

FURTHER INFORMATION ON SPECIFIC ISSUES RELATING TO THE GRIZZLY BEAR RECOVERY PLAN

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In September 1993 the Fish and Wildlife Service approved a revision of the Grizzly Bear Recovery Plan. The Plan was originally approved in January 1982. In May 1994 The Fund For Animals and 22 other organizations and individuals filed suit in the U.S. District Court for the District of Columbia over the adequacy of the Plan. Later in May 1994 the National Audubon Society and 19 other organizations and individuals also filed suit in the same court. The two cases were eventually consolidated. In September 1995 the court issued an opinion. The motions for summary judgment of both the plaintiffs and the defendants were granted in part and denied in part. The court ordered the Service to reconsider certain portions of the Plan and to provide supplemental information. The information presented in this document includes the supplemental information that the Service was to provide and the results of its reconsideration.

This document is being made available for public review. After considering comments received, the Service will finalize the document. Once the document is approved by the Regional Director, appropriate portions of it will be appended to, and become part of, the Plan.

FURTHER INFORMATION ON MONITORING GENETIC DIVERSITY IN GRIZZLY BEAR POPULATIONS - A SUMMARY OF KNOWLEDGE AND OPTIONS

INTRODUCTION

The 22 year history of grizzly bear (*Ursus arctos horribilis*) conservation actions under the Endangered Species Act has taken place in parallel to an explosion of new information and techniques in conservation genetics. The species' 1975 listing as threatened predated publication of seminal works in conservation genetics (Soule and Wilcox 1980; Schonwald-Cox et al. 1983) as well as initial efforts at modeling persistence of bear populations based on demographic and genetic attributes (Shaffer 1978). Even the Grizzly Bear Compendium (NWF 1987), a more recent, encyclopedic examination of grizzly bear ecology and management, devotes only a bit over 1 page to genetic considerations.

Basic techniques for evaluation of genetic attributes at the population level have been available for many years. For example, the use of protein electrophoresis to describe the genetic structure of wild populations predates grizzly bear listing by several decades (Lewontin 1991). However, use of the diverse suite of genetic tools available today, ranging from DNA fingerprinting using micro- and mini-satellites to

mitochondrial DNA analysis, was either in its infancy or undiscovered in 1975. The U.S. Fish and Wildlife Service (Service) has incorporated genetic concerns into recovery plans and environmental review documents for numerous species, including the grizzly bear (USFWS 1993) and gray wolf (Canis lupus)(USFWS 1994). The Service also sponsored a workshop on grizzly bear genetics in 1985 (Harris 1985; Allendorf and Servheen 1986). The grizzly bear recovery plan reviews the threats of genetic impoverishment in isolated populations and advocates genetic management through periodic translocation into the isolated Yellowstone population.

The Service recognizes the need for continuing genetic monitoring of listed grizzly bear populations, all of which experience cumulative effects of varied human activities that may act to reduce genetic variability. This review summarizes the rationale for managing for genetic diversity, available assay techniques, and current findings in bear genetics. Using responses from a questionnaire submitted to prominent geneticists, it concludes with options for implementation of a continuing genetic monitoring program for grizzly bears in the contiguous United States.

WHY MANAGE FOR GENETIC DIVERSITY?

Wildlife scientists have long recognized the values of maintaining genetic variability in wild populations. Hunter (1996) places these values in 3 categories: evolutionary potential, loss of fitness, and utilitarian values.

Genetically variable populations retain evolutionary potential because greater genetic diversity results in greater likelihood of evolution in response to changing environmental conditions, and possibly greater dispersal ability.

Similarly, genetically variable populations are thought to maintain fitness, because recessive deleterious alleles are less apt to be expressed and because they may possess more adaptive phenotypic characteristics.

Finally, the utilitarian values of genetic variation can be exemplified by the essential roles played by wild genetic material in development of disease-resistant varieties of crops and productive domestic animals.

Concern for loss of genetic variability is greatest in cases of small and/or isolated populations. Genetic variability in isolated populations is determined largely by the combined actions of natural selection and genetic drift (Nunney and Campbell 1993). Small or fluctuating populations can be expected to lose genetic variability through decrease in the percentage of genes that are polymorphic (heterozygous), and through changes in allele frequency with loss of rare alleles (Awise 1994; Gyllensten 1985). Small, isolated populations may also be subject to increased mating between close relatives ("inbreeding"), with increased possibility of expression of normally recessive, deleterious alleles in a homozygous state (Ralls et al. 1986). Inbreeding depression has been hypothesized as a contributor to decreased fitness in many natural and domesticated animal populations (Allendorf and Leary 1986; Roelke et al. 1993; O'Brien et al. 1985). Early work (Frankel and Soulé 1981) suggested that inbreeding causing only a 10% decrease in genetic variation in small populations could result in an up to 25% reduction in reproductive performance.

Other negative consequences of reduced genetic diversity have been hypothesized to include outbreeding depression (mating among divergent genotypes resulting in weak or sterile offspring), and loss of evolutionary flexibility (loss of alleles conferring advantages in adaptation to changing environmental conditions)(Primack 1993). (Outbreeding depression is not a negative consequence of reduced genetic diversity. It is a potential negative effect of breeding between evolutionarily divergent individuals that may have genes which are adapted for the local environment and incompatible. This is very different from inbreeding depression. An example would be that breeding between Kodiak Is. brown bears and Yellowstone grizzly bears could lead to outbreeding depression since both mtDNA and nuclear DNA studies have demonstrated that these populations have evolved independently for over 1,000 bear generations.)

Adverse physiological effects with apparent genetic associations have been documented in both in situ (Roelke et al. 1993) and ex situ (Ralls and Ballou 1983) populations. The cheetah (*Acinonyx jubatus*) and Florida panther (*Felis concolor coryi*) are oft-cited examples of possible deleterious inbreeding effects. While recent studies have advanced alternative explanations for cheetah declines, including predation, demographic, and toxic effects (Caughley 1994; Caro and Laurenson 1994), some workers (Roelke et al. 1993) trace a genetic link to the suite of physiological abnormalities prominent in Florida panther populations, including cardiac, spermatozoal, and reproductive tract defects.

Overall, however, empirical evidence for fitness declines in wild populations due to genetic impoverishment has been relatively scarce. Nuclear and mitochondrial genetic analyses of brown bears on Kodiak Island, where the brown bear population has been isolated from mainland populations for an estimated 10,000 years, have revealed little or no genetic variability (Waits et al. undated; Paetkau et al. in press); however, no apparent loss of fitness has been reported and vital rates appear strong (NWF 1987). Some authors have recently argued that demography, rather than genetics, may be of more immediate importance to conservation of most wild populations (Lande 1988). While debate as to the relative importance of genetic and demographic aspects of small population biology continues, managers increasingly advocate attention to genetic considerations in design of reintroduction schemes (Leberg 1990; IUCN 1995). The inevitable reduction in genetic variability to be expected in isolated grizzly populations (using the Cabinet-Yaak population as an example, reduction in variability has been estimated at 9-17% over 5 generations (Harris 1985)) argues for continuing monitoring, whether or not fitness loss has been detected.

MEASURES AND TECHNIQUES IN CONSERVATION GENETICS

Genetic variation in natural populations can be measured in 2 broad fashions: mean heterozygosity and allelic diversity. Mean heterozygosity measures the proportion of loci (chromosomal positions) at which an individual is heterozygous (has more than one allele). Allelic diversity measures the number of alleles per locus

(Leberg 1990; Hunter 1996).

Genetic assay techniques have been developed to examine both nuclear and cytoplasmic genes. The most familiar technique is protein electrophoresis, which indirectly identifies alleles through visualization of the enzymes they code for. At the other end of the spectrum is the most direct family of techniques, DNA sequencing, which identifies the sequence of the 4 nucleotide forming the DNA molecule. Several intermediate techniques cleave DNA using restriction enzymes and characterize the fragments (Hunter 1996; Avise 1994).

Cytoplasmic techniques in animals focus on variability in mitochondrial DNA. Mitochondrial DNA accounts for only a small fraction of the genome (Klug and Cummings 1991; Paetkau and Strobeck in press), but exhibits several characteristics that make it desirable as a genetic marker, including more rapid evolution than nuclear DNA (note: the average rate of sequence evolution in the mitochondrial genome is higher than the nuclear genome, but there are regions of nuclear DNA such as microsatellite loci that evolve more rapidly than some conserved mtDNA gene regions), maternal inheritance, and lack of recombination (Hutchinson et al. 1974). Thus, it has been advocated as a favored vehicle for phylogeographic analyses (Avise et al. 1987 in Avise 1995).

Several aspects of ursid biology suggest a special role for mitochondrial techniques. Because female bears are generally more sedentary than males, because females and young are spatially associated over time, and because bear populations retain significant demographic autonomy over time, the maternal mitochondrial pathway may offer ways to assay genetic structure in the absence of distinction among autosomal genes (Avise 1995).

Blood and/or tissue have traditionally been used as sources of genetic material from wild populations. Acquisition of these materials has been hindered by the need to live-capture and anesthetize individuals, resulting in substantial cost and risk. However, recent advances in amplifying minute quantities of DNA through the polymerase chain reaction (PCR) have allowed reliable genotyping, with a 99% confidence level, after extraction of only a few picograms of nuclear DNA from hair, feces and forensic or ancient samples (Taberlet et al. 1996; Taberlet and Bouvet 1992).

