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Estimation of population size for wolverines *Gulo gulo* at Daring Lake, Northwest Territories, using DNA based mark-recapture methods

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This paper presents the results of the first substantive DNA mark-recapture sampling effort for wolverines *Gulo gulo* using hair-snag sampling. In the spring of 2004, 284 bait posts were sampled in 3×3 km cells for four sessions in the Daring Lake area of the Northwest Territories, Canada. Bait posts were baited with caribou and scent lures. As well, a fish lure was dragged around by snowmobiles during bait post setup. One hair sample was genotyped from each post for each session. Results suggested a high degree of attraction to bait posts by wolverines with capture probabilities of > 0.5 for both sexes and very precise estimates for females. Males displayed substantial closure violation whereas females did not. Investigation of reduced effort designs suggests that a 2-session sampling design with moderate densities of bait posts is adequate for estimation of population size for wolverines due to high capture probabilities. A longer-term monitoring effort is recommended to allow better understanding of wolverine populations in the area.

Key words: DNA, hair-snag sampling, mark-recapture, population estimation, program MARK, wolverine

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The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) has identified the wolverine *Gulo gulo* a conservation priority. In Canada's Low Arctic tundra, on the central barrens, human-caused mortality is increasing due to increasing levels of resident and sport hunting, as well as resource development activity. There is a concern with the potential cumulative impacts of habitat loss, disturbance and increasing mortality pressures that may lead to a decline in wolverine abundance.

Snow track surveys used to index wolverine abundance are prone to observer bias, variable snow conditions and error rates that are difficult to assess. Given these limitations, the Government

of the Northwest Territories initiated a research project to develop a better sampling protocol to assess the relative abundance of wolverines across broad landscapes. By snagging hair samples and identifying individuals using DNA, the intent is to utilize more reliable trend information for wildlife monitoring programs. Ultimately, our goal is to model and assess the cumulative impacts of anthropogenic activity at a regional scale.

Estimation of population size using DNA mark-recapture methods has been used extensively for grizzly bears *Ursus arctos* (Woods et al.1999, Boulanger et al. 2002, Mowat et al. 2005), marten *Martes americana* (Mowat & Paetkau 2002), and other carnivore species. Recently, Flagstad et al. (2004) used scat-based sampling to obtain an estimate of population size for wolverines across a broad geographic area.

Our paper details the first attempt to apply DNA hair-snag sampling to a wolverine population using a new method of capturing hair from wolverines. Given this, the objectives of the analysis were to assess optimal mark-recapture estimation methods for wolverines and explore potential issues with the technique. Wolverines were sampled intensively during the 2004 field effort creating a rich data set. This provided the opportunity to explore alternative cost-efficient sampling designs through randomly re-sampling the full data set.

Material and Methods

Field methods

In April 2003, we conducted a pilot study to the northwest of Lac de Gras, within a 40-km radius of our research camp (64°52' × 111°35') at Daring Lake, Northwest Territories, Canada. We tested four hair-snagging devices: 1) 50-cm vertical stand made of 10-mm rebar, 2) 38 × 80 cm cylinder of ¾" expanded steel, 3) 5-gallon plastic pail, and 4) 1.5-m vertical spruce post. Each snagging device was fitted with double stranded barbed wire, and baited with caribou, beaver and commercially prepared lures. We deployed 192 stations over an area of 1,500 km^2 for a period of 14 days. Of the four designs tested, the vertical wooden post snagged the largest quantities of wolverine hair (Fig. 1). Posts provided less hair from non-target species than the other snagging devices positioned closer to the ground. The trial in 2003 led to the identification of twenty wolverines (12 males and eight females), so we proceeded with an expanded and more extensive sampling effort in 2004, involving only wooden posts.

Figure 1. Wolverine consuming caribou bait at top of wooden post used to snag hair samples during the 2004 Daring Lake Wolverine DNA Mark-recapture Project, Northwest Territories, Canada.

In April of 2004 we sampled a larger grid (2,556 km^2) to obtain population estimates of wolverines. We sampled 284 3 × 3 km cells for four sessions during 22 March - 9 May, 2004. Within each cell, one bait post was set up for each session with a caribou meat 'reward' and two scent lures. The bait sites were moved to the approximate center of each cell quarter for four 10-day sessions total. A fish bait lure was dragged behind the snowmobile to further attract wolverines to the bait sites. For bait posts that collected hair on one or more barbs in a given session, hair from one barb was selected for genetic analysis, biasing selection towards better quality samples (more hairs with visible roots) and away from samples that obviously came from non-target species (e.g. red hair from foxes *Vulpes vulpes*).

We selected seven microsatellite markers (Gg-4, Gg-7, Ggu101, Ggu216, Ma-2, Mvis-75, Ba-4) based on the results of Kyle & Strobeck (2002). Samples that were missing data for > 1 marker were excluded from analysis of individual identity. Expected heterozygosity of these markers averaged 0.68 in our final data set of 74 individuals, with a mean of 4.9 alleles observed per marker. Quality assurance methods of Paetkau (2003) were used to ensure the accuracy of individual identifications. Briefly, any 7-locus genotype that matched another

