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Density and population size estimates for North Cascade grizzly bears using DNA hair-sampling techniques

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Abstract

We used non-invasive DNA hair-sampling and catch per unit effort (CPUE: grizzly bears detected per 1000 trap nights) to estimate relative density and population size for a threatened grizzly bear population in the North Cascade Ecosystem of Washington and British Columbia. We used linear, logistic, and linear through the origin regression analyses to estimate the relationship between catch per unit effort and grizzly bear density for seven other grizzly populations. One grizzly bear was detected during 5304 trap nights (CPUE = 0.19) over 3 years in the North Cascades. This CPUE was much lower than in the other seven populations, including two threatened grizzly populations in the Cabinet-Yaak and Selkirk Mountain Ecosystems. The logistic model (curvilinear relationship) best fit the data ($R^2 = 0.927$), and yielded density and population size estimates of 0.15 bears/100 km^2 (90% CI = 0.03–0.71) and six bears (90% CI = 1–27), respectively. Natural recovery seems unlikely for the North Cascade grizzly bear population because the population has a high likelihood of extinction due to demographic and environmental stochastic effects associated with extremely small population numbers. We recommend population augmentation. DNA hair-sampling and catch per unit effort models can be a useful method to evaluate relative densities and numbers of animals in small, threatened grizzly bear populations when sample sizes are too small to yield traditional mark–recapture analysis.

Keywords: Grizzly bear; DNA hair-sampling; Catch per unit effort; Small population; North Cascade Ecosystem

1. Introduction

Grizzly bears in the North Cascades Ecosystem (NCE) have been protected on both sides of the international border for decades, but the population has not recovered from extremely low numbers. Although grizzly bear population numbers in the North Cascades are unknown, biologists estimated approximately 20 animals in BC (Gyug, 1998; BCMELP, 2001) and <50 animals in Washington state (Almack et al., 1993) based on sighting information over several decades. These putative population estimates for the NCE are similar to estimated population sizes in the Cabinet-Yaak Ecosystem (CYE) of northwest Montana (Kasworm et al., 2000) and the Selkirk Mountain Ecosystem (SME) of northern Idaho (Wakkinen and Johnson, 1999). If these estimates are accurate, the remnant NCE population might have a reasonable chance of eventual recovery with minimal (CYE; Servheen et al., 1995) or even no (SME) augmentation efforts. If the NCE population is considerably smaller than the CYE and SME, augmentation may be necessary to achieve recovery. In this paper we estimate the NCE population size relative to the CYE and SME using DNA hair-snag techniques, to help direct management and recovery efforts.

The use of non-invasive DNA sampling methods by collecting hair from animals in the field has become a widely used and accepted technique in wildlife management,

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especially in bear *Ursus* spp. biology (Taberlet et al., 1997,1999; Woods et al., 1996,1999). Using a scent lure to attract bears to a site, a perimeter fence of barbed wire snags hair from an animal. Resulting hair roots contain enough DNA to identify species (Woods et al., 1999), sex (Mowat and Strobeck, 2000; Taberlet et al., 1993), and genotypic profiles of individuals without seeing or handling the animals (Kohn and Wayne, 1997; Foran et al., 1997; Gagneux et al., 1997; Goossens et al., 1998). The use of hair-sampling techniques is also a potential tool for monitoring smaller populations (USFWS, 1997). We tested hair-sampling methods as a way to detect bears and estimate density and population size of the small, threatened population of grizzly bears *Ursus arctos* in the North Cascade Ecosystem (NCE) of British Columbia (BC) and Washington (WA).

Traditional mark–recapture designs for population estimates using non-invasive techniques (Woods et al., 1999; Mowat and Strobeck, 2000) may be unusable in this case because of very small sample sizes (Seber, 1982) and very low capture-recapture rates in this small, remnant population. A possible alternative is catch per unit effort models (CPUE; Seber, 1982; DeLury, 1947; Ricker, 1975) which may be more appropriate than traditional mark–recapture estimates for our analysis because of the relaxed assumptions and inherently small sample sizes associated with very small populations. Therefore, we used CPUE and consistent hair-sampling methods for geographic comparisons of relative abundance, similar to fisheries techniques to determine relative stock sizes among different areas (King, 1995; Gulland, 1969; Wooster, 1998).

We compared hair-sampling CPUE from the North Cascades to CPUE results from seven other grizzly hair-sampling projects (Woods et al., 1999; Mowat and Strobeck, 2000; Poole et al., 2001; Proctor, 1998 unpublished data). We developed a regression model based on these datasets to determine the relationship between CPUE and estimated population density. Our objective was to use this regression model to estimate relative grizzly bear population density and size within the NCE study area. Implications of our results may be important for other extremely small populations of grizzly bears, where capture–recapture data is difficult to obtain, and presence–absence information is focal to future management and population recovery decisions.

2. Study area

The NCE study area covered 3750 km^2 (11%) of the 35,150 km^2 international North Cascades Grizzly Bear Ecosystem (Fig. 1). In 1998, 2400 km^2 were hair-sampled in British Columbia's North Cascades Grizzly Bear Population Unit. In 1999–2000, 1250 km^2 were sampled each year throughout Washington's North Cascades National Park, Pasayten Wilderness, Okanogan National Forest, and the Glacier Peak Wilderness. Elevations ranged from 150 to 500 m in the riparian lowlands of the western portion North Cascades, and from 1300 to 3200 m in the mountainous terrain along the North Cascade crest. Climate was maritime in the west, with annual rainfall from 170 to 300 cm, and continental in the east, with annual precipitation from 25 to 50 cm falling mostly as snow (Franklin and Dyrness, 1973). Vegetation in the NCE was classified into 12 major vegetation zones (Franklin and Dyrness, 1973), which created a diversity of habitats from riparian bottomlands (*Thuja plicata, Alnus rubra, Acer circinatum*) and wet montane forests (*Abies amabilis, Tsuga mertensiana, Psuedotsuga menziesii*) on the west side, to subalpine forests (*Abies lasiocarpa, Picea engelmannii, Pinus contorta*) and herbaceous meadow complexes (V*accineum* spp, *Valariana sitchensis, Lupinus* spp, *Equisetum arvense*, sedges and grasses) on the east side.

The US portion of the CYE covered 6500 km^2 of northwestern Montana and northeast Idaho (Fig. 2). Our study area focused on grizzly bear home ranges within 600 km^2 of the Yaak River drainage located north of Troy, MT, bounded to the east by the Yaak River, to the west by the Kootenai Valley, and to the north by Highway 3 in BC. Elevations in the CYE ranged from 550 to 2350 m. Short summers and heavy snowfall characterized the Pacific Maritime climate in the winter, and weather patterns produced 100–150 cm annual precipitation (Kasworm et al., 2000). Topography and vegetation in the study area was dominated by forested peaks and ridges (*Thuja plicata, Tsuga heterophylla, Pinus contorta, Shepherdia canadensis*), and low to mid elevation open meadow complexes (*Pseudotsuga menziesii, Xerophyllum tenax, and Vaccineum spp*). Much of the vegetative diversity was caused by extensive wildfires and forestry practices in the area. Population estimates were 20–30 grizzly bears in the Yaak portion of the CYE (Kasworm et al., 2000) and 12 grizzlies in Management Unit 4-4 in the B.C. Kootenay region (Simpson et al., 1995). Population densities in our study area were estimated at 1.19 bears/100 km^2 (conservative estimate calculated from Kasworm et al,. 1998 and Simpson et al., 1995).