THE STATE OF KNOWLEDGE OF BEAR GENETICS

Allozyme markers were used in the late 1970's to examine genetic structure of polar bear (*Thalarctos maritimus*) populations. These studies observed little or no variation in 13 enzyme loci (Allendorf et al. 1979). Data on genetic variation in grizzly bears were first presented in the mid-1980's (Knudson and Allendorf 1985). These initial results, also based on protein electrophoresis, confirmed that substantial genetic divergence existed between Montana and Alaska populations. Recently completed analyses using nuclear microsatellite markers (Paetkau et al. in press) found high levels of within-population variation in brown bear populations at the core of the North American range, and substantially lower diversity at the southern fringe of the range, in the Northwest Territories, and in southwest Alaska.

Paetkau et al. commented on the apparent substantial drop in heterozygosity in the Yellowstone grizzly population in historic time. Assuming that historic levels of diversity in the population were similar to levels currently found in northwest Montana grizzlies (an assumption that is not confirmable with any existing data), they estimated that H_e had dropped by 15% to 20% since the population was isolated less than a century ago.

Paetkau et al. also attempted to estimate effective population size (N_e) based on heterozygosity. Their calculated N_e for the Kodiak population corresponded to only 3.7% to 18.7% of the total population size, a fraction much smaller than earlier estimates of N_e/N based on demographic parameters, which had approached 32% (Harris and Allendorf 1989). Recently, much emphasis has been placed on molecular approaches to determining evolutionary history and taxonomic relationships between the 8 extant bear species. Evolutionary trees, which are based on steady but random chromosomal mutation over time, with associated DNA sequence divergence ("genetic distance"), have been constructed that generally agree that the bears diverged from the Procyonidae (raccoons and allies) approximately 30 million years ago. Within the ursid line, the giant panda (*Ailuropoda melanoleuca*) and spectacled bears (*Tremarctos ornatus*) next diverged at 18-25 million years BP and 12-15 million years BP, respectively. The lineages of the remaining 6 ursid bears became distinct between 5-7 million years BP (O'Brien 1993). Within the ursid line, evidence suggests that the sloth bear (*Melursus ursinus*) emerged 7 million years BP, and the line leading to the Asiatic black bear (*Ursus thibetanus*) and North American black bear (*Ursus americanus*) diverged approximately 6 million years BP, with the sun bear (*Helarctos malayanus*) line diverging soon afterward (5 million years BP).

Close phylogenetic relationships between brown and polar bears have been reported based on mitochondrial DNA analyses; most workers believe that the polar bear originated from a clade of brown bears during the Pleistocene (Talbot and Shields 1996a,b; Shields and Kocher 1991; Waits et al. in press). Estimates of date of divergence of polar bears from brown bears range from 300-400,000 years BP (Talbot and Shields 1996b) to 146,000-185,000 years BP (Waits et al. in press).

Recent work has also addressed long-standing questions of intraspecies taxonomy in brown bears. Using mitochondrial sequence data revealing substantial sequence divergence and near total geographic distinctness, Waits et al. (in press) have proposed the existence of 4 major phylogeographic clades of North American brown bears. Clade evolution has been attributed to divergence in Pleistocene glacial refugia, multiple migrations across Beringia, and low levels of female dispersal. The proposed clades differ markedly from traditional North American brown bear taxonomies based on morphological features; Waits et al. suggest that morphological differences historically used to define subspecies in brown bears may represent phenotypic plasticity rather than long term genetic isolation.

Similar genetic structures based on mitochondrial analyses have been identified among European brown bear populations (Taberlet and Bouvet 1994; Randi et al. 1994). Conversely, little evidence of population structure has been demonstrated in mitochondrial DNA restriction enzyme analysis of black bear populations (Cronin et

al.1991).

Genetic tools have applications in bear conservation that go well beyond taxonomy. Mitochondrial techniques have been published for distinguishing scat and hair from brown and black bears (*Ursus americanus*) on sympatric range (Waits and Ward 1995). Nuclear techniques are also available allowing the study of gene sequences from bear scat (Kohn et al. 1995, also can add Taberlet P, Camarra J-J, Griffin S, Uhres, E., Hanotte, O., Waits, L. P., Paganon, C., Burke, T., Bouvet, J. (in press). And recent nuclear microsatellite analysis has demonstrated multiple paternity in brown bears (Craighead et al. 1995, also Waits et al in prep.).

Applications such as these point to expanded future roles for genetics in census techniques for many wildlife species.

GENETIC GOALS OF THE 1993 GRIZZLY BEAR RECOVERY PLAN

Genetic diversity is discussed in 2 sections of the 1993 Grizzly Bear Recovery Plan(USFWS 1993) -- Management of Genetic Diversity (pp.27-28) and Long-term Strategy for Yellowstone Population (Appendix E; p.168). The former section discusses that the reduction in grizzly range has resulted in elimination of historic levels of gene flow. It cited work by Harris (1985) and Harris and Allendorf (1989) on the likelihood that effective population sizes (N_e) of existing populations are not large enough to avoid genetic impoverishment in the short term. Based on these concerns, the 1993 Recovery Plan suggested proactive genetic management of the isolated Yellowstone population through placement of one individual into the population each generation (10 years). Although not specifically stated in the 1993 Recovery Plan, the concern of possible loss of genetic diversity is only a potential problem to the two isolated grizzly bear populations - the Yellowstone and North Cascades ecosystem populations.

The 1993 Plan does not specifically call for development of a continuing program to monitor genetic status of all 5 grizzly bear populations. Such a program, however, appears important to assure that the unique attributes and threats experienced by each population will be evaluated over time, and appropriate management actions initiated in a timely fashion.

METHODS FOR MONITORING THE GENETIC STATUS OF BEAR POPULATIONS

Methods for implementing genetic monitoring programs are not explicitly addressed in the expanding genetics literature. With this in mind, we elected to forward a brief questionnaire (Appendix I) to individuals that have published articles dealing specifically with bear genetics, and who are familiar with small bear population genetics, and comparative genetics between bear populations from standpoints of conservation, taxonomy, or phylogeography.

Key points garnered from responses from 6 individuals are summarized below:

1. Monitoring programs should focus on nuclear microsatellite markers.

Respondents emphasized the high sampling variance among genetic loci, and the consequent need for examination of multiple loci. Increasing the number of loci scored reduces sampling variance more than increasing sample size of individuals once approximately 30 individuals have been sampled. This factor may be especially important in analysis of small grizzly populations, such as those in the Cabinet Yaak and Selkirks. This need for sampling multiple loci reduces the value of mitochondrial DNA approaches, which in effect sample a single, fixed locus.

Other drawbacks of mitochondrial approaches are their high relative cost, and the difficulty of interpreting results from a maternally-inherited gene in a species such as the grizzly where gene flow is male-biased. Finally, inbreeding depression and associated fitness loss -- primary concerns of a monitoring program -- are related to the expression of rare, deleterious alleles in nuclear, rather than mitochondrial, genes.

Given these attributes, and acknowledging the value of mitochondrial DNA techniques for phylogeographic studies, our respondents were in agreement that a genetic monitoring program should focus on nuclear DNA techniques -- specifically analysis of multiple DNA microsatellites. Nuclear microsatellites are inherited from both parents, are highly variable, and are independent. Statistical power for detecting bottlenecks improves as the number of loci scored increases; respondents advocated using a minimum of 6 to 8 loci, and optimally 20 to 40. PCR primer sequences derived from black bears (note: 12 new loci are from a European brown bear DNA library and work is underway to isolate additional loci from a Yellowstone grizzly bear DNA library, Waits and Ward, unpublished) are rapidly becoming available (one respondent indicated that 8 are presently available, while another indicated that 23 are available, demonstrating the rapid evolution of this field of research).

2. Blood and tissue remain the most reliable sources of genetic materials.

Respondents detailed the diversity of materials currently being investigated as sources of genetic material, including blood, tissue, hair, scat, saliva, and urine, and the decreasing DNA yield from each source (generally in the order listed, with the last 3 sources being similar). Genotyping errors may be experienced with low yield sources; for example, isolations using less than 10 hairs may have to be repeated 2 to 6 times to avoid errors. An advantage of tissue and blood samples is that they can provide enough material for archival purposes.

Respondents also pointed out the ancillary information that active versus passive sample acquisition may provide, such as confirmation of individual age, sex, and possible relatedness to others in the population -- all factors that assist interpretation of results. For example, a sample of anonymous hair snags biased toward adults might mask incipient loss of genetic variation for several years. Of course, passive sampling

might be advantageous if a goal is to minimize animal handling.

3. Genetic status assessments should be conducted each decade.

Genetic assessments should be conducted at minimum once per generation (approximately 10 years in grizzlies), and ideally more often if adequate samples (>30) were available. Optimally, genotypes would be recorded by birth year, or samples could be pooled from 2-3 cohorts to increase sample size. Obvious trade-offs would be, on the one hand, the desirability to gather samples as seldom as possible and, on the other hand, the desirability of rapidly detecting loss of variation and/or population decline. Respondents pointed out that the more frequently samples are done, the less strong the associated genetic signal will be.

Thus, more frequent sampling would entail larger individual and loci sample size in order to achieve desired levels of statistical power.