genotype at all-but-one or two markers ('1MM-' and '2MM-pairs') were flagged as potentially containing errors, and were reanalyzed. Since errors generally affect just one, or less often two, markers, the scrutiny of such pairs is an efficient method to detect and correct genotyping error (Paetkau 2003). In our study, the error-checking reduced the number of 1MM-pairs from 10 to 0, and the number of 2MM-pairs from seven to four. In addition to genotyping error, high match probabilities can cause inaccuracies in genetic assignments of individual identity when > 1 individual is sampled with a given multilocus genotype. The low number of 2MM-pairs, each of which were confirmed through data replication, and absence of 1MM-pairs in the final error-corrected data set indicated that the probability of any pair of individuals matching at all seven markers (i.e. the match probability) was small enough to effectively guarantee that each individual that we sampled had a unique genotype (Paetkau 2003). Gender analysis involved the co-amplification of segments of the SRY and ZFX/ZFY genes. The ZFX/ZFY primers were P1-5EZ (Aasen & Medrano 1990) and an unpublished primer (CTCCTTTTTCCTTATGCACC) that is conserved in hyena (Schwerin & Pitra 1994) and mouse (Marden et al. 1990). The SRY primers were 121R (Taberlet et al. 1993) and an unpublished primer (CATTGTGTGGTCTCGTGATCAAA) that was designed from pilot whale sequence (Griffiths & Tiwari 1993). This particular combination of primers, which were used at a concentration of 55 nM for each ZFX primer and 160 nM for each SRY primer, was identified by testing a variety of primers that were on hand at the lab. They amplify well in several mustelid species that we have tested, including fisher *Martes pennanti*, badger *Meles meles* and pine marten *Martes martes*.

Analysis of mark-recapture data

Data summary

Data was summarized in terms of overall frequencies of captures for individuals. For traditional mark-recapture analysis, multiple captures of individuals are pooled into one capture per session. Summary statistics were generated for the pooled data set.

Assessment of closure violation

The Pradel model (Pradel 1996) in program MARK (White & Burnham 1999) was used to assess the data set for closure (Boulanger & McLellan 2001). The main premise for this test is that, if closure violation was occurring, wolverines that were near the grid edge ('edge' wolverines) would have lower recapture rates due to a reduced trap encounter rate compared to wolverines farther from the edge ('core' wolverines). In addition, if wolverines moved from the grid for some of the sampling sessions, then edge wolverines would exhibit a lower apparent survival estimate than core wolverines. Also, wolverines that immigrated into the grid area during sampling would be more prone to be captured near the grid edge. The distance from edge of capture was the shortest distance from the grid edge to the mean location of hair-collection posts where a wolverine was identified during the entire project.

The Pradel (1996) model as incorporated in program MARK (White & Burnham 1999), which estimates apparent survival (ϕ), recruitment (f) and recapture probability (p), was used for this analysis. The estimates for recapture rate are for the exact sampling period, whereas the estimates for the apparent survival rate (ϕ) and recruitment (f) correspond to the interval before the given sampling period.

We assumed that the population of wolverines was demographically closed for this analysis. The duration of sampling was $\approx$ 1 month so this assumption was reasonable. Apparent survival (ϕ) equals true survival (S; due to mortality) times the fidelity of wolverines (F) to the sampling grid ($\phi = SF$). Because the population was demographically closed, we assumed that true survival equaled one ($S = 1$) and therefore relative changes in f reflect wolverine fidelity to the sampling grid rather than actual mortalities, i.e. $\phi = F$. The Pradel recruitment rate estimates 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 zero and therefore measures of recruitment reflected permanent immigration or 'additions' of wolverines into the sampling grid. Wolverine females typically have offspring in late February or early March. Since young do not hunt or scavenge with females during early development, they were not captured at bait posts and are therefore not part of the sampled population. For the sake of simplicity we will refer to ϕ as the rate of 'Fidelity' and *f* as the rate of 'Additions' in the rest of the report.

As an initial appraisal of population closure we evaluated the goodness of fit of Pradel models con-

strained to only allow certain forms of closure violation as first proposed by Stanley & Burnham (1999). We emulated the approach of Stanley & Burnham (1999) by fixing parameters to appropriately constrain the Pradel model as detailed in Boulanger & McLellan (2001).

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 the logistic curve, which is used to model covariates in MARK, could accommodate 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 program MARK by the mean and standard deviation of observed distances (White et al. 2002). 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 score was considered the most parsimonious, thus minimizing estimate bias and optimizing precision (Burnham & Anderson 1998). Delta AIC_c (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 < 2 was treated as worth considering.

If a segment of core animals was identified by the Pradel analysis, then population estimates were calculated for this segment and extrapolated to the entire grid area (Boulanger & McLellan 2001). This extrapolation was based on the assumption that differences in population size estimates were due to closure rather than differences in densities of wolverine on the trapping grid. A test of uniform density (Otis et al. 1978) was therefore conducted to test whether density was reasonably uniform between core and extrapolated areas.

Population estimates

We primarily used the Huggins closed models (Huggins 1991) for model selection and population estimates. Sexes of wolverines were entered as groups in this analysis, testing whether sexes displayed differing forms of capture probability variation. Models with time, heterogeneity and behaviour variation were considered in the analysis. Mixture model heterogeneity estimators (Pledger 2000) as incorporated in program MARK were used to model heterogeneity variation. This is modeled by letting capture probabilities come from more than one capture probability distribution. There are three parameters with the 2-distribution mixture model. The parameters are the probability that a given capture probability will come from the first distribution (π), the mean capture probability of the first distribution (θ_1), and the mean capture probability of the second distribution (θ_2; Pledger 2000). As with the Pradel analysis, AIC_c model selection was used to assess parsimonious estimation models.