The SME covered 5700 km^2 in northern Idaho, northeastern Washington, and southern BC (Fig. 2). Our study area covered grizzly bear home ranges within 450 km^2 of north central Idaho, bordered to the east by the Kootenai Valley, to the north by the international Canada/US border, and to the west by upper Priest Lake/Priest River. The terrain was mountainous, elevations ranged from 550 to 2500 m. Climate was similar to that of the NCE with lesser extremes of precipitation and heat. Vegetation in the SME was Engelmann spruce/subalpine fir (*Picea engelmanni*/*Abies lasiocarpa*) and cedar/western hemlock (*Thuja plicata*/*Tsuga heterophylla*)

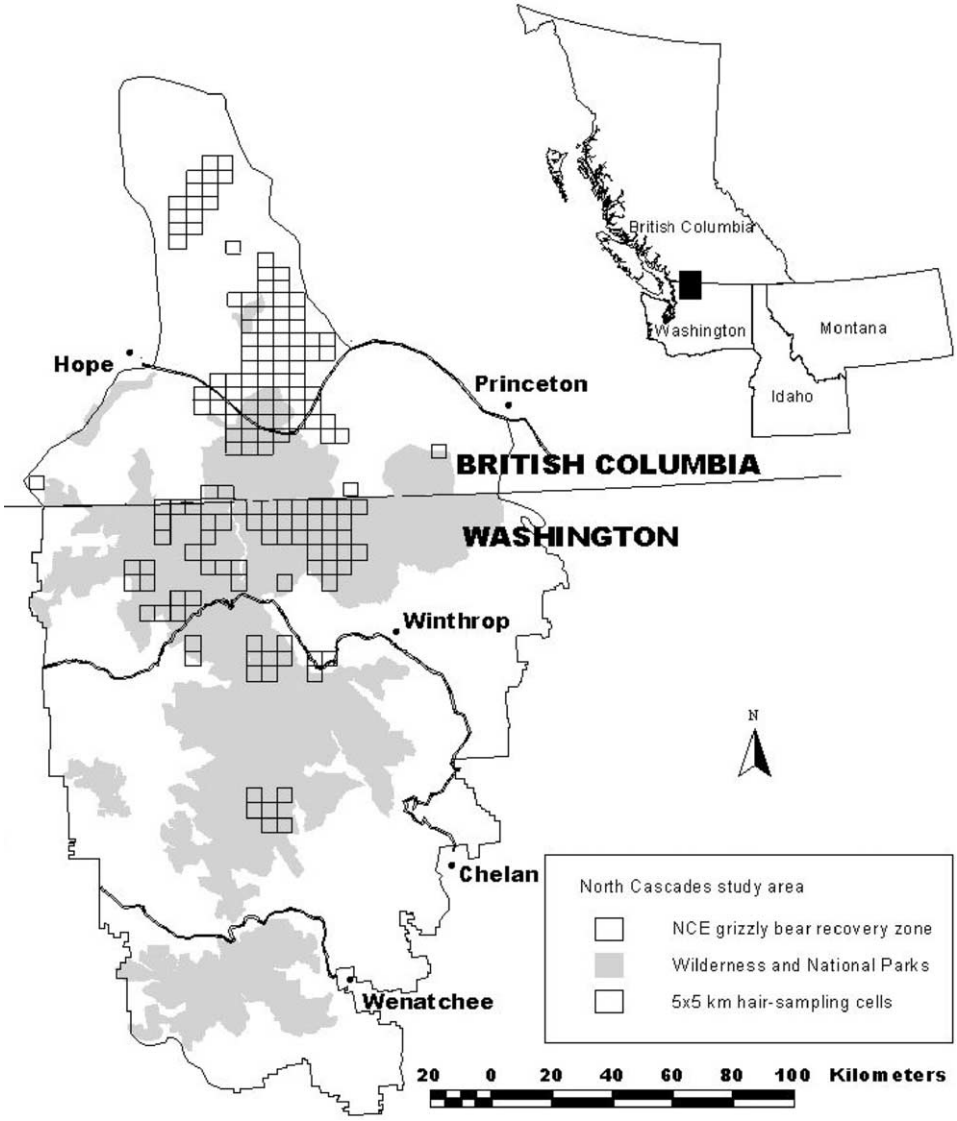

Fig. 1. Study area and 5×5 (25 km^2) sampling cells in the North Cascade Ecosystem of Washington and British Columbia, 1998–2000.

(Parish et al., 1996). It was estimated that 30–55 grizzly bears inhabit the SME; population densities were estimated at 1.41 bears/100 km^2 (Wielgus et al., 1994).

3. Methods

3.1. Hair-sampling

We sampled for bear hair within the NCE for 3 years, (1998–2000) using standardized non-invasive DNA hair-sampling methods (Woods et al., 1996, 1999; Boulanger, 1998a; Mowat and Strobeck, 2000). In 1998, 97 5×5 km hair-sampling sites were established in 2400 km^2 of the BC NCE between 22 June and 27 July (Mowat and Davis, 1998). In 1999 and 2000, a total of 97 hair-sampling sites were established in 1350 km^2 of the WA NCE between 15 May and 30 September. Hair-sampling site locations in 2000 overlapped but were not limited to sites employed in 1999, in order to increase our confidence of detection with repetitive years of sampling. In all 3 years, we trapped two to four sessions, each session equal to 28 days. The trapping grid and sampling strategy in 1999–2000 in WA was the same as that established by the B.C. Ministry of Environment, Lands and Parks in 1998, to allow pooling of datasets (Fig. 1).

Our hair-sampling design used an irregular-shaped grid, which did not meet the assumption of geographic closure (Otis et al., 1978; White et al., 1982) normally required for mark-recapture studies. Demographic closure was minimized using a standardized short time period per sampling site in which mortality and migration were negligible (Seber, 1982). We used adaptive systematic sampling (Thompson et al., 1992; Krebs, 1999) to optimize grizzly bear detection success, and

sampled areas of previous grizzly bear sightings (Gyug, 1998; Almack et al., 1993) and/or prime grizzly bear habitat identified by local biologists and the North Cascades Interagency Grizzly Bear Committee (IGBC, 1987). We subdivided these areas into a grid design for evenly spaced sampling across these habitats. Hair-sampling sites were employed near the center of each 5×5 km cell, in areas considered attractive to bears to maximize bear encounters and capture probabilities (Woods et al. 1999).

We used 5×5 km cells and sampled for multiple sessions suggested by Boulanger (1999) as the most efficient and effective sampling regime for the NCE. A 5×5 km sampling cell also approximates a minimal female grizzly bear home range during a 2 week time interval (Mace and Waller, 1997) which increases the probability of females as well as males encountering a hair-sampling site (Boulanger, 1998b). We sampled low elevation, riparian areas and avalanche chutes during the early season (15 May–15 July), and mid to high elevation, avalanche chutes and berry patches during the late season (15 July–30 September).

Each hair-sampling site consisted of a perimeter fence of single-strand barbed wire and an elevated non-reward scent of liquid fish and cattle blood and/or rotted meat, consistent with Woods et al. (1996). To minimize human scent, leather gloves were worn when handling barbed wire and personal supplies were left at least 100 m from sampling sites. All sites were placed at least 250 m from any trail, and at least one km from campgrounds to comply with human-bear safety protocols in the area.

Sampling sites were visited 14 days after initial set-up to remove hair samples and replenish the site with fresh lure. Final hair samples were collected 2-weeks after rescenting, for a total of 28 days per site. Each barb that garnered hair (regardless of the amount) was classified as one sample. Samples were collected with forceps, placed in envelopes, labeled, and dried in desiccant to minimize handling effects and reduce degradation of DNA until analyzed in the laboratory (Taberlet and Luikart, 1999; Murphy et al., 2000). Details on lure, site preparation, and hair collection methods are described in Mowat and Strobeck (2000) and Woods et al. (1999). All sites were taken down after the 28-day sampling period and removed or relocated to another location to prevent behaviorally dominant animals from influencing the attractiveness of the sampling site to other potential individuals in the area.