4. Genetic monitoring programs should be tailored to individual population needs and status.

It will eventually be desirable to monitor all grizzly populations non-invasively using scat or hair, assuming that DNA yield from these methods will improve over time. In the near term, however, monitoring programs should take advantage of ongoing research activities providing samples of blood and tissue. For example, an adequate sample of juvenile bears (approximately 30) is currently being captured on an annual basis in the Yellowstone Grizzly Bear Ecosystem (If a sample was inadequate, year groups could be pooled.). However, given the small number of individuals currently captured in the smaller grizzly populations (Cabinet-Yaak, Selkirk, Mission), non-invasive approaches may need to use existing, imperfect methods. The small sample sizes that are likely to be available in these populations necessitate careful study design and a clear understanding of data limitations (see 6).

Should additional funding become available, pedigree reconstruction could be undertaken for populations of approximately 200 or less. This approach would entail documentation of adult female-offspring relationships through direct observation, scat or hair collection, and examination of 30-50 microsatellite loci. Pedigree maintenance would require sampling of nearly all new cubs.

5. Better understanding of genetic interchange between peninsular populations in the U.S. and adjacent Canadian populations is needed.

The present level of genetic interchange between U.S. peninsular populations (Cabinet-Yaak and Selkirk) and adjacent Canadian populations is not well documented. Because sample size of bear genetic materials that are likely to be collected from these populations will be small under any scenario, augmentation determinations are likely to

rely heavily upon modeling techniques. Model reliability will rest upon correct assumptions regarding transborder interchange.

6. Power analyses and/or modeling techniques to better understand the relationship between observed and actual loss of genetic variability should be undertaken concurrently with acquisition of genetic samples.

Respondents listed similar factors suggesting loss of genetic variation that would call for increased management attention to a grizzly population, including demographic (increased cub mortality, observed sibling matings) and physiologic (declining sperm counts, low hormone levels, increased levels of infectious disease) abnormalities.

It was noted that "biologically significant" loss of variation is difficult to determine, as examples of populations with low genetic variation (e.g., Kodiak bears) and no resultant fitness loss are available. However, respondents still pointed out that rapid declines in effective population size, particularly those that persist for more than 1 generation, can be assumed to increase extinction risk.

A drawback of management decision-making based on documented loss of genetic variability (versus decision-making based on modeling) is that the difficulty of statistically detecting loss of variation may delay management actions until populations are already in jeopardy (For example, in some situations, a loss of as much as 30% of genetic diversity may occur before the loss can be statistically proven). Respondents mentioned the growing interest in power analysis in the biological sciences; power analysis attempts to define either prospectively or retrospectively a level of confidence that a failure to detect a decline is in fact not a decline.

Prospective power analysis is increasingly being advocated as an integral part of research design (Steidl et al. 1997; Reed and Blaustein 1997). In this case, power analyses could help in determining sample sizes and number of loci to be scored.

7. Recurring genetic assays would be relatively inexpensive.

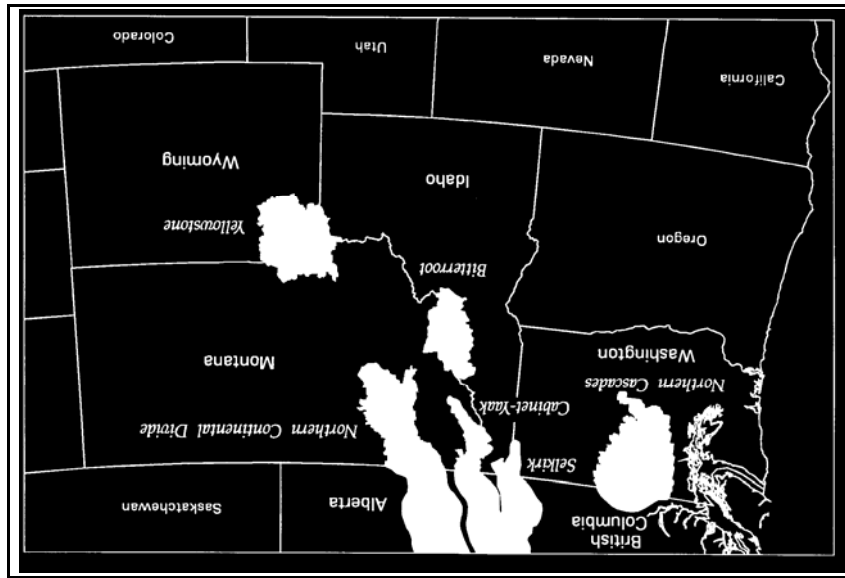
Respondents suggest that costs per individual for monitoring 6-20 loci would be in the range of \$200. Thus total cost for a population sample of 30 would be approximately \$6,000. If protocols called for repeated genotyping (as is recommended for low yield DNA sources such as scat, hair, and urine), costs would be higher.

Respondents did not comment on the costs associated with simulation modeling.

SYNTHESIS

Of the 5 existing grizzly bear populations, only the Yellowstone and the North Cascades populations are genetically isolated from larger contiguous populations. Genetic isolation is the lack of natural movement of breeding animals into a population

due to physical barriers or a lack of dispersal behavior to facilitate such movement. If bears were reintroduced into the Bitterroot ecosystem, these bears would be genetically isolated until and if they might eventually be joined with adjacent populations through movements. However, this linkage is questionable considering the lack of movement



between populations to date. The NCDE, Cabinet-Yaak and Selkirk grizzly bear populations are all contiguous with larger populations in Canada. This larger contiguous Canadian population consists of thousands of grizzly bears in an unbroken population extending northward from the NCDE, Cabinet-Yaak and Selkirks in the U.S. through the Canadian Rockies and eventually joining the grizzly bear populations in the Yukon and Alaska. The U.S. portions of these populations are subsets of larger populations that extend southward across the border into the U.S. (Figure 1). Because the NCDE, Cabinet-Yaak, and Selkirk populations are not genetically isolated, a loss of genetic diversity is not a threat to these populations. This alleviates the need to establish objective measurable criteria for genetics for these three contiguous populations.

The Yellowstone population has been isolated from contiguous populations to the north for perhaps 80 years. Perhaps because of this isolation, the Yellowstone population has less genetic diversity than the NCDE population (Table 1). However, since there are no historic data on genetic diversity in places like Yellowstone, we do not know that the existing level of genetic diversity is not natural. At this time there are no data to demonstrate any physical problems associated with a reduction in fitness in the Yellowstone grizzly bears that might be due to a loss of genetic diversity. In fact, the Yellowstone population shows every indication of a healthy population including high litter size (an average of 2.16 cubs per litter from 1991-1996), a high number of females seen with cubs (an average of 23 females with cubs seen each year from 1991-1996), high numbers of cubs seen (an average of 49 cubs seen each year from 1991-1996), almost no evidence of disease, high survivorship, an equal sex ratio, normal body size

and physical characteristics, and a population increasing at between 4 and 7% per year for the past 8-10 years. These population fitness factors will continue to be monitored as part of normal research and monitoring activities so as to be sure and recognize any possible impacts on fitness related to changes in genetic diversity. Given the healthy nature of the Yellowstone population, the Service believes the current level of genetic diversity (Table 1) is adequate. Because the population is isolated, the Service believes that it is important that the genetic diversity not deteriorate by a significant amount.

As evidenced by Table 1, no information on the genetics of the North Cascades population is available. The North Cascades population is not a healthy population because it is low in numbers and the specific reasons for these low numbers such as low cub production and/or poor survival are as yet unknown. Also, the genetic heterozygosity of the North Cascades population is unknown and we cannot assume at this time that it is sufficient without genetic sampling.

There is a need to monitor the change in genetic diversity over time in order to make sound decisions on the need for augmentation of new individuals to increase diversity if diversity is being lost. If genetic diversity is being lost in any of the isolated populations, this will be detected by the monitoring program and measurement protocol established in this recovery task. Augmentation will likely be the only way that genetic material can be transferred to an isolated population because there is no evidence of any movement of bears between existing populations. In fact, since 1975 more than 500 different grizzly bears have been captured and monitored and not one of these bears has ever moved across the unoccupied habitats between the existing ecosystems.

Table. 1. Genetic variability within healthy North American brown bear populations based on nuclear DNA microsatellite analysis averaged over 8 loci (from Waits et al. in press).

Population	Alleles	Diversity	Sample size
Kodiak Island, Alaska	2.1	26.5%	34
Kluane National Park, Canada	7.4	76.2%	24
East Slope, Alberta, Canada	6.4	65.6%	30
NCDE, Montana, USA	6.8	70.3%	35
Yellowstone, USA	4.4	55.5%	46

Diversity is calculated by $h = (1 - \sum x_i^2) / (n-1)$, where x_i is the frequency of the i th lineage (allele) and n is the

sample size.

This review highlights the need for a proactive strategy for assuring that there is no significant loss of genetic diversity in the isolated grizzly bear populations in the conterminous United States, and continued baseline monitoring of all populations and those contiguous populations in Canada. Isolation of the Yellowstone population makes this the population of most concern because it is the only isolated population, other than the North Cascades (where no data are currently available). Given that the data on population fitness indicates that current levels of genetic diversity are adequate, but concern that an ongoing loss below current levels could lead to detrimental conditions, maintenance of the existing levels of diversity is desirable. Table 1 shows current levels of genetic diversity in the NCDE and Yellowstone as compared to some other healthy North American brown bear populations (from Waits, et al. (in press)).