Population estimates from the program MARK models and program CAPTURE (Otis et al. 1978) models were considered. Estimates from all of the Huggins MARK models were model-averaged, allowing population estimates that were influenced by all the estimation models considered in the analysis.

Exploration of cost-efficient sampling methods

A key objective of our study was to determine optimal sampling protocols for the estimation of population size and trend of wolverines. The sampling design for 2004 efforts was spatially intensive. This allowed detailed assessment of areas in which wolverine traverse during sampling as well as exploration of alternative sampling designs.

To explore alternative sampling strategies we subsampled the data set in terms of the number of sessions used for estimates and the spatial configuration of bait site posts. The entire genetic data set was used as a basis for simulations including multiple captures of individual wolverines at different sites within each session. Grids were subsampled by pooling data from adjacent cells in the full data set grid. For example, 36 km^2 cells were created by pooling four adjacent 3×3 km cells into a 6×6 km cell. Sites (bait posts) were then selected randomly from within each larger pooled cell for each session to create a simulated data set at a lesser sampling intensity. In some cases incomplete pooled cells were created due to the number of rows and columns available in the full data set. In this case, the cell was subsampled with an intensity equal to other full cells (by adjusting the number of sessions it was sampled). This approach allowed the full grid to be considered for all simulations. It did not bias simulation results, since each grid cell received the same degree of sampling effort.

Population estimates were then generated from these pseudo data sets to provide an indication of

Table 1. Grid designs compared in random subsampling analysis for the 2004 Daring Lake Wolverine DNA Mark-recapture Project, Northwest Territories, Canada.

Data set	Grid cell size[1]	Sessions	Traps employed per session	Total traps employed
Capture frequencies	9 (3 × 3 km)	1	284	284
Capture frequencies	18 (3 × 6 km)	1	142	142
Capture frequencies	36 (6 × 6 km)	1	71	71
MR data set	9 (3 × 3 km)	2	284	568
MR data set	18 (3 × 6 km)	2	142	284
MR data set	36 (6 × 6 km)	2	71	142
MR data set	9 (3 × 3 km)	4	284	1136
MR data set	36 (6 × 6 km)	4	71	284
MR data set	81 (9 × 9 km)	4	35	140

[1] One bait site was placed in a grid cell for each session.

estimator performance if sampling intensity was lessened. For most data sets, the process of randomly resampling the grid was repeated 1,000 times to provide full coverage of potential data sets. The exception to this was the full data set (four sessions and 9-km^2 cells). In this case, data from all the bait sites were used for estimates, and therefore subsampling was not possible. The degree of effort for each sampling design was indexed by the total number of unique bait sites that were sampled for the entire duration of sampling (Table 1). All data subsampling was conducted using programs written in SAS statistical software (SAS Institute 2000).

A 'capture frequencies' estimation method that uses data from only one session was also considered in simulations. This estimator basically uses the number of unique captures of wolverines at different sites to build a distribution of capture frequencies. For example, during session 1, five females were caught at only one site, five were caught at two sites and two were caught at four sites. MARK model M_{h2} π(.) p1&2 (sex) was used to obtain population estimates for 1-session and 4-session data sets. Previous work with this estimator has suggested that it is biased by extreme 'trap happy' animals that display 'outlier' capture frequencies (John Boulanger, unpubl. data). Therefore, capture frequencies of wolverines beyond the 95th percentile were not considered in estimates. This truncated the maximum number of captures within a session to $\leq$ 16. The Lincoln-Petersen (LP) estimator (Lincoln 1930) was used for 2-session data sets and MARK Huggins mixture models (described previously) were used for 4-session data sets.

The performance of estimators was evaluated in terms of capture probabilities, precision, mean population estimates and number of unique wolverines captured for a given sampling effort. Capture probability was estimated as the mean number of wolverines captured per session divided by the superpopulation estimate from the full data set. Precision was indexed by the coefficient of variation or the standard deviation of repeated resamplings divided by the mean estimate. A coefficient of variation of less than 15% was considered acceptable. The coverage, or proportion of the superpopulation sampled provided an approximation of the yearly capture probability for a monitoring project. Coverage levels above 50% were considered optimal for population monitoring purposes.

Results

Data summary

In 2003, we identified 112 captures of 20 wolverines, and in 2004 we had 780 captures of 53 wolverines. Each individual was defined using a sample with a complete 6- (2003) or 7-locus (2004) genotype. In 2003, four samples were missing data for one out of six markers used for genetic identification. In 2004, 14 samples were missing data for one of seven markers used for genetic identification. Samples that were missing data for $>$ 1 marker were excluded from analysis of individual identity.

For standard mark-recapture analysis, data for individual captures was pooled for each session. For example, a wolverine caught 10 times at unique bait sites in a session was considered one capture for that session. Summary statistics were then tallied from pooled data for each sex class (Table 2). The number of animals caught n(j) was approximately constant for all four sample sessions. This suggested minimal temporal variation in capture probabilities. In total, 29 males and 24 females were identified. The number of newly caught wolverines (uj)

Table 2. Summary statistics for mark-recapture analysis for the 2004 Daring Lake Wolverine DNA Mark-recapture Project, Northwest Territories, Canada.