We tested the efficacy of this hair-sampling technique using radio-collared grizzly bears in the CYE and SME. Stratification was similar to that used in the NCE (Fig. 2). A grid composed of 5×5 km sampling cells was placed over lifetime home ranges of currently radio-collared grizzly bears rather than sightings and "best habitat assessments" as in the NCE. Three radio-collared grizzly bears in the CYE, and six radio-collared grizzly bears in the SME were available for this study. Home ranges were delineated using program CALHOME to determine each animal's available 50 and 80% seasonal lifetime adaptive kernel home range. The number and placement of sampling cells was finally determined by plotting early and late season radio-telemetry locations. These seasonal sampling cells were overlaid onto orthophoto maps to determine accessibility to each cell, and hair-sampling sites were placed in the center of each cell. Hair-sampling sites in the CYE and SME were deployed and scented beginning in late June through August in 2000 and 2001. We used the same standard protocol for visitation, baiting, collection and storage of hair samples as in the NCE.

3.2. Genetic analyses

DNA was extracted from all samples using a Qiagen DNAEasy extraction kit (Qiagen Inc., Valencia, CA) or Chelex methods (Walsh et al., 1991); DNA was resuspended in 400 μL of Te^{-4} or 200 μL of Chelex solution, respectively. We used 2–10 hair roots per sample (maximum amount used when available) for DNA amplification using polymerase chain reaction (PCR). To minimize potential for contamination, DNA extractions were conducted in a laboratory dedicated to low quantity (hair, feces, and bone) DNA, which was spatially separated from PCR products and concentrated DNA sources. All DNA extractant and PCRs contained negative controls (water only) to monitor for contamination.

We determined species identification (SID) of each sample collected in the field using standardized PCR methods (Murphy et al., 2000; Shields and Kocher, 1991; Paetkau and Strobeck, 1996). All DNA extracts genotyped as grizzly bear were PCRed at least two times to rule out spontaneous contamination for definitive SID. We determined gender from each individual grizzly bear identified using methods described in Woods et al. (1999). PCR conditions for gender identification used the same procedures as were used for SID, including positive and negative controls.

Grizzly bear samples were genotyped using microsatellite methods described by Paetkau and Strobeck (1994) and Paetkau et al. (1995). PCR cocktail and amplification techniques are described in Woods et al. (1999). We used 6 highly variable microsatellite loci (G1A, G10B, G10C, G10L, G10M, and G10P) (Paetkau and Strobeck, 1994; Paetkau et al., 1995) as a conservative number of loci for determining individuals (Hughes and Queller, 1993) since we expected these small populations to show signs of depauperate genetic variation. All PCR fragments for SID, gender identification and microsatellites were separated by size on 6%- polyacrylamide gels using ABI 377 fluorescent dye

system/DNA Sequencer. GeneScan and Genotyper software (Perkin Elmer-Applied Biosystems. Foster City, CA) were used to score genotypes.

Genotypes from all known and currently radio-collared individuals (previously handled animals in the field) from the CYE and SME were collected from several databases (M. Proctor, L. Waits, unpublished data). We obtained blood, tissue, or hair samples from any individuals missing from the database, and samples were replicated to calibrate results. Blood samples were extracted in a different facility to separate concentrated DNA extract from pre-PCR hair and tissue extract. The correct consensus genotype (CCG; Goossens et al., 2000), or the most likely genotype, was defined as a true heterozygote when the same two alleles were obtained at least twice. For a true homozygote, CCG was defined when a single allele was observed at least three times. Multiple PCR amplifications for each allele accounted for technician error, stochastic sampling, the possibility of allelic slippage, generation of false alleles, and the risk of contamination (Taberlet et al., 1996, 1999; Taberlet and Luikart, 1999; Waits and Leberg, 2000; Gagneux et al., 1997).

Genotype matches and similarities between all samples were objectively identified and designated as unique genotypes after calculating the probability of identity (PI) for all identical genotypes (Mills et al., 2000, Waits and Leberg, 2000, Waits et al., 2001). Allele frequencies used for these calculations were obtained from individual genotypes from each designated population, such as the CYE (46 samples) or the SME (55 samples) grizzly bear populations (M. Proctor, unpublished data; K. Romain-Bondi, unpublished data). Accurate matches used a conservative probability of siblings equation (PI

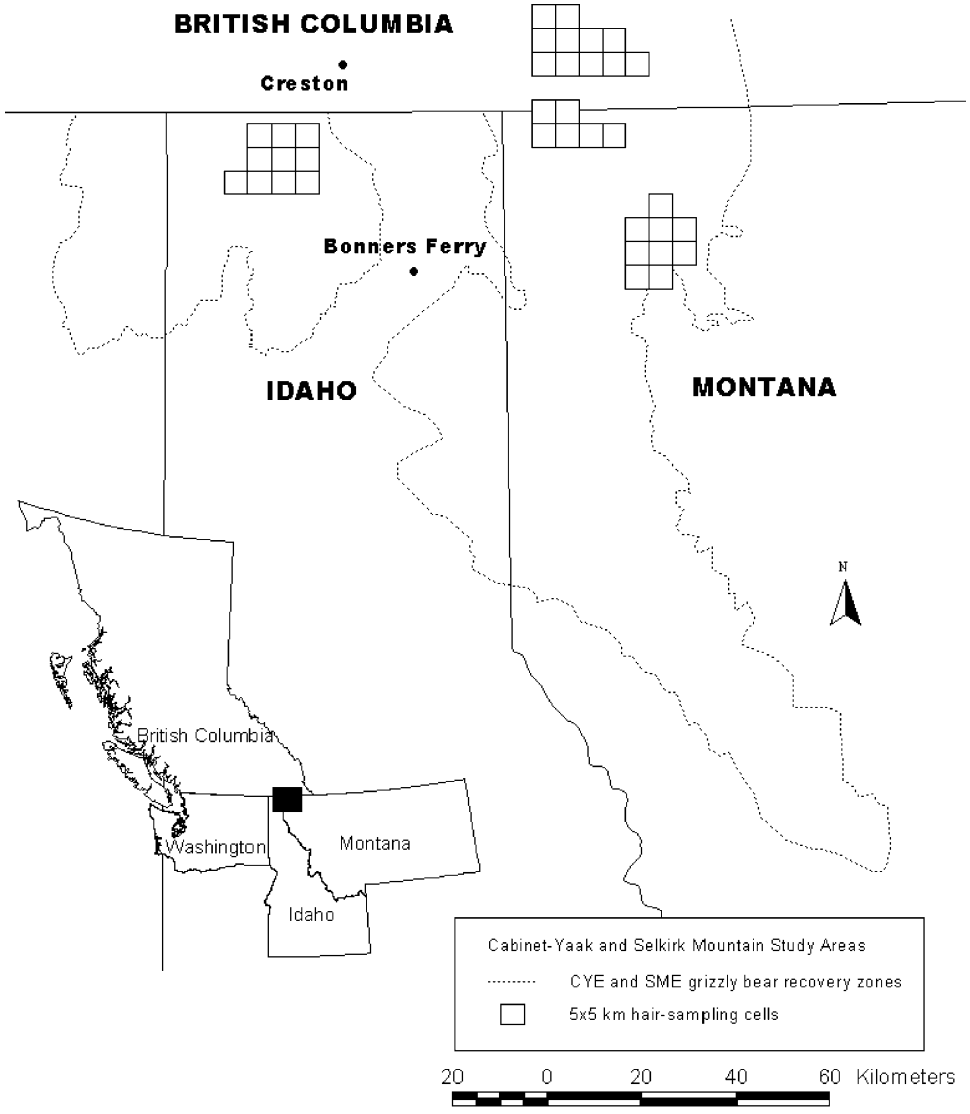

Fig. 2. Study areas and 5×5 (25 km^2) sampling cells in the Cabinet-Yaak Ecosystem of Montana and British Columbia, and the Selkirk Mountain Ecosystem of north Idaho, 2000–2001.

sibs) and were accepted only when PI sibs probabilities were less than 0.01 (Woods et al., 1999; Waits et al., 2001).