Efforts in the near term should attempt to balance the need for prompt data acquisition and possible management action with a recognition of the limitations and rapid evolution of current science. This calls for an adaptive, management-oriented approach to data gathering and evaluation. A reasonable approach for the next 10 years would include genotyping of available samples from all populations, and opportunistic acquisition of additional samples (in amounts suitable for archival purposes) from ongoing research and/or management captures. Comparisons of diversity change using microsatellite loci in each population should be made on 10 year intervals to assure adequate sample sizes for statistical comparison. Concurrently, modeling research should be continued to identify research designs offering optimum statistical power and effective non-invasive sampling techniques for future monitoring. This research could also weigh the relative advantages of modeling versus non-invasive genetic sampling approaches for the smaller populations. The results of these research projects could influence later decisions as to whether additional actual sampling will be required in the smaller populations.

Finally, the problems identified above point out that augmentations may have to be undertaken as a result of data confirming diversity loss but not necessarily confirming fitness loss. Given this potential problem, future revisions of the Grizzly Bear Recovery Plan will include expanded discussions of the challenges of maintaining genetic variation and will incorporate the findings of the monitoring program. This will allow the public to better understand future decisions regarding augmentation.

LITERATURE CITED

Allendorf, F. W., and C. Servheen. 1986. Genetics and conservation of grizzly bears. *Trends in ecology and evolution* 1:88-89.

Allendorf, F.W., and R.F. Leary. 1986. Heterozygosity and fitness in natural

- populations of animals, pp. 57-76 in M. E. Soule, ed., *Conservation Biology. The science of scarcity and diversity*. Sinauer Assoc., Sunderland, MA.
- Allendorf, F.W., F.B. Christiansen, T. Dobson, W.F. Eanes, and O. Frydenberg. 1979. Electrophoretic variation in large mammals. I. The polar bear. *Hereditas* 91:19-22.
- Avise, J.C. 1995. Mitochondrial DNA polymorphism and a connection between genetics and demography of relevance to conservation. *Conservation Biology* 9(3):686- 690.
- Avise, J.C. 1994. *Molecular markers, natural history and evolution*. Chapman and Hall, New York, NY.
- Avise, J.C., J. Arnold, R.M. Ball, E. Bermingham, T. Lamb, J.E. Neigel, C.A. Reeb, and N.C. Saunders. 1987. Intraspecific phylogeography: The mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics* 18:489-522.
- Caro, T.M., and M.K. Laurenson. 1994. Ecological and genetic factors in conservation: A cautionary tale. *Science* 263:485-486.
- Caughley, G. 1994. Directions in conservation biology. *J. of Animal Ecology*. 63:215-244.
- Craighead, L., D. Paetkau, H.V. Reynolds, E.R. Vyse, and C. Strobeck. *J. of Heredity* 86:25-261.
- Cronin, M.A., S.C. Amstrup, G.W. Gardner, and E.R. Vyse. 1991. Interspecific and intraspecific mitochondrial DNA variation in North American bears (*Ursus*). *Canadian J. of Zoology* 69:2985-2992.
- Frankel, O., and M. Soule. 1981. *Conservation and evolution*. Cambridge University Press. Cambridge, UK.
- Gyllensten, U. 1985. Temporal allozyme frequency changes in density fluctuating populations of willow grouse. *Evolution* 39(1):115-121.
- Harris, R.B., ed. 1985. *Results of the workshop on grizzly bear population genetics*. U.S. Fish and Wildlife Service. Missoula, MT
- Harris, R.B., and F.W. Allendorf. 1989. Genetically effective population size of large mammals: An assessment of estimators. *Conservation Biology* 3:181-191.
- Hunter, M.L. 1996. *Fundamentals of Conservation Biology*. Blackwell Science.

Cambridge, MA.

Hutchinson, C.A., J.E. Newbold, S.S. Potter, and M.H. Edgell. 1974. Maternal evidence of mammalian mitochondrial DNA. *Nature* 251:536-538.

IUCN. 1995. Guidelines for reintroductions. Reintroduction Specialist Group. World Conservation Union. Gland, Switzerland.

Klug, W.S., and M.R. Cummings. 1991. *Concepts of Genetics*. Third Edition. Macmillan Publishing Company. New York, NY.

Knudson, K.L., and F.W. Allendorf. 1985. Preliminary survey of genetic variation in grizzly bears. University of Montana. Missoula, MT.

Kohn, M., F. Knauer, A. Stoffella, W. Schroder, and S. Paabo. 1995. Conservation genetics of the European brown bear - a study using excremental PCR of nuclear and mitochondrial sequences. *Molecular Ecology* 4:95-103.

Lande, R. 1988. Genetics and demography in biological conservation. *Science* 241:1455-1460.

Leberg, P.L. 1990. Genetic considerations in the design of introduction programs. *Trans. 55th N.A. Wildl. & Nat. Res. Conf.:*609-619.

Lewontin, R.C. 1991. 25 years ago in genetics - electrophoresis in the development of evolutionary genetics - milestone or millstone. *Genetics* 128:657-662.

National Wildlife Federation. 1987. *Grizzly bear compendium*. Washington, DC.

Nunney, L., and K. Campbell. 1993. Assessing minimum viable population size: Demography meets population genetics. *Trends in Ecology and Evolution* 8(7).

O'Brien, S.J. 1993. The molecular evolution of the bears, pp.26-33 in I. Stirling, ed., *Bears: Majestic creatures of the wild*. Rodale Press. Emmaus, PA.

O'Brien, S.J., M.E. Roelke, L. Marker, A. Newman, C.A. Winkler, D. Meltzer, L. Colly, J.F. Evermann, M. Bush, and D.E. Wildt. 1985. Genetic basis for species vulnerability in the cheetah. *Science* 227:1428-1434.

Paetkau, D, and C. Strobeck. in press. Ecological genetic studies of bears using microsatellite analysis. *International Conference on Bear Research and Management* 10.

Paetkau, D., L. Waits, P.L. Clarkson, L. Craighead, E. Vyse, R. Ward, and C. Strobeck.

in press. Variation in genetic diversity across the range of North American brown bears. *Conservation Biology*.

- Primack, R.B. 1993. *Essentials of conservation biology*. Sinauer Associates. Sunderland, MA.
- Ralls, K., P. H. Harvey, and A. M. Lyles. 1986. Inbreeding in natural populations of birds and mammals, pp 35-56 in M. Soule, ed., *Conservation Biology*. Sinauer Assoc., Sunderland, MA.
- Ralls, K., and J. Ballou. 1983. Extinction: Lessons from zoos, pp 164-184 in Schonewald-Cox et al., eds., *Genetics and conservation*. Benjamin Cummings. Menlo Park, CA.
- Randi, E., L. Gentile, G. Boscagli, D. Huber, and H.U. Roth. 1994. Mitochondrial DNA sequence divergence among some west European brown bear (*Ursus arctos* L.) populations. *Lessons for conservation*. *Heredity* 73:480-489.
- Reed, J.M., and A.R. Blaustein. 1997. Biologically significant population declines and statistical power. *Conservation Biology* 11(1):281-282.
- Roelke, M. E., J. S. Martenson, and S. J. O'Brien. 1993. The consequences of demographic reduction and genetic depletion in the endangered Florida panther. *Current Biology* 3(6):340-350.
- Schonewald-Cox, C.M., S.M. Chambers, B. MacBryde, and W.L. Thomas (eds.). 1983. *Genetics and Conservation*. Benjamin/Cummings. Menlo Park, CA.
- Shaffer, M.L. 1978. Determining minimal viable population sizes: A case study of the grizzly bear (*Ursus arctos horribilis*). Ph.D. Diss. Duke University. Durham, NC.
- Shields, G.F., and T.D. Kocher. 1991. Phylogenetic relationships of North American ursids based on analysis of mitochondrial DNA. *Evolution* 45(1):218-221.
- Soule, M.E., and B.M. Wilcox (eds.). 1980. *Conservation biology: An evolutionary - ecological perspective*. Sinauer Associates. Sunderland, MA.
- Steidl, R.J., J. Hayes, and E. Schaubert. 1997. Statistical power analysis in wildlife research. *J. of Wildlife Management* 61(2):270-279.
- Taberlet, P., and J. Bouvet. 1994. Mitochondrial DNA polymorphism, phylogeography, and conservation genetics of the brown bear *Ursus arctos* in Europe. *Proceedings Royal Society of London B* 255:195-200.
- Taberlet, P., and J. Bouvet. 1992. Bear conservation genetics. *Nature*

358:197.

Taberlet, P., S. Griffin, B. Goossens, S. Questiau, V. Manceau, N. Escaravage, L.P. Waits, and J. Bouvet. 1996. Reliable genotyping of samples with very low DNA quantities using PCR. *Nucleic Acids Research* 24(16):3189-3194.

Talbot, S.K., and G.F. Shields. 1996a. Phylogeography of brown bears (*Ursus arctos*) of Alaska and paraphyly within the Ursidae. *Molecular Phylogenetics and Evolution* 5(3):477-494.

Talbot, S.K., and G.F. Shields. 1996b. A phylogeny of the bears (Ursidae) inferred from complete sequences of three mitochondrial genes. *Molecular Phylogenetics and Evolution* 5(3):567-575.

U.S. Fish and Wildlife Service. 1994. Final environmental impact statement: The reintroduction of gray wolves to Yellowstone National Park and central Idaho. Helena, MT.