Statistic	Session (j) 1	2	3	4	Total
Males					
Animals caught n(j)	17	18	19	23	
Total individuals caught M(j)	0	17	21	24	29
Newly caught u(j)	17	4	3	5	
Frequencies f(j)	9	4	4	12	
Females					
Animals caught n(j)	18	20	23	19	
Total individuals caught M(j)	0	18	23	24	24
Newly caught u(j)	18	5	1	0	
Frequencies f(j)	1	3	7	13	

decreased for both sex classes with session. However, new males were still being captured in the latter sessions, suggesting new males may have entered the grid. The capture frequencies of both sexes suggested high capture probabilities with many animals being captured four times. This is especially the case with females where only one animal was caught once. In contrast, nine males were captured once, suggesting closure violation or heterogeneity of capture probabilities.

Assessment of closure violation

A plot of the spatial distribution of captures suggested that wolverines in the center of the grid area were captured more often than wolverines on the edge of the grid. One plausible reason for this was that wolverines on the edge of the grid were not on the grid area during the entire project and therefore encountered fewer traps. In general, wolverines showed a large degree of attraction to bait sites as indicated by the frequency of unique captures at bait sites (Fig. 2). These frequencies are a tally of unique sites encountered for individual wolverines over the course of sampling. For example, one male wolverine was identified at 57 different sites during the course of the study.

For the Pradel model closure analysis, goodness of fit tests in program RELEASE (Burnham et al. 1987) suggested minimal overdispersion ($\chi^2 = 3.01$, df = 6, P = 0.81), so AIC_c was used for model selection. AIC_c model selection results suggested that male wolverines displayed varying fidelity and rates of addition as a function of distance from grid edge (Table 3, Model 1). Females did not ex-

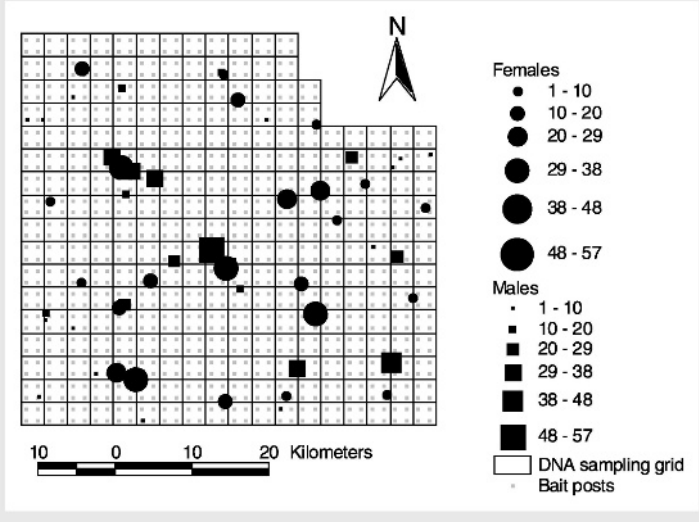

Figure 2. Spatial distribution of mean capture locations and the corresponding number of captures for individual male and female wolverines for the 2004 Daring Lake Wolverine DNA Mark-recapture Project, Northwest Territories, Canada. Each point corresponds to the mean capture location of an individual wolverine. The size of the point corresponds to the number of unique sites that an individual was captured. Each 3 × 3 km square grid cell received one bait site for four sampling sessions.

Table 3. AIC_c model selection for Pradel model analysis for the 2004 Daring Lake Wolverine DNA Mark-recapture Project, Northwest Territories, Canada. Akaike Information Criteria (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), number of parameters (K) and deviance are presented.

	Fidelity (ϕ)		Additions (f)		Recapture					
No	Male	Female	Male	Female	probability	AIC_c	ΔAICc	w_i	K	Deviance
1	(.)[A]+ld[B]	1[C]	(.)+ld	0	sex	195.9	0.0	0.54	6	183.3
2	sex[D]+ld	sex+ld	sex+ld	sex+ld	sex	197.5	1.6	0.19	8	180.5
2	(.)+ld	(.)	(.)+ld	(.)	sex	198.1	2.2	0.18	7	183.3
3	(.)+ld,ld^2	1	(.)+ld,ld^2	0	sex	198.5	2.6	0.15	8	181.5
4	D	1	d	0	sex	199.3	3.4	0.10	6	186.8
6	(.)+ld	(.)+ld	(.)+ld	(.)+ld	sex	202.0	6.1	0.02	10	180.4
5	(.)	1	(.)+ld	0	sex	202.9	7.0	0.02	5	192.5
6	(.)+ld	1	(.)	0	sex	205.2	9.3	0.01	5	194.8
7	(.)	1	(.)	1[C]	sex	207.0	11.1	0.00	4	198.7
8	(.)	(.)	(.)	(.)	sex	211.3	15.4	0.00	6	198.7
9	(.)	(.)	(.)	(.)	(.)	217.8	21.9	0.00	3	211.7
10	time	time	time	time	sexXtime	224.0	28.2	0.00	15	190.6
11	1	1	0	(.)	sex	225.3	29.4	0.00	3	219.1
12	1	1	(.)	(.)	(.)	229.7	33.8	0.00	2	225.7
13	(.)	(.)	0	0	(.)	244.2	48.3	0.00	2	240.1
14	1	1	0	0	(.)	244.3	48.4	0.00	1	242.2

[A] Parameter was held constant, assuming that it did not vary with trapping period.
[B] log-transformed distance of mean capture from grid edge.
[C] Parameter was fixed at 1 (ϕ) or 0 (f) under the assumption of no emigration or immigration.
[D] Model assumed different intercept but similar slopes for distance from edge curves.

hibit substantial closure violation (Model 1) as suggested by support for model 1, which assumed no emigration or additions of females during sampling. A model that assumed different levels of fidelity and addition of male and female wolverines, but similar slopes for distance of grid edge and fidelity and additions was marginally supported ($\Delta AIC_c = 1.6$). Inspection of plots from this model suggested that additions for females became 0 and fidelity became 1 when the mean distance of capture from the grid edge was > 2 km. Therefore, this model also suggested minimal violation of closure for female wolverines. The Pradel method is mainly sensitive to permanent rather than temporary movement of wolverines from the sampling area (Boulanger & McLellan 2001). So it is still possible that closure violation occurred for females to a lower degree.