3.3. Density estimates

We calculated CPUE, defined as the number of hair-samples collected and/or individuals detected per number of trap nights, for the NCE, SME, CYE, and for five other hair-sampling projects in the US and Canada. These other hair-sampling projects were in: (1) the South Selkirk Mountains (SSM) in BC (Proctor, 1998, unpublished data); (2) the Central Selkirk Mountains (CSM) in BC, (Mowat and Strobeck, 2000); (3) Waterton National Park (WNP) in Alberta (Mowat and Strobeck, 2000); (4) the West Slopes (WS) Rocky Mountains in BC (Woods et al., 1999); and (5) Prophet River (PR) in BC (Poole et al., 2001; Boulanger and McLellan, 2001). Population density estimates using stringent mark-recapture analyses were obtained for each area based on radio telemetry (CYE, SME, and SSM), or program CAPTURE's best-fit population estimation models (CSM, WNP, WS, and PR; Table 1).

All hair-sampling studies used the standardized protocol developed by Woods et al. (1996) including a blood-based non-reward bait; a perimeter of single stranded barbed wire for hair collection; and similar grid cell sizes ranging from 5×5 to 9×9 km (Table 1) which maximized population closure for mark–recapture population estimates (Boulanger and McLellan, 2001). Each study left bait sites up for a 12–14 day sampling period to minimize human disturbance to the site, and moved each sampling site after 28 days to avoid intraspecific behavioral biases. Furthermore, all of the studies we used in our analyses were conducted by closely cooperating individuals.

We tested our relative trapping efficiency in the NCE by calculating the total number of hair samples collected per trap night ($CPUE_{total}$) and the total number of black bear hair samples collected per trap night ($CPUE_{black}$) in the NCE, CYE, and the SME. We used a one-sample t-test to test the null hypothesis that NCE 'hair sampling efficiency' was equal to $CPUE_{total}$ and $CPUE_{black}$ for all other populations. Since sampling protocol was similar between all study areas, we expected little difference in hair-sampling efficiency ($CPUE_{total}$ and CPUEblack). If overall hair sampling efficiency in the NCE was lower than in other study areas, it may not be appropriate to compare grizzly detection success to all other populations. We calculated grizzly bear detection success (number grizzly bears detected per 1000 trap nights, $CPUE_{grizzly}$) for all populations and used a scatter plot of the $CPUE_{grizzly}$ data to identify the relationship between estimated density and CPUE. We used three linear regressions in SYSTAT 9.0 (Systat Inc., 1999). The simple linear regression model assumed that a density of bears exists even when CPUE is zero. The logistic (curvilinear) model assumed that zero CPUE approximates zero density, and the linear regression through the origin models assumed that a zero CPUE corresponds to a zero density of bears.

To corroborate which model fit the data best, we plotted home range size against population density for 19 male (IGBC, 1987) and 25 female grizzly bear populations (data from McLoughlin et al., 2000) in North America, to test for a linear or curvilinear relationship between density and home range. If linear, a simple linear regression model may be most appropriate for

Table 1
Summary of hair-sampling projects in eight grizzly bear populations

Ecosystem	NCE	CYE	SME	SSM	PR	CSM	WNP	WS
Study area (km^2)	3650	600	450	325	8527	9866	5030	4096
Grid size (km)	5×5	5×5	5×5	5×5	9×9	8×8	8×8	8×8
Sex ratio (M:F)	0:1	1:2	3:1	0.9:1	1:1	0.8:1	1.4:1	1.2:1
Density[a]	–	1.19a	1.41b	2.33c	2.24d	2.66e	1.47f	2.54g
Hits (%)[b]	0.69	0.56	0.59	0.87	–	0.73	0.48	
Effort (trap nights)	5304	817	586	804	6180	3810	4494	2653
Total hair samples	1708	282	136	331	–	4245	635	1753
Black Bear hair samples	1378	252	126	–	–	661	469	1091
Grizzlies detected	1	6	6	19	98	109	37	54
$CPUE_{black}$[c]	0.26	0.31	0.22	–	–	0.17	0.10	0.41
$CPUE_{total}$[c]	0.32	0.35	0.22	0.41	–	1.11	0.14	0.66
$CPUE_{grizzly}$[d]	0.19	7.34	9.80	23.63	15.86	28.61	8.23	20.35

Population densities obtained from the following information: (a) CYE (Kasworm et al., 1998; Simpson et al., 1995), telemetry data; (b) SME (Wielgus, 1996), telemetry data; (c) SSM (Wielgus, 1996), telemetry data; (d) PR (Boulanger and McLellan, 2001), CAPTURE model AICc; (e) CSM (Mowat and Strobeck, 2000), CAPTURE model Mh; (f) WNP- (Mowat and Strobeck, 2000), CAPTURE model Mh; (g) WS (Woods et al., 1999), CAPTURE model Mh.

[a] Bears/100 km^2.

[b] ⩾One sample per site.

[c] Hair samples collected per trap night.

[d] Grizzly bears detected per 1000 trap nights.

determining density of grizzly bears based on CPUE. If non-linear, a log-transformed curvilinear model may be most appropriate since home range size should be inversely proportional to detection probabilities or CPUE.

We used the three regression equations to estimate population density for the NCE, and extrapolated density to our sample area to obtain population size estimates. We used Zar (1984) to estimate the standard error and 90% confidence intervals for the linear and log linear models (p. 274, Eq. 17.26), and for a linear through the origin model (pp. 284–285, Eqs. 17.48–17.56). We tested the hypothesis that the NCE grizzly bear density was significantly less than densities of other threatened grizzly bear populations (CYE and SME; Zar, 1984).

4. Results

We sampled 3750 km^2 of the NCE (11% of the entire ecosystem), and collected a total of 1708 hair samples in 5304 trap nights which yielded an overall hair-capture success $CPUE_{total} = 0.32$. We obtained $\geqslant$one hair sample from 69% of the sites. In comparison, we sampled 600 km^2 (9%) of the CYE, collected 282 hair samples in 817 trap nights which yielded a $CPUE_{total} = 0.35$, and 56% of the sites collected $\geqslant$one hair sample. Finally, we sampled 450 km^2 (8%) of the SME, collected 136 hair samples in 586 trap nights which yielded a $CPUE_{total} = 0.23$, and 59% of the sites collected $\geqslant$one hair sample (Table 1). Hair-sampling success in the NCE did not differ from the mean $CPUE_{total}$ (0.482 ± 0.127 (SE); $df = 5$, $P = 0.32$) for all populations (Table 1). Black bear hair sampling success in the NCE also did not differ from the mean $CPUE_{black}$ (0.242 ± 0.01 (SE); $df = 4$, $P = 0.76$) for all populations (Table 1). These results suggest that sampling efficiencies were similar in all areas, allowing comparisons of $CPUE_{grizzly}$.