U.S. Fish and Wildlife Service. 1993. Grizzly bear recovery plan. U.S. Fish and Wildlife Service, Missoula, MT.

Waits, L.P., S.L. Talbot, R.H. Ward, and G.F. Shields. in press. Phylogeography of the North American brown bear and implications for conservation. *Conservation Biology*.

Waits, L.P., and R.H. Ward. 1995. Unambiguous genetic identification of bear species by taxon specific sequences (TSSs). *Proceedings of the 10th International Conference on Bear Research and Management* (abstract).

Waits, L.P., D. Paetkau, C. Strobeck, and R.H. Ward. in press. A comparison of genetic variability in brown bears (*Ursus arctos*) from Alaska, Canada, and the lower 48 states. *Proceedings of the 10th International Conference on Bear Research and Management* 10.

Appendix I. Questions asked of scientists knowledgeable about genetic issues in small bear populations in general or in the Rocky Mountains in particular and who have published bear-related articles in the genetics literature.

Numerous techniques for examining genetic variation at the DNA sequence level have been refined in the last decade. Journal articles typically focus on either nuclear or mitochondrial analysis, but generally acknowledge the value of using the 2 approaches in tandem. Given your knowledge of grizzly bear status in the western states (6 populations ranging from several dozen to several hundred individuals, each experiencing a varying level of historic isolation), how would you structure an on-going genetic monitoring program?

- a) What combination of nuclear and mitochondrial techniques would you use?
- b) What biological materials (hair, blood, tissue) would need to be collected for these procedures?
- c) How often would you re-assess the genetic status of these populations?
- d) What "triggers" would you use to advocate genetic augmentation, if any, of these existing populations?
- e) Would your monitoring procedures vary between populations depending on population size or other factors?
- f) Using today's costs as a benchmark, what might such a program cost on an annual basis for the lab work? We can estimate the costs to gather the samples from bears if we get some ideas on how many and how often samples should be collected.

Respondents:

Steve Forbes, University of Montana
Michael Kohn, University of California at Los Angeles
Gordon Luikart, Universite Joseph Fourier (France)
David Paetkau, University of Alberta
Pierre Taberlet, Universite Joseph Fourier (France)
Lisette Waits, Universite Joseph Fourier (France)

PROPOSED SUPPLEMENT TO THE GRIZZLY BEAR RECOVERY PLAN
CONCERNING GENETIC MONITORING

Part 3, Recovery (begins on page 33)

Insert the following recovery criteria on page 34:

- (3) Assure no significant decline from current levels of genetic heterozygosity in the Yellowstone population.
 - (a) If a statistically significant decline in genetic heterozygosity is detected below current levels (55.5%) in the Yellowstone population over any 10-year period starting in 1998, augmentation of that population will be initiated to improve genetic heterozygosity. Changes in genetic heterozygosity will be measured comparing at least 16 of the same DNA microsatellite loci from each bear sampled (heterozygosity will be calculated by $h = (1 - \sum x_i^2) / (n - 1)$, where x_i is the frequency of the i th lineage (allele) and n is the sample size). Statistically significant declines will be measured using a paired T-test ($p < 0.05$). Augmentation will require that at least one animal enter the breeding population during any 10-year period to be successful at increasing genetic heterozygosity. This is based on computer simulations of the number and frequency of bears necessary to increase genetic heterozygosity in a grizzly bear population (Harris 1985).
- (4) Determine current levels of genetic heterozygosity in the North Cascades population. Once determined, then measurable, objective genetic recovery criteria will be established for the North Cascades population based on the best available scientific information.

Insert the following recovery tasks in the Step-Down-Outline under task 111 on page 34:

1111. Determine methods for monitoring genetic heterozygosity.
1112. Monitor levels of genetic heterozygosity.
1113. Establish recovery criteria for genetic heterozygosity of isolated grizzly bear populations.

Insert the following recovery task in the Step-Down-Outline under task 112 on page 34:

1121. Establish reporting procedures and systems to gather and evaluate information on genetic heterozygosity of grizzly populations.

Insert the following recovery tasks and descriptive text under task 111 in the appropriate chapters for the six recovery areas:

Determine methods for monitoring genetic heterozygosity. (X1111)

Genetic variability status assessments should be conducted every 10 years (approximately once per grizzly bear generation). Assessments should score multiple nuclear microsatellite loci (optimally 20 - 40 loci). To the extent practicable, assessments should examine mean heterozygosity and allelic heterozygosity within and between all listed grizzly populations, and should be adapted and improved over time as genetic techniques evolve. Levels of genetic heterozygosity should be monitored via allele frequencies. Heterozygosity will be

calculated by $h = (1 - \sum x_i^2) / (n - 1)$, where x_i is the frequency of the i th lineage (allele) and n is the sample size. Statistically significant declines will be measured using a paired T-test ($p < 0.05$).

Simulations of genetic heterozygosity changes per generation should be conducted using data gathered by ongoing sampling and new techniques as available, and the results should be used to aid in interpretation of the allele frequency data from field collections.

Monitor levels of genetic heterozygosity. (X1112)

Monitor levels of genetic heterozygosity using the methods selected under task X1111.

Establish recovery criteria for genetic heterozygosity of isolated grizzly bear populations. (X1113)

Establish recovery criteria for genetic heterozygosity of isolated grizzly bear populations using the methods in tasks X1111 and X1112.

Insert the following recovery task and descriptive text under task 112 in the chapter for each of the six recovery areas:

Establish reporting procedures and systems to gather and evaluate information on genetic heterozygosity of grizzly populations. (X1121)

To maintain a sample of the genetics of all populations, all cooperating agency personnel handling grizzly bears for research or management purposes should provide samples of blood and tissue from each new individual captured and all dead bears not previously sampled to a designated genetic repository. Ages should be determined for each bear sampled. Techniques for collection and handling of samples will be developed and distributed to all agencies by the Grizzly Bear Recovery Coordinator. Changes in genetic monitoring and augmentation criteria will be made as necessary when and if new information becomes available.

Information should be collected on the current level of genetic heterozygosity between U.S. peninsular populations and contiguous Canadian populations by coordinating with Canadian management authorities and continuing the collection of blood and tissue samples for genetic screening from Canadian populations. Heterozygosity will be measured using at least 16 microsatellite loci and

measuring heterozygosity by $h = (1 - \sum x_i^2) / (n - 1)$, where x_i is the frequency of the i th lineage (allele) and n is the sample size.

Change Task Y512 (p. 56) to:

Develop and Test Procedures to Relocate Bears between Areas for Demographic or Genetic Purposes (Y512)

Develop and coordinate interagency agreements and procedures for the introduction of grizzly bears into the Yellowstone population if genetic monitoring demonstrates a statistically significant decline in genetic heterozygosity in this population over a 10 year period. The number, sex, and age(s) of any such bears are to be determined through consultation with genetic experts if and when the need arises. Use of nuisance bears for such purposes shall not be permitted. Ecosystems with populations that are not isolated breeding units shall be the sources for such bears. Responsibility for this effort lies with the Recovery Coordinator in coordination with State and Federal agencies and appropriate universities.

DISEASE AND PARASITES

The recovery plan indicates that parasites and disease do not appear to be significant causes of natural mortality among bears (Jonkel and Cowan 1971, Kistchinskii 1972, Mundy and Flook 1973, Rogers and Rogers 1976). Research in Alaskan grizzly bears has shown previous exposure by some grizzly bears to rangeliferine brucellosis and leptospirosis, though impacts to populations are unknown (Zarnke 1983). The most common internal parasite noted in grizzly bears is *Trichinella* for which 62% of grizzly bears tested positive from 1969-81 (Greer 1982). Effects of these levels of incidence are unknown but monitoring will be continued.

Mortality summaries from the Yellowstone Ecosystem for 1959-87 did not identify disease as a significant factor resulting in mortality (Craighead et al. 1988). Only one of 477 known mortalities was attributed to disease or parasites. Thirty-eight mortalities could not be identified by cause and some of these may have been related to disease or parasites, but these factors do not appear to be significant causes of mortality affecting Yellowstone grizzly bears.

The Montana Department of Fish, Wildlife, and Parks operates a Wildlife Laboratory at Bozeman. One of the Laboratory's objectives is to necropsy wildlife specimens suspected of being diseased, parasitized, or dying of unknown causes to identify the cause of death (Aune and Schladweiler 1995). Tissue samples are examined by Veterinary Pathologists at the State Diagnostic Laboratory. Though disease was not considered a threat at the time of listing, the U.S. Fish and Wildlife Service will continue to have dead grizzly bears processed through a Laboratory to determine cause of death and to maintain baseline information on diseases and parasites occurring in grizzly bears. This action will serve to continue monitoring of these agents as potential monitoring sources. This action will serve to continue monitoring of these agents as potential mortality sources. If disease is later determined to be a threat, the US Fish and Wildlife Service will evaluate and adopt specific measures to control the spread of any disease agent and treat infected animals where possible. These measures will depend on the disease agent identified. At this time, the US Fish and Wildlife Service sees no need to add a specific task or recovery criteria to the Recovery Plan relating to disease.