Inspection of plots of fidelity and rates of addition for male wolverines from model 1 suggested that fidelity was low and rates of addition high for wolverines for mean distances of capture of < 7 km (Fig. 3). At greater distances, fidelity was close to one and additions close to zero suggesting minimal closure violation. Therefore, wolverines captured at a mean distance of > 7 km were most likely 'core' animals that spent most of the time of sampling on the sampling grid.

Model selection and superpopulation estimates

AIC_c model selection results for the Huggins closed N mixture models suggested that models that assumed heterogeneity and behavioural response were most supported (Table 4). Model selection results suggested that much of the heterogeneity was from the male segment of the population. Inspection of sex-specific population estimates for each model suggested little effect of model selection on

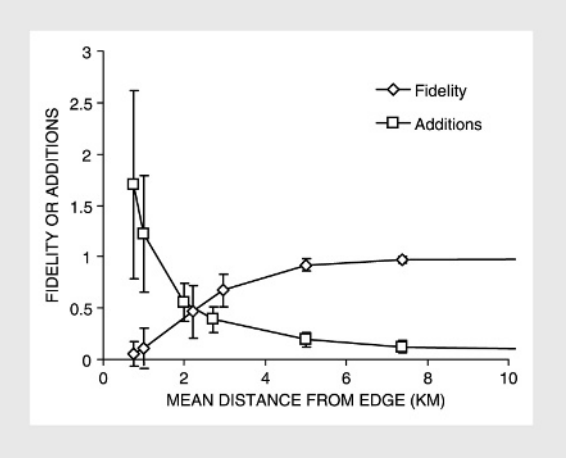

Figure 3. Plots of the relationship between mean distance of capture from grid edge and fidelity and rate of additions for male wolverines from Pradel model analysis (see Table 3, model 1).

Table 4. AIC_c model selection for Huggins closed N mixture model analysis for the 2004 Daring Lake Wolverine DNA Mark-recapture Project, Northwest Territories, Canada. Akaike Information Criteria (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), number of parameters (K) and deviance are presented. Population estimates ($\hat{N}$) and associated standard errors (SE) are given for each model.

							Males		Females	
No	Model	AIC_c	ΔAIC_c	w_i	K	Deviance	$\hat{N}$	SE	$\hat{N}$	SE
1	M_{bh2} π (.) $\theta_{1\&2}$ (sex) $c_1\&c_2$(sex)	218.9	0.00	0.30	9	200.0	40	6.16	24	0.79
2	Males M_{h2}, Females M_o	219.0	0.70	0.29	4	210.8	35	4.90	24	0.13
3	M_{h2} π (.) $\theta_{1\&2}$ (sex)	219.6	0.68	0.21	5	209.3	35	4.89	24	0.33
4	M_{th2} π (.), $\theta_{1\&2}$ (sex) +time	221.4	2.45	0.09	8	204.7	35	4.04	24	0.00
5	M_{h2} π (sex) $\theta_{1\&2}$ (sex)	221.7	2.77	0.08	6	209.3	35	4.90	24	0.53
6	M_{h2} π (.) $\theta_{1\&2}$ (.)	223.3	4.37	0.03	3	217.2	32	2.57	26	2.29
7	M_b π(sex) c(sex)	230.3	11.37	0.00	4	222.1	33	3.61	24	0.28
8	M_o π(sex)	237.9	18.96	0.00	2	233.8	29	0.69	24	0.13
9	M_t	247.1	28.17	0.00	4	238.9	29	0.36	24	0.32

female compared to male superpopulation estimates. Estimates for females were identical to the total number captured (see Table 4) suggesting that the entire population was sampled.

Inspection of model-averaged estimates of capture ($\hat{p}$) and recapture probabilities ($\hat{c}$; probability of capture after initial capture) suggested a slight increase in probability of capture after initial capture (females: $\hat{p} = 0.77$, CV = 14%, $\hat{c} = 0.8$, CV = 13%; males: $\hat{p} = 0.55$, CV= 58%, $\hat{c} = 0.64$, CV = 60%). In addition, higher coefficients of variation (CV) of male estimates suggested a high degree of heterogeneity in capture probability of males compared to females.

The Pradel closure analysis suggested that males violated the assumption of closure, and that males captured at distances of > 7 km from the grid edge were most likely core wolverines. Therefore, population estimates were calculated for this core segment and extrapolated to the entire grid area. Tests for uniform density suggested that densities of marked wolverines were relatively uniform for males ($\chi^2 = 4.08$, df = 4, P = 0.39) and females ($\chi^2 = 1.75$, df = 4, P = 0.89). A sensitivity analysis of core-extrapolated estimates suggested that male estimates were initially high, then declined at distances of around 7 km, the distance predicted by the Pradel model. In contrast, estimates for females were relatively steady with minimal decline (Fig. 4).

