is no variance estimate associated with the 10 km cutoff. Ideally, the core bear – edge bear cutoff could be estimated and the variance introduced by the estimation incorporated into the overall variance of the population estimate. Second, the Pradel analysis estimates of fidelity and addition rates mainly correspond to permanent (i.e., one entry or exit for the entire project) rather than temporary movement across grid boundaries. Therefore, core-extrapolated estimates may not be robust to large-scale temporary movement across grid boundaries. However, it is biologically reasonable to assume that temporary movement is most likely to occur closer to the grid boundaries than permanent movement. As a result, it would be expected that the 10-km cutoff should encompass edge bears exhibiting both temporary and permanent movement across grid boundaries. Finally, extrapolation of core densities relies on the assumption of even densities of bears throughout the sampling grid. Results of the χ^2 tests suggest uniform densities, but this assumption is probably violated to some degree. In conclusion, more theoretical development of this method is needed to ensure its general applicability; however, we feel that core-extrapolated estimates do give a useful approximation of the potential difference between superpopulation and grid-based ("average N") estimates that complements the results of other closure tests.

The core-extrapolation method differs from previous data-based methods of obtaining density estimates, such as the nested-subgrid routine in CAPTURE (Otis et al. 1978). The key distinction is that the core-extrapolation method defines an inner strip width based upon the results of the Pradel analysis, rather than defining an outer strip width based upon changes in population-size estimates as a function of grid size (the nested-subgrid approach). The selection of inner strip width is based upon the Pradel covariate analysis of the entire data set, instead of a comparison of nested grids, which could circumvent sample-size issues associated with the nested-subgrid approach (Wilson and Anderson 1985). We stress that core-extrapolated population estimates are not a substitute for the use of radio-collared bears to explicitly estimate average populations of bears on sampling grids using the joint mark–recapture telemetry methods of Powell et al. (2000), Eberhardt (1990), Miller et al. (1997), or Garshelis (1992). While radio-tagging bears presents a financial and logistical challenge in remote areas, it does yield a more direct estimate of movement across grid boundaries and therefore will provide a more reliable density estimate than DNA methods alone.

Genetic issues

An inherent assumption of this analysis is that the degree of error caused by genetic typing is minimal. Of the potential problems that can occur, we were most concerned with laboratory-based genetic errors. However, recent work by D. Paetkau (in preparation) suggests that the rate of genetic errors can be minimized if proper laboratory protocols and screening procedures are used, and that a system of genetic markers with the variability of the ones we used will typically resolve all individuals.

Conclusions

The Pradel analysis using MARK provides further inferences concerning closure violation that should be of value for analyzing any mark–recapture data set. For example, the importance of assessing capture locations relative to the edge of the grid was demonstrated; this is a parameter that is ignored in tests based on naïve data sets. More development using simulation modeling is needed to further test the properties of proposed population estimators; however, the general technique of detecting closure violation is valid, as it is a special-case analysis of the tested Pradel (Pradel 1996), Huggins (Huggins 1991), and CAPTURE (Otis et al. 1978) mark–recapture estimators.

Our results suggest that it is difficult to eliminate closure violation when using DNA-based mark–recapture of grizzly bears in an open system. The Prophet River grid area was 8527 km^2 and it is unlikely that many large-scale projects such as this can be conducted. Given these constraints, censuses should be designed not only to minimize closure bias through grid shape and placement but also to ensure that sample sizes are large enough to allow tests for closure violation (sensu Otis et al. 1978; White et al. 1982; Arnason et al. 1998; Stanley and Burnham 1999). This analysis shows that placing suspected areas of high animal density near the center rather than the edge of census grids should be a design priority.

Accurate and precise estimates of large carnivore populations using DNA-based mark–recapture are difficult to obtain unless sampling is conducted in areas of topographic confinement, which are uncommon, or sample sizes are large enough to allow the use of existing closure tests, open models, and the newer methods presented in this paper. We suggest that in many circumstances, the most appropriate use of DNA-based mark–recapture projects may be repeated sampling or monitoring of grid areas on an annual or biannual basis to allow the use of open models (Anderson et al. 1995) to estimate apparent survival and population trends, a technique that is more robust to closure violation and associated sampling problems.

Acknowledgements

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