Literature Cited:

- Aune, K. and P. Schladweiler. 1995. Wildlife laboratory annual report, 1994. Montana Fish, Wildlife, and Parks, Helena. 83pp.
- Craighead, J.J., K.R. Greer, R.R. Knight, and H. Ihsle Pac. 1988. Grizzly bear mortalities in the Yellowstone ecosystem, 1959-1987. Montana Fish, Wildlife, and Parks, Helena. 103pp.
- Greer, K.R. 1982. Grizzly bear mortality studies 1981. Fed. Aid Wildl. Rest. Proj. W-120-R-13, Job No. 2, Study No. WI-1.0. Mont. Dep. Fish, Wildl. Parks, Helena.

20pp.

Jonkel, C.J. and I. McT. Cowan. 1971. The black bear in the spruce-fir forest. Wildl. Monogr. 27. 57pp.

Kistchinskii, A.A. 1972. Life history of the brown bear (*Ursus arctos L.*) In northeast Siberia. Pages 67-73 in S. Herrero ed. Bears--their biology and management. IUCN Publ. New Series 23.

Mundy, K.R.D. and D.R. Flook. 1973. Background for managing grizzly bears in the national parks of Canada. Can. Wildl. Serv. Rep. Series No. 22. 35pp.

Rogers L.L. and S.M. Rogers. 1976. Parasites of bears; a review. Pages 411-430 in M.R. Pelton, J.W. Lentfer, and G.E. Folk ed. Bears--their biology and management. IUCN Publ. New Series 40.

Zarnke, R.L. 1983. Serologic survey for selected pathogens in Alaskan wildlife. J. Wildl. Dis. 19:324-329.

FURTHER INFORMATION ON RELIANCE ON CANADA

Four grizzly bear populations span the international border with Canada (USFWS 1993). The Northern Continental Divide, Cabinet-Yaak, and Selkirk ecosystems are extensions of contiguous grizzly bear populations in British Columbia. The North Cascades recovery area may be isolated from other grizzly bear populations in British Columbia. The court ruling (p. 31) questions the reliance on Canada in setting the recovery targets for the Cabinet-Yaak and Selkirk ecosystems and asks the US Fish and Wildlife Service to explain this reliance on Canada and the setting of the population targets in these two ecosystems. In this response we address issues relating to the Cabinet-Yaak and Selkirk populations and also add some information on cooperation with Alberta even though Alberta does not directly effect these ecosystems

Population targets for the Selkirk and Cabinet-Yaak ecosystems relied on the fact that these populations were adjacent to Canadian grizzly bear populations. These targets were based on: (1) the maximum density of grizzly bears that could live in the available habitat each ecosystem on the US side of the contiguous population; and (2) the fact that the US side of the border was only a subset of a larger, contiguous population that ranged far to the north in Canada. Such reliance is sound and biologically reasonable because grizzly bear populations are contiguous across the US-Canada border (McLellan 1989a, Kasworm and Servheen 1995, Kehoe 1995, Wakkinen and Allen-Johnson 1996), and continued management actions in both the US and Canada strive to maintain these contiguous populations. Although grizzly bear populations across the US-Canada border in the Selkirks and Cabinet-Yaak ecosystems are lower in density than populations in some other areas in British Columbia, and although Canadian populations are subject to the same threats that grizzly bear populations in the US face such as human development, there is ongoing management action in British Columbia to assure the continued existence of grizzly bears across the border in both ecosystems. The statements in the Recovery Plan concerning the small numbers of bears and ongoing threats to their habitat are forthright statements of fact that identify potential problems. The issue is not being ignored, but is being increasingly addressed by Canadian authorities because they, like US management authorities, wish to assure the future of their grizzly bear populations. The fact that these contiguous Canadian grizzly bear populations are relatively low in density does not mean they do not exist. In fact, there is increasing and ongoing action to assure the continued existence of these Canadian populations as detailed below.

The presence of contiguous US-Canada grizzly bear populations is the foundation of pursuit of recovery of the species in the Selkirk and Cabinet-Yaak ecosystems. The US portion of these contiguous populations is not sufficient in area or extent to maintain a viable population in and of itself. Recovery of the species in these ecosystems in isolation from Canada would likely not be possible given limited available habitat. If there were no grizzly bears and/or no assurance of continued contiguous populations with Canada in the Selkirk and Cabinet-Yaak ecosystems, the population targets for these ecosystems would have been different and, in fact, the very idea of recovery in these areas might have been questioned due to the limited habitat on the

US side. However, grizzly bears do exist as contiguous populations in both ecosystems and ongoing efforts in both countries help to assure the existence of these contiguous populations.

Thus, reliance on Canada to maintain populations and set recovery goals on that basis is reasonable and justified as detailed below. In the case of the Selkirk Ecosystem, the recovery zone was extended into British Columbia by international agreement. The Recovery Plan does acknowledge threats to grizzly bear populations in Canada, but these threats are not uncontrolled (see Court opinion, page 31). There are several grizzly bear population and habitat management plans and agreements that apply to these threats (as described below) that make reliance on Canada a reasonable approach based upon past and expected cooperation and coordination in recovery and management.

Recovery Plan Task 82 (pages 79, 98, and 116) discusses coordination and information exchange with Canada concerning bear research and management. Cooperation and coordination between the United States and the provinces of British Columbia and Alberta are an essential part of grizzly bear recovery efforts. Fortunately, both countries have worked closely together to maintain and increase grizzly bear populations in the four transboundary recovery areas. Several efforts were ongoing at the time of the recovery plan revision, but numerous other grizzly bear conservation efforts have been initiated since 1993 under the direction provided in the recovery plan and in Alberta and British Columbia management plans.

International cooperation and coordination have been furthered by Canadian participation in the Interagency Grizzly Bear Committee. This body is composed of agency representatives that have jurisdiction and responsibility for grizzly bear habitat or populations at the state and federal levels. Canadian participation was formalized in a Conservation Strategy document among various Ministries of the Province of British Columbia and the Interagency Grizzly Bear Committee (IGBC 1989). The intent of this agreement was to "effect cooperative and compatible grizzly bear management programs that will support the cooperative management objectives for grizzlies...".

In 1990 the Alberta provincial government developed a Management Plan for grizzly bears in Alberta (AFLW 1990). The plan lists goals of increasing the provincial grizzly bear population by 25%, increased management for viewing and nonconsumptive uses, and coordinated management of shared grizzly populations with neighboring wildlife agencies. The management plan emphasizes reduction of total man-caused mortality to no more than 6% of estimated populations, prevention of nuisance bear situations, and habitat management through inventory, protection, and enhancement. The U.S. Grizzly Bear Recovery Coordinator was asked to critique the document prior to release.

In 1995 the British Columbia provincial government developed a grizzly bear conservation strategy (BCMELP 1995a). The strategy's mandate is to ensure the continued existence of grizzly bears and their habitats for future generations. The strategy has four goals:

1. To maintain in perpetuity the heterozygosity and abundance of grizzly bears and the

ecosystems.

2. To improve the management of grizzly bears and their interactions with humans.
3. To increase public knowledge of grizzly bears and their management.
4. To increase international cooperation in management and research of grizzly bears.

As part of this plan, British Columbia is adopting measures to protect grizzly bear habitat through land use planning and land use codes, reduce or eliminate grizzly bear hunter harvest, increase fines for illegal kills of grizzly bears, establish a trust fund and other mechanisms to provide funds for habitat conservation, improve public information programs to reduce human/bear conflicts that may result in loss of bears or habitat (BCGBCS 1996).

Another part of the Strategy is to establish a British Columbia Grizzly Bear Science Committee which will make recommendations directly to the Minister of Environment concerning grizzly bear policy and management. This committee is composed of government and independent grizzly bear scientists from Canada and a scientific representative from the United States (USFWS Grizzly Bear Recovery Coordinator) who review all aspects of grizzly bear management and research policy in British Columbia. The Committee is presently making significant recommendations to improve grizzly bear management in British Columbia such as the identification and management of linkage zones to assure population connectivity for grizzly bear habitat adjacent to the Northern Continental Divide, Selkirk, and Cabinet-Yaak recovery zones (Apps 1997, BCGBCS 1997).

Another outcome of US involvement in the Grizzly Bear Science Committee has been creation of the Canadian Rocky Mountains Grizzly Bear Planning Committee in 1997. This committee is composed of representatives from Alberta, British Columbia, Canadian National Parks, and representatives of the US Interagency Grizzly Bear Committee. The purpose is to identify grizzly bear conservation areas in the Rocky Mountain portion of grizzly bear habitat across Canada and the US, develop management objectives for these areas, facilitate interagency cooperation in management, integrate land use objectives, identify research and inventory needs, cooperatively monitor grizzly bear status and trends, prevent and react effectively to human-bear conflicts, and consider new management programs and strategies. A similar cooperative US/Canada working group is being initiated for portions of British Columbia and the US within and adjacent to Selkirk and Cabinet-Yaak recovery zones.

British Columbia has cooperated in population augmentation in the Cabinet Mountains by providing grizzly bears from the southeast portion of the province (Kasworm et al. In press). Four females were transplanted from British Columbia to the Cabinet Mountains during 1990-94. This experimental program was the first occasion that non-nuisance grizzly bears were moved internationally from one area to another with the intent of increasing the target population.