Estimates of superpopulation size compared to closure corrected population size were 68 and 9% higher for males and females, respectively (Table 5). The difference for females could be due to random variation in densities rather than closure violation. Coefficients of variation for superpopulation and closure-corrected population estimates for males were relatively high compared to females. Females showed very high levels of precision, which was presumably due to high capture probability levels combined with minimal capture probability variation.

Exploration of cost-efficient sampling methods

Wolverines displayed very high capture probability levels when the cell size was 3×3 km (Fig. 5). The performance of sampling designs for male wolverines was influenced greatly by closure violation (Table 6). Sample designs that sampled for one or two sampling periods displayed population estimates closer to the closure-corrected population size and higher levels of precision. The most likely explanation for this is that these designs were less influenced by transient wolverines appearing on the sampling grid for short periods, therefore inflating population estimates and reducing precision. The Lincoln-

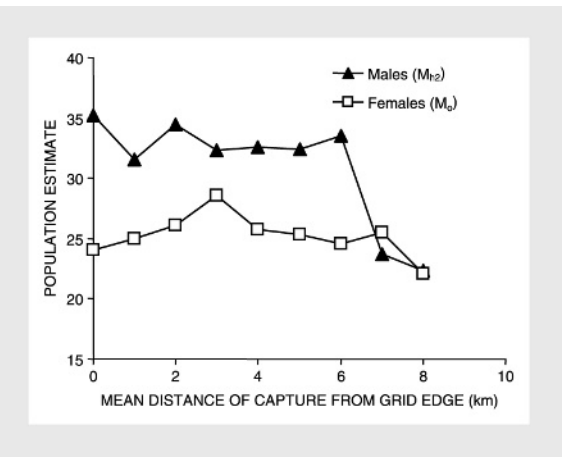

Figure 4. Core-extrapolated estimates of population size for male and female wolverines as a function of mean capture from distance of edge cutoff. Estimates are from the MARK Huggins mixture model (see Table 4, model 2).

Table 5. Superpopulation and closure corrected population estimates ($\hat{N}$) for wolverines in the 2004 Daring Lake Wolverine DNA Mark-recapture Project, Northwest Territories, Canada.

Parameter	$\hat{N}$	SE	95% C.I.		CV (%)
Superpopulation					
Males					
MARK model average	37	5.64	25	48	15.4
M_h (jackknife)	36	4.60	32	51	12.8
M_{bh} (Pollock & Otto 1983)	44	7.74	35	67	17.6
Females					
MARK model average	24	0.54	23	25	2.2
M_h (jackknife)	26	1.17	25	29	4.5
M_o	24	1.13	24	24	4.7
M_{bh} (Pollock & Otto 1983)	24	0.27	24	24	1.1
Closure corrected (core extrapolated at 8 km)					
Males					
M_{h2} π (.)$\theta_{1\&2}$ (sex)	22	0.36	20	40	16.2
M_h (jackknife)	22	0.26	22	33	11.9
Females					
M_{bh2}	22	0.00	22	22	0.4
M_o	22	0.02	22	22	0.1

Petersen (LP) 2-session estimator with an 18 km^2 grid cell size showed the best overall performance, with higher levels of precision and population estimates closest to the closure corrected population size. However, it is emphasized that the fact that the LP estimator was close to closure-corrected N estimates could be an anomaly of simulations. As discussed later, the only method to ensure closure corrected estimates is the testing and modeling of closure violation.

The degree of bias in closed N estimates for males was close to zero when grid cell size was 81 km^2 and four sessions were sampled. However, this result should not be interpreted to mean that closure bias will be negligible at this cell size. The reduction in bias as cell size increased was due to increased heterogeneity of capture probabilities rather than reduction of closure bias, given that the same overall grid size was used for all grid cell sizes that were subsampled.

The performance of estimators and sampling designs for females was influenced more by heterogeneity variation (at larger grid cell sizes) than closure bias (see Table 6). As cell size increased, precision and estimated population sizes decreased. One exception to this was stable population size estimates for the 4-session heterogeneity M_{h2} estimators. In this case, there was still enough information to allow the modeling of heterogeneity leading to unbiased performance (in terms of superpopulation estimates), even when cell size was large. However, the degree of precision did decrease to unacceptable levels when cell size was greater than 36 km^2. In general, the 2-session LP estimator design showed the best performance of all designs and estimators as long as cell size was 18 km^2 or less. The 1-session designs and estimators also displayed reasonable performance as long as cell size was 9 km^2. The 4-session design did not show a good performance with males but reasonable performance with females (Table 7). One issue with the evaluation of the 81 km^2 cell size 4-session design was that it was

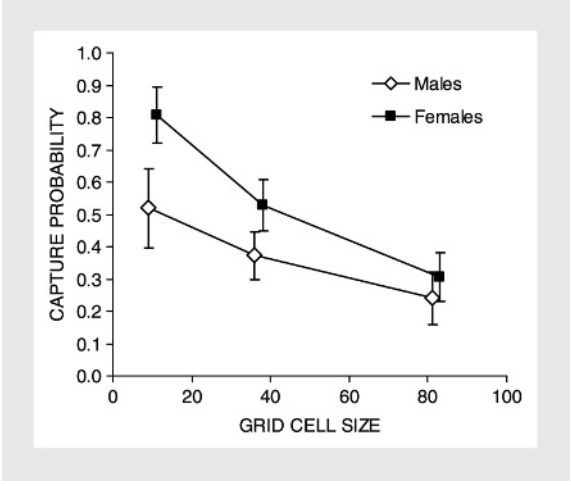

Figure 5. Estimates of capture probability for different grid cell sizes (from subsampling analysis) for wolverines for the 2004 Daring Lake Wolverine DNA Mark-recapture Project, Northwest Territories, Canada. Superpopulation estimates for the grid were used to estimate capture probabilities, and therefore estimates are conservative for males where closure was violated.