Species identification (SID) identified 1378 black bear samples and four grizzly bear samples with an overall 1999–2000 SID success rate of 95% in the NCE study area. We identified 252 black bear and 21 grizzly bear samples in the CYE study area (97% SID success rate), and 126 black bear and six grizzly bear samples in the SME study area (97% SID success rate). Any amplification failures were potentially due to insufficient degraded DNA. No samples were genotyped as multiple species. Black bear samples were not analyzed for individual or sex ID.

We identified one female grizzly bear in the NCE, six grizzlies in the CYE (four females and two males), and six grizzlies in the SME (one female, two males, three unknowns; Table 1). $CPUE_{grizzly}$ was estimated at 0.19, 7.34, and 9.80 grizzlies captured per 1000 trap nights in the NCE, CYE, the SME, respectively (Table 1). $CPUE_{grizzly}$ for the other five study areas are also presented in Table 1. The simple linear regression model (Table 2, Fig. 3a) predicted a density of 0.879 ± 0.201 (SE) grizzlies per 100 km^2 (Table 3) in the NCE. The log linear model (Table 2, Fig. 3b and c) predicted a density of 0.153 ± 0.312 (SE) grizzlies per 100 km^2 (Table 3). The linear regression through the origin model (Table 2, Fig. 3d) predicted a density of 0.021 ± 0.170 (SE) grizzlies per 100 km^2 (Table 3). The curvilinear model was supported by a non-linear relationship between home range and density (Fig. 4; IGBC, 1987; McLoughlin et al., 2000).

Density estimates for the NCE were significantly less than that of the next smallest density of bears in the CYE for all three models (linear: $t = 2.636$, $P < 0.025$; curvilinear: $t = 7.776$, $P < 0.0001$; linear through the origin: $t = 4.928$, $P < 0.005$). Similarly, density estimates for the NCE were significantly less than mean density estimates for all other populations (linear: $t = 5.467$, $P < 0.0025$; curvilinear: $t = 8.06$, $P < 0.0001$; linear through the origin: $t = 4.941$, $P < 0.005$). Mean predicted absolute number of bears in the NCE study area ranged between 1 and 33 animals for the three different models, with a mean of six animals (3–11, 90% C.I.) for the best fit model (Table 3).

5. Discussion

The unexpected low number of grizzly bear detections ($N = 1$) and very low $CPUE_{grizzly}$ (0.19 grizzlies captured/1000 trap nights) in the NCE suggest that population densities in the NCE are considerably lower than in the threatened CYE and SME populations. However, even though we sampled probable grizzly bear habitat recommended by local experts, we sampled a relatively small proportion (11%) of the North Cascades Ecosystem, and grizzlies outside our study area would not have been detected. Since past efforts to capture and radio-collar these extremely mobile and wide-ranging animals in the NCE have been unsuccessful, managers have suspected an extremely low population density. Based in part on our results, the BC Ministry of Water, Land and Air Protection is currently planning augmentation for recovery of the North Cascades grizzly bear population (BCMELP, 2001). Our results support augmentation, since natural recovery seems unlikely at such small population sizes because of the high likelihood of extinction due to demographic and environmental stochastic effects (Wielgus et al., 2001; Wielgus, 2002).

We extrapolated three regression lines from our catch-effort models to include the North Cascade $CPUE_{grizzly}$, which falls outside the observed range of all other populations' CPUE and density values. We extrapolated beyond the CPUE range because there is no data present to predict the y-intercept value for such

Table 2
Analysis of variance (ANOVA) for density of grizzly bears using hair-sampling techniques in seven different ecosystems

Source	Sum-of-squares	df	Mean-square	*F*-ratio	*P*-value
Model 1: Linear ($n=7$, $R^2=0.879$)					
CPUE	1.913	1	1.913	36.470	0.002
Error	0.262	5	0.052		
Model 2: Logistic-Curvilinear ($n=7$, $R^2=0.927$)					
CPUE (log)	0.593	1	0.593	63.615	0.000
Error	0.047	5	0.009		
Model 3: Linear through origin ($n=7$, $R^2=0.959$)					
CPUE	28.326	1	28.326	140.108	0.000
Error	1.213	6	0.202		

Model 1: density = 0.866 + 0.068 * CPUE. Model 2: ln density = −0.911 + 0.580 * ln CPUE. Model 3: density = 0.112 * CPUE.

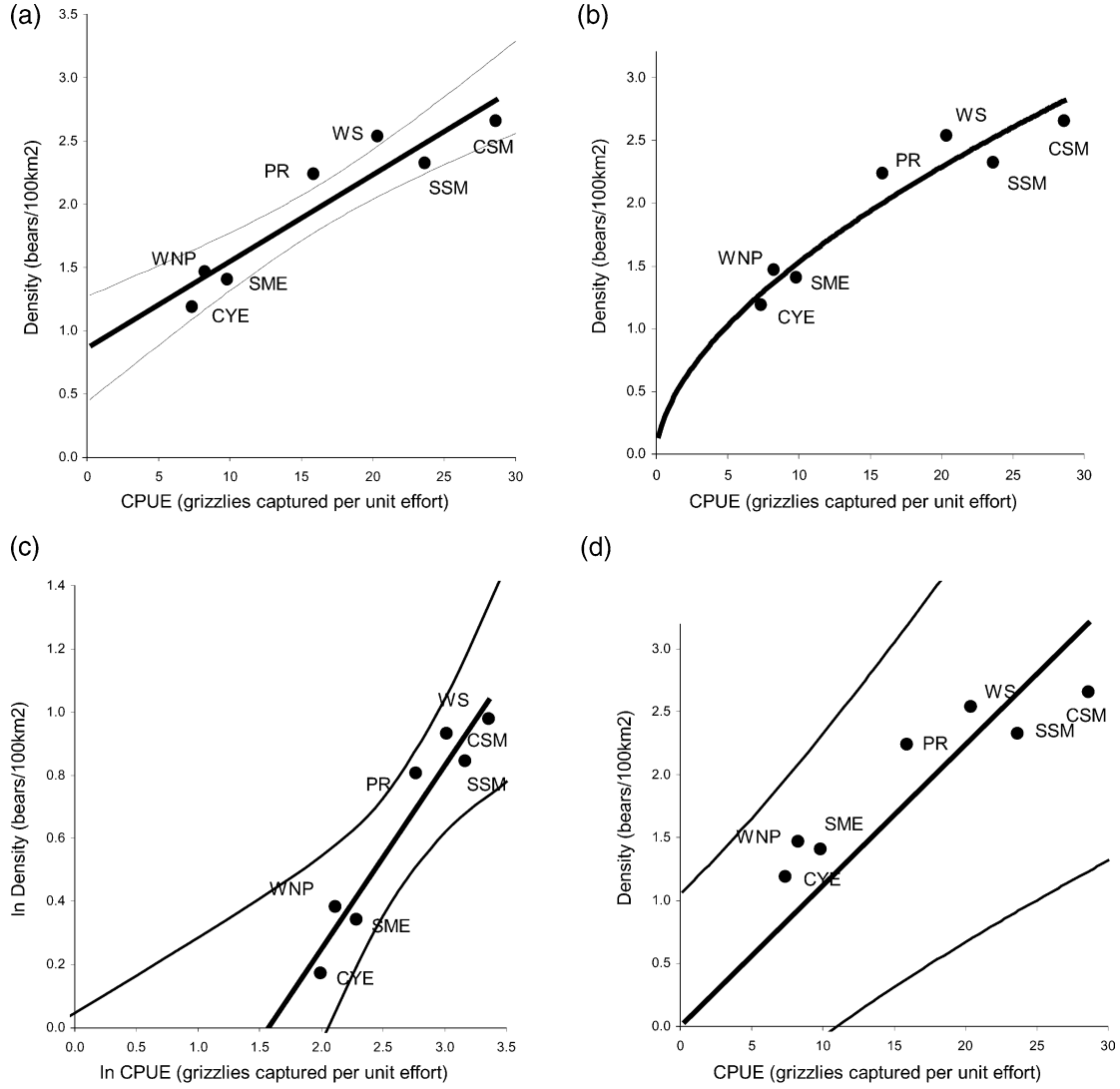

Fig. 3. Regression and 90% confidence intervals for catch per unit effort (grizzlies captured per 1000 trap nights) and density (grizzlies per 100 km^2). (a) Model 1: linear; (b) Model 2: curvilinear; (c) Model 2: Log transformed curvi-linear; (d) Model 3: Linear through the origin.