Hunting has occurred in the British Columbia portion of several recovery zones in the past. British Columbia closed the hunting season in the Selkirk grizzly bear recovery area in 1995, thereby reducing the potential for human-caused mortalities. There has not been a hunting season for grizzly bears directly north of the Cabinet-

Yaak recovery zone since the 1970s. Hunting north of the Northern Continental Divide recovery zone is a regulated permit hunt in both British Columbia and Alberta. Both provinces set hunting quotas to harvest no more than 4% of the estimated population (AFLW 1990, BCMELP 1995b).

The US and Canada have cooperated by having US trapping crews access the British Columbia portion of the Northern Continental Divide, Selkirk, and Cabinet-Yaak recovery areas to radio collar and monitor grizzly bears (Kehoe 1995, Wakkinen and Allen-Johnson 1996, Kasworm et al. In press). British Columbia has an active grizzly bear research program adjacent to the Northern Continental Divide recovery zone (McLellan 1989a). All of these studies have documented transboundary movements by radio collared grizzly bears and demonstrate international cooperation in research efforts, monitoring the effects of habitat management, and monitoring mortality. No grizzly bears have been captured and monitored as yet within the North Cascade recovery area.

Several Canadian and U.S. research programs have been supported by the USFWS to gain information on grizzly bears in the Canadian portions of the Northern Continental Divide ecosystem (McLellan 1989a, McLellan 1989b, McLellan 1989c, McLellan and Shackleton 1988, McLellan and Shackleton 1989a, McLellan and Shackleton 1989b, McLellan and Hovey 1995, Kehoe 1995, Hovey and McLellan 1996), Selkirk Ecosystem (Knick and Kasworm 1989, Wielgus et al 1994, Wakkinen and Allen-Johnson 1996, Wakkinen and Kasworm 1997) and Cabinet-Yaak ecosystem (Kasworm and Servheen 1995, Kasworm et al in press). Canadian scientists have participated in U.S. research efforts focusing on population trend calculation and evaluation of ecosystem wide methods for population trend estimation utilizing standard capture techniques and DNA analysis (Servheen et al. 1994, Hovey and McLellan 1996, Servheen et al. 1996).

The Service therefore determines that, given the history of positive cooperation and increasing coordination with British Columbia and Alberta, sufficient evidence exists to justify as biologically sound the reliance on Canada grizzly bear populations and management programs in setting the population targets for the Selkirk and Cabinet-Yaak ecosystems.

Literature Cited

- Alberta Forestry, Lands, and Wildlife. 1990. Management plan for grizzly bears in Alberta. Wildlife Manage. Planning Ser. No. 2. Edmonton, Alberta. 164pp.
- Apps, C. 1997. Identification of grizzly bear linkage zones along the highway 3 corridor of southeast British Columbia and southwest Alberta. B.C. Ministry of Environ., Lands, and Parks. 45pp.
- British Columbia Grizzly Bear Conservation Strategy. 1996. Fact sheet #1.

British Columbia Grizzly Bear Conservation Strategy. 1997. Fact Sheet #2.

British Columbia Ministry of Environment, Lands, and Parks. 1995a. British Columbia grizzly bear conservation strategy. Victoria, BC, Canada. 15pp.

British Columbia Ministry of Environment, Lands, and Parks. 1995b. Conservation of grizzly bears in British Columbia background report. Victoria, BC, Canada. 70pp.

Hovey, F.W. and B.N. McLellan. 1996. Estimating population growth of grizzly bears from the Flathead River drainage using computer simulations of reproductive and survival rates. *Can. J. Zool.* 74:1409-1416.

Interagency Grizzly Bear Committee. 1989. Conservation strategy for grizzly bear populations shared between British Columbia and the states of Washington, Idaho, and Montana. Missoula, MT. 4pp.

Kasworm, W.F. and C. Servheen. 1995. Cabinet/Yaak ecosystem grizzly bear and black bear research 1994 progress report. U.S. Fish and Wildlife Service, Missoula, Mont. 42pp.

Kasworm, W.F., T. J. Thier, and C. Servheen. In press. Grizzly bear recovery efforts in the Cabinet/Yaak ecosystem. *Int. Conf. Bear. Res. And Manage.*

Kehoe, N. M. 1995. Grizzly bear distribution in the North Fork of the Flathead River valley: a test of the linkage zone prediction model. M.S. thesis, Univ. Mont., Missoula. 55pp.

Knick, S.T. and W. Kasworm. 1989. Shooting mortality in small populations of grizzly bears. *Wildl. Soc. Bull.* 17:11-15.

McLellan, B.N. 1989a. Dynamics of a grizzly bear population during a period of industrial resource extraction. I. Density and age-sex composition. *Can. J. Zool.* 67:1856-1860.

McLellan, B.N. 1989b. Dynamics of a grizzly bear population during a period of industrial resource extraction. II. Mortality rates and causes. *Can. J. Zool.* 67:1861-1864.

McLellan, B.N. 1989c. Dynamics of a grizzly bear population during a period of industrial resource extraction. III. Natality and rate of increase. *Can. J. Zool.* 67:1865-1868.

McLellan, B.N. and F.W. Hovey. 1995. The diet of grizzly bears in the Flathead River

drainage of southeastern British Columbia. *Can. J. Zool.* 73:704-712.

McLellan, B.N. and D.M. Shackleton. 1988. Grizzly bears and resource extraction industries: effects of roads on behavior, habitat use and demography. *J. Appl. Ecol.* 25:451-460.

McLellan, B.N. and D.M. Shackleton. 1989a. Immediate reactions of grizzly bears to human activities. *Wildl. Soc. Bull.* 17:269-274.

McLellan, B.N. and D.M. Shackleton. 1989b. Grizzly bears and resource extraction industries: habitat displacement in response to seismic exploration, timber harvesting, and road maintenance. *J. Appl. Ecol.* 26:371-380.

Servheen, C., F. Hovey, B. McLellan, R. Mace, W. Wakkinen, W. Kasworm, D. Carney, T. Manley, K. Kendall, and R. Wielgus. 1994. Report of the northern ecosystems researchers on grizzly bear population trends in the North and South Forks of the Flathead and Blackfoot Indian Reservation of the NCDE; the Cabinet-Yaak ecosystem; and the Selkirk ecosystem; and future data needs to improve trend estimates. Report to the Interagency Grizzly Bear Committee. Missoula, Mont. 14pp.

Servheen, C., R. Mace, R. Harris, W. Kasworm, W. Wakkinen, B. McLellan, D. Carney, A. Sokkalla, T. Wittinger, S. Gniadek, K. Kendall, T. Manley, and J. Waller. 1996. Report on methods to determine population size and rate of change for grizzly bears at the ecosystem scale. Report to the Interagency Grizzly Bear Committee. Missoula, Mont. 22pp.

Wakkinen, W.L. and B. Allen-Johnson. 1996. Selkirk ecosystem project, December 1995-December 1996. Idaho Dept. Fish and Game. Boise. 72pp.

Wakkinen, W.L. and W.F. Kasworm. 1997. Grizzly bear and road density relationships in the Selkirk and Cabinet-Yaak recovery zones. U.S. Fish and Wildlife Service, Missoula, Mont. 28pp.

Wielgus, R.B., F.L. Bunnell, W.L. Wakkinen, and P.E. Zager. 1994. Population dynamics of Selkirk Mountain grizzly bears. *J. Wildl. Manage.* 58:266-272.

U.S. Fish and Wildlife Service. 1993. Grizzly bear recovery plan. Missoula, MT. 181pp.

FURTHER INFORMATION ON LIVESTOCK PREDATION BY GRIZZLY BEARS

All grizzly bears that die as a result of conflicts with livestock are counted as human caused mortalities and become part of the human caused mortality limit. Specifically, the recovery plan states that human caused mortality may not exceed 4 percent of the population based on the most recent 3-year sum of females with cubs in each ecosystem. Furthermore, no more than 30 percent of this 4 percent mortality limit shall be females. These mortality limits cannot be exceeded during any 2 consecutive years for recovery to be achieved. Grizzly bear mortality or removal related to livestock conflicts has always been counted as a human-caused mortality and calculated in the recovery criteria. For example, 68 records of instances of human caused mortality occurred in the Northern Continental Divide Ecosystem from 1987-92 (page 63 of the Grizzly Bear Recovery Plan, USFWS 1993). Six of these bears were livestock depredation related mortalities and applied to the 4 percent mortality limit. None of the livestock related grizzly bear mortalities were females and therefore did not apply to the female mortality limit.

By meeting the human-caused mortality criteria (including grizzly bear mortalities relating to livestock) the FWS is addressing this statutory delisting factor and measuring whether threats to the grizzly bear have been ameliorated.

Literature Cited

U.S. Fish and Wildlife Service. 1993. Grizzly bear recovery plan. Missoula, MT. 181pp.

FURTHER INFORMATION ON METHODS TO MONITOR GRIZZLY BEAR POPULATIONS

INTRODUCTION AND BACKGROUND ON THE POPULATION PARAMETERS USED IN THE 1993 GRIZZLY BEAR RECOVERY PLAN

INFORMATION ON POPULATION PARAMETERS CONSIDERED BUT NOT SELECTED IN THE 1993 GRIZZLY BEAR RECOVERY PLAN

NEW POPULATION MONITORING METHODS SINCE THE RECOVERY PLAN WAS RELEASED

A REVIEW OF NEW APPROACHES TO POPULATION MONITORING OF GRIZZLY BEAR POPULATIONS DEVELOPED SINCE THE 1993 RECOVERY PLAN WAS COMPLETED

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INTRODUCTION AND BACKGROUND ON THE POPULATION PARAMETERS USED IN THE 1993 GRIZZLY BEAR RECOVERY PLAN

The 1993 Grizzly Bear Recovery Plan proposed the use of three parameters to assess the status of a grizzly population. These three parameters were unduplicated counts of females with cubs of the year, the number of human-caused bear mortalities, both the total number and the number of those killed by humans that were females, and the distribution of family groups of grizzly bears. These three parameters were chosen in lieu of any other applicable methods available at the time the recovery plan was written in the late 1980s and early 1990s.