Table 6. Summary of design and estimation model performance for male and female wolverine populations from random subsampling analysis. Superpopulation estimates (N_s) corresponds to estimate for grid and surrounding area whereas closed population estimates (N_c) correspond to the estimated average number of wolverines on the grid at any one time (see Table 4). Average population estimates ($\hat{N}$) and the coefficient of variation from average population estimates from random subsamples are given for each design and estimation model (see Table 1).

			Males (N_s=36, N_c=22)		Females (N_s=24, N_c=22)	
Design Sessions	Cell area	Estimation model	$\hat{N}$	CV	$\hat{N}$	CV
1	9	M_{h2} π(sex) $\theta_{1\&2}$(sex)	22.2	12.7	21.5	6.6
1	18	M_{h2} π(sex) $\theta_{1\&2}$(sex)	20.5	18.0	21.4	15.6
1	36	M_{h2} π(sex) $\theta_{1\&2}$(sex)	19.8	28.6	21.6	31.6
2	9	Lincoln-Petersen	24.6	6.9	23.0	2.9
2	18	Lincoln-Petersen	21.2	9.2	21.5	6.6
2	36	Lincoln-Petersen	18.4	13.0	19.5	15.2
4	9	M_{h2} π(sex) $\theta_{1\&2}$(sex)	36.0	16.2	24.2	5.9
4	36	M_{h2} π(sex) $\theta_{1\&2}$(sex)	24.9	18.1	24.2	13.7
4	81	M_{h2} π(sex) $\theta_{1\&2}$(sex)	21.8	24.1	23.8	27.3

based on the sample size of wolverines on the Daring Lake grid (superpopulation sizes of 37 and 24 males and females, respectively). Presumably, the grid size could be increased to allow more wolverines to be sampled therefore offsetting decreased precision caused by lower capture probabilities. To explore this, we conducted simulations in program MARK using heterogeneity parameters from the M_{h2} model (see Table 4, model 3). We ran simulations at different population sizes with mean capture probabilities for the 81 km^2 cell size (see Table 6) and found that a CV of 15% for females could be achieved, if population size was greater than 50 females (a total population size of 100 wolverines assuming an even sex ratio) and four sessions of sampling were conducted. The CV for males was 28% when the population size of males was 50. The lower degree of precision was most likely due to the higher degree of heterogeneity variation associated with males. Some of the heterogeneity variation due to closure might be diminished at larger grid sizes, in which case precision would be higher than suggested by simulations.

Discussion

The Daring Lake data set suggests that bait posts are a highly efficient method of sampling wolverine populations. The levels of precision attained for female wolverines have not been achieved for grizzly bears (Boulanger et al. 2002) or any other large carnivores using DNA sampling. It is extremely important to consider the exact methodologies and environmental conditions used for the Daring Lake Project when interpreting these results and implementing recommendations for other projects. For example, this project occurred in early spring when alternative sources of food were minimal and DNA preservation was enhanced due to cold and dry conditions. It is likely capture probabilities would decrease in later spring when alternative food sources are available and conditions are wetter. Scent lures were dragged behind snowmobiles further attracting wolverines to sites compared to scents at sites only. A reward was given at bait posts and bait posts were moved after each session which may have increased wolverine capture probabilities after

Table 7. Recommended sampling designs for wolverines for estimation of population size for the 2004 Daring Lake Wolverine DNA Mark-recapture Project, Northwest Territories, Canada.

	Sessions				
Sampling intensity	Males	Females	Sites per session	Total sites	Population size needed
9 km^2 (3 × 3 km)	1-2	1-2	284	284	≥50[A]
18 km^2 (3 × 6 km)	2	2	142	284	≥50
36 km^2 (6 × 6 km)	2	4	71	284	≥50
81 km^2 (9 × 9 km)	4	4			≥100

[A] Assuming an even sex ratio of wolverines, i.e. 25 males and 25 females.

initial capture. Other factors, such as genotyping only one sample at a post and sampling in early spring when females with young may utilize smaller home ranges, could potentially reduce capture probabilities. It is also possible that the high density of traps saturated the wolverine populations therefore maximizing initial encounter of wolverines and creating a larger degree of 'trap happy' behavioural response than if less intense designs were used. In this case, the capture probabilities estimated by the subsampling simulation analysis might be biased high. We suggest that bait dragging and other methods be used to ensure a high degree of initial encounter of wolverine at bait sites, especially if less spatially intense sampling designs are used.

Estimation of population size for male wolverines is much more challenging than for female wolverines. Male wolverines displayed a greater degree of closure violation, which was most likely due to transient wolverines only spending a portion of the time of sampling on the grid. The consequence of this was reduced capture probabilities, reduced precision, and a substantial difference between superpopulation and closure corrected estimates. In contrast, females displayed minimal violation of closure resulting in higher capture probabilities and enhanced precision.

These estimates provide a 'snapshot' of wolverines during early spring. Females producing young typically give birth in late February or early March, and may initially limit their excursions from the natal den. By April, adult females ($\geq$ 2 years of age) are believed to be utilizing their available home ranges (Mulders 2000). Subadult wolverines (12 and 24 months of age) likely utilize a broader area than established females (Mulders 2000, Inman et al. 2007). As discussed later, a multi-year sampling approach is needed to ascertain the actual dynamics and longer-term status of the population.