Table 3
Summary of three models to predict North Cascade Ecosystem grizzly bear density and absolute number of bears within the 1998–2000 study area

Model	Regression equation	R^2	Predicted Density			Absolute Numbers		
			lo	mean	hi	lo	mean	hi
1	$y=0.866+0.068x$	0.88	0.47	0.88	1.28	18	33	48
2[a]	$y=-0.9107+0.580x$	0.93	0.08	0.15	0.29	3	6	11
3	$y=0.112x$	0.96	0	0.02	0.35	1[b]	1	13

NCE predicted densities are based on the NCE catch per unit effort of 0.19 bears per 1000 trap nights and each model's regression line fit to seven other hair-sampling projects. Absolute numbers of animals based on the predicted density of animals within the 3750 km^2 NCE study area. Model 1: simple linear regression. Model 2: log transformed linear regression. Model 3: linear regression through the origin. Models plotted with surrounding confidence intervals in Fig. 3a, c, d.

[a] Model 2, predicted density and absolute numbers recorded as the antilog of actual values.

[b] Low absolute value is 1 and not the predicted negative value, because 1 grizzly bear was found in the NCE.

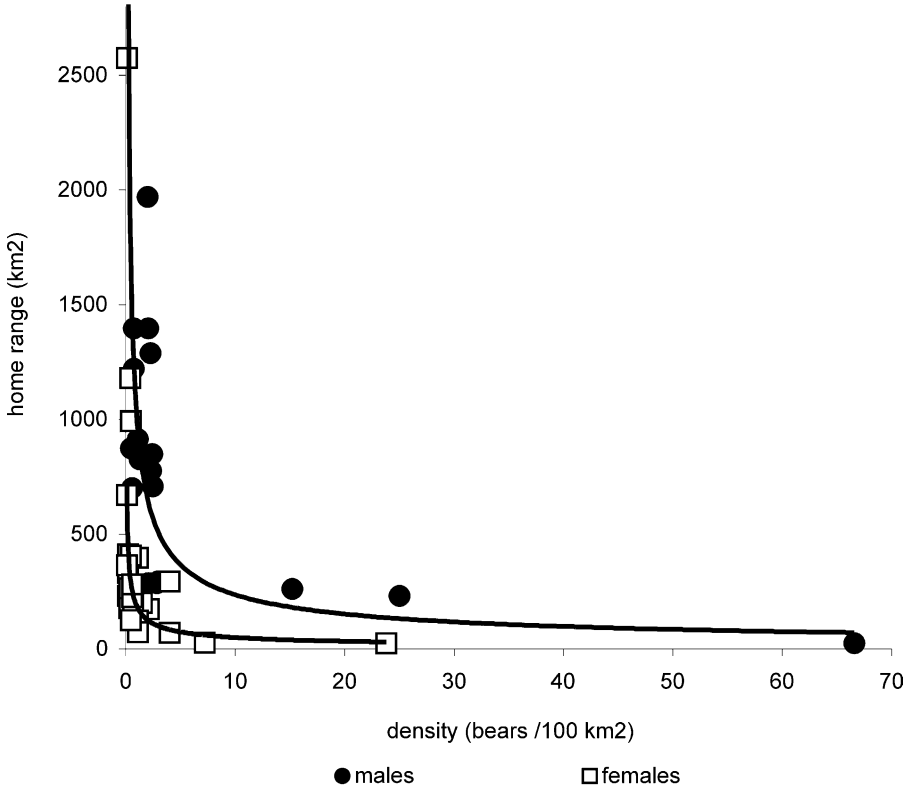

Fig. 4. Non-linear relationship between annual home range and estimated density for 19 populations of adult male grizzly bears in North America (IGBC, 1987), and 25 populations of adult female grizzly bears in North America (McLoughlin et al., 2000).

extremely small populations, but there is no reason to assume the linear or curvilinear relationships do not hold true throughout the entire range. This model is advantageous over minimum population estimates ($N=1$) because it provided some insight into the possible density and range of relative numbers of grizzly bears in the NCE population ($N=1$–27).

The linear regression model fit the data fairly well ($R^2=0.88$), but it assumes there is a relatively high density of bears even where bears are known not to exist. This model predicted a mean density (0.88 bears/100 km^2) and a mean number of bears in the NCE ($N=33$) to be considerably higher than the two other model predictions. The curvilinear regression model fit the data slightly better ($R^2=0.93$). This model assumes a nearly zero density when CPUE is zero, and that CPUE decreases more rapidly at very low population densities. The curvilinear model yielded a grizzly bear density in the NCE of 0.15 bears/100 km^2, resulting in a very small mean population estimate of $N=6$. Finally, the linear regression through the origin model yielded the best fit regression at $R^2=0.96$, resulting in the smallest predicted mean density of 0.02 bears/100 km^2 and population size ($N=1$), but the regression may not pass through the population means and the R^2 value is inflated because of the lack of an intercept (Zar, 1984).

While the assumption of zero CPUE equals zero density may seem reasonable for models 2 and 3, it is not known a priori (Zar, 1984) that density will be zero when CPUE is zero. For example, six radio-collared bears in the SME were monitored and encountered hair-sampling sites during the 2000–2001 study, but none were detected in the hair samples. These models would falsely estimate that no bears existed in the study area, given that our CPUE for these individuals equaled zero. However, we suspect that previously captured and collared bears may have a lower probability of hair-sampling capture than uncollared bears (unpublished data, recognized by Boulanger, 1996), which would not affect capture probabilities in our NCE study area.

The curvilinear model seems to best fit the dataset. Justification for this model is supported by the curvilinear relationship between home range size and density plotted in Fig. 4. McLoughlin et al. (2000) found the same non-linear correlation between low densities of grizzly bears and large home range size, based on habitat quality. Habitat is unlikely limiting the grizzly bear population in the NCE (Almack et al., 1993), however the general relationship of decreasing density resulting in rapidly increasing home range size is one possible explanation for a very low CPUE in the North Cascades. For example, extremely small populations of grizzlies may travel very large distances looking for breeding opportunities, hence individual encounter probabilities to hair-sampling sites would decrease as a function of home range size. This non-linear relationship mirrors our CPUE/density curvilinear regression model.

The linear models do not seem as likely because they do not fit the curvilinear home range relationship. The simple linear model also predicts that the NCE population density is similar to extant brown bear population densities in the Swan Hills, Alberta, Tuktoyaktuk Peninsula, Canadian Arctic, and the Eastern Brooks Range, Alaska (IGBC, 1987) where bears were easily seen and captured, unlike in the NCE.

We used catch-effort models not as an alternative to more rigorous capture–recapture methods, but as a suitable analysis for very small populations of grizzly bears since we expected small sample sizes (Seber, 1982) and violations of stringent mark-recapture assumptions. In our analyses we compare relative population sizes using CPUE, not point estimates of population size based on marks and recaptures, on these assumptions: (1) the probability of each individual being caught is constant throughout the experiment; (2) the population must be closed; and (3) all individuals must have the same probability of being caught in the sample (DeLury, 1947; Ricker, 1975).

The first assumption of constant probabilities of capture was met because our trapping effort and detection techniques were collaborated upon by leading scientists in BC and the US using the same standardized methods as presented in Woods et al. (1996). Additional studies that use different capture methods and sampling gear should not attempt to compare their results with our analysis (Seber, 1982; Gulland, 1988; Rounsfell, 1975). Detection techniques and success were assessed across all populations by overall hair samples ($CPUE_{total}$) and black bear hair samples ($CPUE_{black}$) collected per unit time, which verified that hair sampling efforts in the NCE did not differ from the other seven study areas.

The second assumption of *geographic* population closure is not required for CPUE indices of relative population size (Rounsfell 1975; Tanner 1978; Gulland 1988). Sampling efforts did not encompass an entirety of an ecosystem due to limitations in funding and personnel time. Grid edges significantly contributed to immigration and emigration (Boulanger and McLellan, 2001), but were corrected for in mark–recapture density estimates. However, *demographic* closure biases should be small for bears using hair-sampling methods since the length of sampling sessions and duration of hair-sampling studies are restricted (Seber, 1982; Mowat and Strobeck, 2000), minimizing population changes in numbers due to birth or death occurrences, or permanent immigration/emigration.

The third assumption, that all individuals have an equal probability of capture is not a problem in CPUE as it is in mark–recapture studies. CPUE indices of relative population size require constant capture throughout sampling by species random distribution and equal vulnerability throughout the study area (King, 1995). Individual heterogeneity is difficult to measure in grizzly bears because behavior and trap shyness differs between sex and reproductive status, although it can be and was controlled using correction coefficients in cited population density estimates for our analysis. Trap shyness as a result of extreme wariness may be associated with small, remnant grizzly bear populations, but data necessary to test this is presently unavailable.

Unidirectional capture biases may exist, for example if recapture probabilities were less than capture probabilities across areas, or if females were less likely captured than males. However, then the behavior of different sex and age classes of animals towards scent lures should remain relatively constant across areas. Capture probability biases among geographical areas would have increased the randomness or error of the data, thus increasing our type two error rates. This would lead to difficulty obtaining a statistically significant linear relationship between density and CPUE when such a relationship existed. Our type one error rates are given in our alpha probabilities, which were quite small. The statistically significant linear relationship suggests that differently biased estimates were not problematic, and we therefore assumed that biases

associated with our data are most likely present and should affect all populations equally, which allowed for comparisons among CPUE

Future studies of genetic hair-sampling techniques for small populations should include variation of grid density and duration of hair-sampling sites. Grid sizes may require adjustment to effectively increase encounter probabilities of animals with large home ranges. Duration may require adjustment to effectively sample a study area for a long enough time period to increase encounter probabilities. However, since DNA degrades rapidly in field conditions, we recommend collecting samples <14 days (Waits, personal communication), but increasing the number of sampling sessions. We recommend using CPUE as a relative index (Southwood, 1978) of population density. If CPUE $\leqslant 1.0$ grizzly captured per 1000 trap nights, the grizzly population may be considered imperiled (density estimated at 0.40 bears/100 km^2 using the curvilinear model). This density estimate is equivalent to the lowest density estimate on the Tuktoyaktuk Peninsula (IGBC, 1987), and is significantly lower than the CYE density estimate ($P<0.0005$). At such low densities, managers may want to redirect resources to direct recovery efforts such as augmentation, instead of further minimum population estimates.

Because small sample sizes associated with small populations of grizzly bears preclude mark–recapture analyses, catch-effort data can be useful in evaluating relative density and population sizes. Recognizing there are few other means to sample and estimate density and population sizes for extremely small, threatened and endangered grizzly bear populations, we advocate hair-sampling techniques and CPUE models as a potentially useful method to obtain such data.

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References

Almack, J.A., Gaines, W.L., Naney, R.G., Morrison, P.H., Eby, J.R., Wooten, G.F., Snyder, M.C., Fitkin, S.H., Garcia, E.R., 1993. North Cascades Grizzly Bear Ecosystem Evaluation: Final Report. Interagency Grizzly Bear Committee, Denver, Colorado.

BC Ministry of Environment, Land and Parks (BCMELP), 2001. Recovery plan for grizzly bears in the North Cascades of British Columbia—Draft. North Cascades Grizzly Bear Recovery Team. Victoria, BC.

Boulanger, J., 1996. Preliminary Report: Inference from Bears Radio-collared During the West Slopes 1996 DNA Mark–recapture Inventory Project. Wildlife Branch, Ministry of Environment, Land and Parks, Victoria, BC.

Boulanger, J., 1998a. DNA Mark–recapture Methods for Inventory of Grizzly Bear Populations in British Columbia: Elk Valley (1996) Case Study. Wildlife Branch, Ministry of Environment, Land and Parks, Victoria, BC.

Boulanger, J., 1998b. An assessment of optimal DNA mark–recapture inventory of grizzly bear populations in British Columbia—Draft. Wildlife Branch, Ministry of Environment, Land and Parks. Victoria, BC.

Boulanger, J., 1999. Comparison of two potential designs for North Cascades Grizzly Bear Project. Wildlife Branch, Ministry of Environment, Land and Parks, Victoria, BC.

Boulanger, J., McLellan, B., 2001. Closure violation in DNA-based mark-recapture estimation of grizzly bear populations. Canadian Journal of Zoology 79, 642–651.

DeLury, D.B., 1947. On the estimation of biological populations. Biometrics 3, 145–167.

Foran, D.R., Minta, S.C., Heinemeyer, K.S., 1997. DNA-based analysis of hair to identify species and individuals for population research and monitoring. Wildlife Society Bulletin 25, 840–847.

Franklin, J.F., Dyrness, C.T., 1973. Natural Vegetation of Oregon and Washington, Pacific Northwest Forest and Range Experiment Station, Forest Service. US Dept. of Agriculture, Portland, Oregon.

Gagneux, P., Boesch, C., Woodruff, D.S., 1997. Microsatellite scoring errors associated with noninvasive genotyping based on nuclear DNA amplified from shed hair. Molecular Ecology 6, 861–868.

Goossens, B., Waits, L.P., Taberlet, P., 1998. Plucked hair samples as a source of DNA: reliability of dinucleotide microsatellite genotyping. Molecular Ecology 7.

Goossens, B., Chikhi, L., Utami, S.S., Ruiter, F.D., Bruford, M.W., 2000. A multi-samples, multi-extracts approach for microsatellite analysis of faecal samples in an arboreal ape. Conservation Genetics 1, 157–162.

Gulland, J.A., 1969. Manual of methods for fish stock assessment. FAO manuals in Fisheries Science No. 4. Food and Agriculture Organization of the United Nations, Rome.

Gulland, J.A., 1988. Fish and Population Dynamics, second ed. John Wiley and Sons, London, UK.

Gyug, L.W., 1998. Forest Development Plan Blue-listed Species Inventory for Mammals: Assessment of Grizzly Bear Populations, Habitat Use and Timber Harvest Mitigation Strategies in the North Cascades Grizzly Bear Population Unit, British Columbia, BC. Ministry of Environment, Southern Interior Region, Kamloops, Canada.

Hughes, C.R., Queller, D.C., 1993. Detection of highly polymorphic microsatellite loci in a species with little allozyme polymorphism. Molecular Ecology 2, 131–137.

Interagency Grizzly Bear Committee (IGBC), 1987. Grizzly Bear Compendium. National Wildlife Federation, Washington DC.

Kasworm, W. F., Their, T. J., Servheen, C., 1998. Grizzly bear recovery efforts in the Cabinet-Yaak Ecosystem. Ursus 10, 147–153.