One potential estimation issue is the 'trap-happy' behavioural response of wolverines to bait posts. A slight increase in capture probabilities after initial capture was detected for both male and female wolverines. Estimates of population size will be negatively biased from non-behavioural response models when trap-happy response occurs (Williams et al. 2002). However, we speculate that continuous sampling of DNA sites (unlike traditional live-traps) and the pooling of data from multiple samplings into one session probably minimizes the degree to which behaviour response affects capture probability estimates. Namely, the event of initial trap encounter and re-encounter probably occurs within a single session and counts as one capture event when the data are pooled. Pooling robustness and higher overall capture probabilities probably explain why non-behavioural models produced reasonable estimates even when behavioural response was detected.

One other study has estimated wolverine population size using DNA from scat samples (Flagstad et al. 2004). Unlike this study, samples were collected across a broad geographic area between February and June. Given the difference in scale of sampling and different method of sample collection, it is difficult to compare population estimates from these studies. One potential issue with scat sampling at larger scales is meeting the assumption that every individual in the population has a non-zero probability of capture. If the scats of some individuals had no probability of being collected then population estimates would be negatively biased. In addition, the actual time of deposition of scat was unknown which therefore extended the actual time frame of sampling to an indefinite longer time period. Finally, the temporal time frame of sampling from this Norwegian study was long (five months) making the assumption of geographic and demographic closure questionable. In contrast, in our study hair sampling was conducted within a defined time period and a more defined sampling area. We suggest that the methods presented in this manuscript can be applied to allow more critical evaluation of the assumptions of scat sampling.

Conclusions

Estimation of population size

The relatively high capture probabilities of wolverines make it possible to consider reduced effort designs that do not involve many ($\geq$ 4) sampling sessions. The reason for this is that mark-recapture estimators, such as the Lincoln Peterson, are relatively robust to capture probability variation when capture probabilities are > 0.5 (Menkens & Anderson 1988, Pollock et al.1990). The 2-session sampling design has the added advantage that the overall duration of trapping is shorter, therefore minimizing the degree of closure violation for males. However, results of the data subsampling analysis suggest that trapping intensity must be relatively intense (see Fig. 5) to ensure higher capture

probabilities. As trapping intensity decreased, capture probabilities decreased leading to reduced precision and estimator performance (see Table 6). Once capture probabilities were below 0.5 (at lesser trapping intensities), a 4-session sampling design is needed to allow the use of robust estimation models (see Table 7).

If estimation of male density is of great importance, it may be argued that the 4-session approach is better than assuming negligible bias with the Lincoln-Petersen estimator. Methods such as the Pradel model/core-extrapolation provide a method to test for and correct for closure violations. This approach requires > 2 sessions of sampling to allow estimates of Pradel model parameters. Session length could potentially be shortened to reduce closure bias with the 4-session design. Alternatively, a multi-year open model estimation approach (McDonald & Amstrup 2001, Boulanger et al. 2004,) may be the best method to estimate male population size.

The 1-session capture frequencies approach to estimating population size worked well at higher bait post densities. However, the degree of precision decreased markedly when bait post density was reduced. The reason for this is that this method relies on multiple samplings of individuals within a single session for population estimates. In addition, heterogeneity estimators used for capture frequency-based estimation assume no behavioural response (Miller et al. 2005). Therefore, they are potentially biased if capture probabilities change after initial capture. It is probable that this method might still be robust to behavioural response if capture probabilities and frequencies are high enough. Further simulation study is needed to determine the general performance of the capture frequency estimator especially its robustness to potential behavioural response to sampling. The other issue with a 1-session sampling design is that it relies on a high degree of capture success for the single session. Weather or other factors can often reduce success rates, so it may be prudent to sample for two sessions as a form of insurance.

Estimation of population trend

Estimation of population size only provides a 'snapshot' of actual wolverine status. We argue that a multi-year monitoring effort is the best methodology to allow the understanding of actual wolverine dynamics and status. Recent advances in mark-recapture modeling (Pradel 1996, Nichols & Hines 2002,) allow inference about the effect of environmental conditions and other covariates on population demography (Boulanger et al. 2004). In addition, it is possible to incorporate genetic data from wolverine mortalities due to harvest and other factors (Barker 2001) to allow enhanced estimates of survival and population trend.

It is possible to monitor trend with only one sampling session per year with the Pradel model (Pradel 1996). However, enhanced estimates of survival and population trend can be obtained by combining open and closed models in a robust design framework (Pollock et al. 1990). Simulations can be used to optimize designs for management objectives. In general it can be concluded that any of the recommended designs for population estimation would be adequate for initial population estimation and monitoring purposes. Although wolverine numbers appear to be healthy in the tundra habitat around Daring Lake, we do not know how representative these densities are relative to boreal and mountain habitats in the Northwest Territories.

It is imperative that any monitoring design be standardized so that the same methodology (trap configuration and bait type) is used each year. In addition, mark-recapture methods that estimate the capture probabilities of wolverines be used for trend monitoring rather than count-based indices. The main problem with count-based indices is that change in capture probabilities of wolverines (due to, for instance, weather, seasonality and methodology) could cause changes in counts of wolverines caught, which would be erroneously interpreted as population change (Anderson 2001). Mark-recapture provides a robust method to estimate trend (Anderson et al. 1995) and factors associated with trend.

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