Kasworm, W.F., Carriles, H., Radandt, T.G., 2000. Cabinet-Yaak Grizzly Bear Recovery Area: 1999 Research and Monitoring Progress Report. US Fish and Wildlife Service, Missoula, Montana.

King, M., 1995. Fisheries Biology, Assessment, and Management. Fishing News Books, Oxford, England.

Kohn, M.H., Wayne, R.K., 1997. Facts from feces revisited. Trends in Evolution and Ecology 12, 223–227.

Krebs, C.J., 1999. Ecological Methodology, second ed. Addison Wesley Longman, Inc, USA.

Mace, R.D., Waller, J.S., 1997. Spatial and temporal interaction of male and female grizzly bears in Northeastern Montana. Journal of Wildlife Management 61, 39–52.

McLoughlin, P.D., Ferguson, S.H., Messier, F., 2000. Intraspecific variation in home range overlap with habitat quality: a comparison among brown bear populations. Evolutionary Ecology 14, 39–60.

Mills, L.S., Citta, F.F., Lair, D.P., Schwartz, M.K., Tallmon, D.A., 2000. Estimating animal abundance using noninvasive DNA sampling: promise and pitfalls. Ecological Applications 10, 283–294.

Mowat, G., Davis, H., 1998. North Cascades Grizzly Bear Inventory: Final Progress Report. Fish and Wildlife Division, Timberland Consultants Ltd, Nelson, BC.

Mowat, G., Strobeck, C., 2000. Estimating population size of grizzly bears using hair capture, DNA profiling, and mark–recapture analysis. Journal of Wildlife Management 64, 183–193.

Murphy, M.A., Waits, L.P., Kendall, K.C., 2000. Quantitative evaluation of fecal drying methods for brown bear DNA analysis. Wildlife Society Bulletin 28, 951–957.

Otis, D.L., Burnham, D.P., White, G.C., Anderson, D.R., 1978. Statistical inference for capture data on closed animal populations. Wildlife Monographs 62, 1–133.

Paetkau, D., Strobeck, C., 1994. Microsatellite analysis of genetic variation in black bear populations. Molecular Ecology 3, 489–495.

Paetkau, D., Calvert, W., Stirling, I., Strobeck, C., 1995. Microsatellite analysis of population structure in Canadian polar bears. Molecular Ecology 4, 347–354.

Paetkau, D., Strobeck, C., 1996. Mitochondrial DNA and the phylogeography of Newfoundland black bears. Canadian Journal of Zoology 74, 192–196.

Parish, R., Coupe, R., Lloyd, D., 1996. Plants of Southern Interior British Columbia. Lone Pine Publishing, Canada.

Poole, K.G., Mowat, G., Fear, D.A., 2001. DNA-based population estimate for grizzly bears Ursus Arctos in northeastern British Columbia, Canada. Wildlife Biology 7, 105–115.

Ricker, W.E., 1975. Computation and interpretation of biological statistics of fish populations. Fisheries Research Board of Canada Bulletin 191.

Rounsfell, G.A., 1975. Ecology, utilization, and management of marine fisheries. C.V. Mosby Co, Saint Louis, Missouri.

Seber, G.A.F., 1982. The Estimation of Animal Abundance, second ed.. Charles Griffin and Company, London.

Servheen, C., Kasworm, W.F., Their, T.J., 1995. Transplanting grizzly bears (*Ursus arctos horribilis*) as a management tool—results from the Cabinet Mountains, Montana, USA. Biological Conservation 71, 261–268.

Simpson, K., Regnier, J., Andrusiak, L., 1995. A Grizzly Bear Management Strategy for the Kootenay Region. Keystone Wildlife Research, Ministry of Environment, Canada.

Shields, G. F., Kocher, T. D., 1991. Phylogenetic relationship of North American Ursids based on analysis of mitochondrial DNA. Evolution 45, 218-221.

Southwood, T.R.E., 1978. Ecological Methods. Chapman and Hall, London.

SYSTAT, 1999. Systat for Windows: statistics. Version 9.0. SYSTAT, Evanston, Illinios.

Taberlet, P., Mattock, H., Dubois-Paganon, C., Bouvet, J., 1993. Sexing free-ranging brown bears *Ursus arctos* using hairs found in the field. Molecular Ecology 2, 399-403.

Taberlet, P., Griffin, S., Goossens, B., Questiau, S., Manceau, V., Escaravage, B., Waits, L. P., Bouvet, J., 1996. Reliable genotyping of samples with very low DNA quantities using PCR. Nucleic Acids Research 24, 3189–3194.

Taberlet, P., Camarra, J. J., Griffin, S., Uhres, E., Hanotte, O., Waits, L. P., Dubois-Paganon, C., Burke, T., Bouvet, J., 1997. Noninvasive genetic tracking of the endangered Pyrenean brown bear population. Molecular Ecology 6, 869–876.

Taberlet, P., Luikart, G., 1999. Non-invasive genetic sampling and individual identification. Biological Journal of Linnean Society 68, 41–55.

Taberlet, P., Waits, L. P., Luikart, G., 1999. Noninvasive genetic sampling: look before you leap. Trends in Evolution and Ecology 14, 323–327.

Tanner, J.T., 1978. Guide to the Study of Animal Populations. University of Tennessee Press, Knoxville, TN.

Thompson, S.K., Ramsey, F.L., Seber, G.A.F., 1992. An adaptive procedure for sampling animal populations. Biometrics Journal 48, 1195–1199.

US Fish and Wildlife Service (USFWS), 1997. Further Information on Specific Issues Relating to the Grizzly Bear Recovery Plan. Grizzly Bear Recovery Coordinator, University Hall, Missoula, Montana.

Waits, J.L., Leberg, P.L., 2000. Biases associated with population estimation using molecular tagging. Animal Conservation 3, 191–199.

Waits, L., Luikart, G., Taberlet, P., 2001. Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. Molecular Ecology 10, 249–256.

Wakkinen, W.L., Johnson, B.K., 1999. Selkirk Ecosystem Project: Selkirk Mountains Grizzly Bear Ecology. Idaho Dept. Fish and Game, Boise, Idaho.

Walsh, S.P., Metzger, D.A., Higuchi, R., 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. BioTechniques 10, 506–513.

White, G.C., Anderson, D.R., Burnham, K.P., Otis, D.L., 1982. Capture–recapture and removal methods for sampling closed populations. (LA–8787-NERP).

Wielgus, R.B., Bunnell, F.L., Wakkinen, W.L., Zager, P.E., 1994. Population dynamics of Selkirk Mountain grizzly bears. Journal of Wildlife Management 58, 266–272.

Wielgus, R.B., Sarrazin, F., Ferriere, R., Clobert, J., 2001. Estimating effects of adult male mortality on grizzly bear population growth and persistence using matrix models. Biological Conservation 98, 293–303.

Wielgus, R.B., 2002. Minimum viable population and reserve sizes for naturally regulated grizzly bears in British Columbia. Biological Conservation 106, 381–388.

Woods, J.G., McLellan, B.N., Paetkau, D., Proctor, M., Strobeck, C., 1996. DNA fingerprinting applied to mark-recapture studies. International Bear News 5.

Woods, J.G., Paetkau, D., Lewis, D., McLellan, B.N., Proctor, M., Strobeck, C., 1999. Genetic tagging of free-ranging black and brown bears. Wildlife Society Bulletin 27, 616–627.

Wooster, W.S., 1998. Lecture Notes on Coastal and Estuarine Studies. Fisheries Science and Management. Springer-Verlag, New York Inc., Berlin, Germany.

Zar, J.H., 1984. Biostatistical Analysis. Prentice Hall Inc, New Jersey.