One of the methods identified in the 1993 Recovery Plan, sightings of a minimum

number of animals sighted each year, was suggested by McCullough (1983) after a review of the best ways to assess the status of the Yellowstone grizzly population. Knight and Eberhardt (1985) built on McCullough's suggestion and used sightings of females to project population status in the Yellowstone grizzly population. These studies were fundamental to the development of monitoring a minimum number of females with cubs in the Recovery Plan by using unduplicated sightings of only the most easily recognized and differentiated age cohort (Knight, et al. 1995), females with cubs of the year. The methods in the Recovery Plan were the best methods available to assess population status at that time and were in fact based on the recommended method proposed by McCullough (1983).

The target of occupancy by females with young is designed to demonstrate adequate distribution of the reproductive cohort within the recovery zone. Adequate distribution of family groups indicates future occupancy of these areas because grizzly bear offspring, especially female offspring, tend to occupy habitat within or near the home range of their mother after weaning. The distribution parameter is important to assure that the bears are distributed across the recovery zone. Without this parameter, the target of females with cubs could be met in a small area of the recovery zone in an area of food concentration with no bears living in other areas inside the zone.

Human-caused mortality is the third parameter monitored according to the 1993 Recovery Plan. Human-caused mortality must be monitored so it can be managed within sustainable levels. The goal of the Recovery Plan is zero human-caused mortalities, while the limit on human-caused mortalities is 4% of the minimum population size as calculated using the number of unduplicated females with cubs. The limit of 4% human-caused mortality in the 1993 Recovery Plan is based on the work of Harris (1985) who demonstrated that a 6% human-caused mortality rate resulted in a stable population. The 4% known mortality rate was used to account for an unknown, unreported rate of 33% (for every 2 bears we know are killed there is one bear that is killed that is unknown).

INFORMATION ON POPULATION PARAMETERS CONSIDERED BUT NOT SELECTED IN THE 1993 GRIZZLY BEAR RECOVERY PLAN

Although other methods of animal census such as mark-recapture methods were available in the published literature, such methods were usually applied to easily captured animals such as rodents or animals in a limited sized area where there was high probability of capturing a large proportion of the animals in a short period of time. Such mark-recapture methods could not be feasibly applied to a low density species like the grizzly bear inhabiting thousands of square miles of wilderness habitat without massive, intrusive capture and aircraft resighting efforts (S. Miller, Alaska Fish and Game Dept., pers. comm. Cited in Harris 1986) which are beyond the budgetary limits of the agencies and the social limits of the public living and recreating in grizzly habitat (see also McCullough 1983 p. 29 - Obtaining such (mark-recapture population) estimates over a sufficient period to give reliable trends would be inordinately expensive

and may require an unacceptable intrusion in the bears' lives.

The Fish and Wildlife Service, in an effort to fully explore all possible avenues to sample grizzly bear populations beyond the use of unduplicated minimum counts as suggested by McCullough (1983), contracted Richard Harris to conduct an exhaustive examination of all possible methods to monitor grizzly bear populations (Harris 1986a). The result of Harris' review showed that there was no easy or overlooked method available to census grizzly bears nor to estimate population trend. To quote Harris (1986a):

Many of the best estimators of population size require an effort and expense well beyond the capability of most government agencies to repeat year after year.

The use of direct sightings of females with cubs or any other age/sex cohort as a trend estimator was examined in detail by Harris (1986b), again funded by the U.S. Fish and Wildlife Service. The result was a statement of caution on the importance of equal sightability of the animals seen before reliable trend estimates could be made. Field experience since 1975 by all agencies yielded knowledge that grizzly bears are difficult to see in forested habitats of the ecosystems in the lower 48 states and that sightability of bears varies from year to year and even from month to month depending on climatic conditions and food availability. The scientific work available, and the experience of agencies in looking for bears was the basis for the statement in the Recovery Plan:

The 6-year average number of unduplicated females with cubs is not adequate to characterize population trend or precise population size (Knight and Blanchard 1993). Any attempt to use this parameter to indicate trends or precise population size would be an invalid use of the data. However, this number can be used to derive a minimum population number. Recovery Plan p. 20.

This statement in the Recovery Plan was a realistic and accurate reflection of the body of knowledge of grizzly bear population status assessment methods at the time the Recovery plan was written. The Fish and Wildlife Service had funded independent researchers to carefully evaluate all methods to be sure there were no other methods available before the Recovery Plan was released.

NEW POPULATION MONITORING METHODS SINCE THE RECOVERY PLAN WAS RELEASED

Since the Recovery Plan was written, many new and innovative population monitoring techniques have become available. These methods allow us to estimate the trend of the population with confidence intervals using reproductive rate and survivorship data (Eberhardt et al. 1994; Eberhardt 1995, Hovey and McLellan 1996), and to estimate total population size using sightings and resightings of females with

cubs and using ages of bears handled (Eberhardt and Knight 1996) and cumulative counts of unique individuals identified by sightings or identification with DNA on hair samples (Boyce et al. 1998). These new methods of estimating population trend and population size were not available when the Recovery Plan was written in the early 1990s and represent an advancement in scientific methods available to wildlife managers.

Table 1. A comparison of the Yellowstone Ecosystem grizzly bear population targets with the actual numbers measured since 1992 using the monitoring methods in the Recovery Plan.

Year	Actual distribution of females with young ¹	Recovery Plan target distribution of females with cubs	Actual known human-caused mortality	Recovery Plan limit on known human caused mortality ²	Years	Actual 6-year average unduplicated females with cubs seen	Target of unduplicated females from Recovery Plan
1992	15 of 18	16 of 18	4	9.8	1987-1992	19.8	15
1993	16 of 18	16 of 18	3	9.5	1988-1993	21.0	15
1994	17 of 18	16 of 18	9	8.5	1989-1994	21.2	15
1995	18 of 18	16 of 18	17	7.1	1990-1995	21.3	15
1996	18 of 18	16 of 18	9	8.9	1991-1996	22.8	15

The use of these new monitoring methods on the Yellowstone ecosystem population data allow a comparison of the accuracy and effectiveness of the Recovery Plan monitoring methods to judge the status of grizzly populations. These new methods allow estimates of population trend (Eberhardt et al. 1994; Eberhardt 1995, Hovey and McLellan 1996) and total population size (Eberhardt and Knight 1996, Boyce et al. 1998) with confidence limits. If the monitoring methods in the 1993 Recovery Plan³ are valid estimators of population status, and these targets are being met, the more precise methods available since the Recovery Plan was released should substantiate that the population is increasing and is above the numbers associated with an average of 15

¹Number of Bear Management Units occupied.

²Limit is no more than 4% of the minimum population size based on unduplicated females with cubs seen.

³The Recovery Plan targets are: a minimum number of unduplicated females with cubs observed (at least 15 on a running 6-year average); limits on human-caused mortality (no more than 4% total and no more than 30% of this total may be females, based on a minimum population estimate); and distribution of family groups (at least 16 of 18 Bear Management Units occupied).

females with cubs⁴.

Table 2. Population status indicators for the Yellowstone Ecosystem grizzly bear population from new methods developed since 1993.

Parameter calculated	Time period	Method	Result	Source
Total population size	1989-1994	Marked females and resights of marked and unmarked females	Total population = 390 107 adult females estimated in the population. 4% mortality limit = 15.6	Eberhardt and Knight 1996
Total population size	1990-1994	Distinct family sightings and resights of these distinct families and new families	Total population = 344 4% mortality limit = 13.8	Eberhardt and Knight 1996
Total population size	1994-1996	Maximum likelihood estimator	Total population = 398 109 adult females estimated in the population. 4% mortality limit = 15.9	Boyce et al. 1998
Population trend	1983-1994	Lotka equation using reproduction and survival rates	Population increasing at 5.2% per year	Eberhardt 1995
Population trend	1984-1994	Stochastic estimate of growth	Population increasing at 4.6% per year	Boyce 1995
Population trend	1986-1994	Maximum likelihood estimator	Population increasing at 7.1% per year.	Boyce et al. 1998

The Recovery Plan population targets for Yellowstone have been met for distribution of females with young since 1993 and for a minimum of 15 females with cubs seen on a running 6-year average since 1992. The mortality limit of 4% of the minimum population estimate has been exceeded since 1994. The minimum population estimate based on females with cubs seen from 1992-1996 varied from 178-244, whereas using newer methods developed since 1993, total population size estimates range from 344 to 398 (Tables 1 and 2). If the total population as derived by the new methods was used to calculate the mortality limit of 4% of the total population, as shown in the **Result** column in Table 2, then the actual mortality level would have been met in

⁴ An average a minimum of 15 females with cubs seen over 3 years would equate to 164 bears at a minimum (15 x 3 = 45; 45) .274 = 164 bears).

each year except 1995.

Of consequence to the fate of any wildlife population is the trend or trajectory of the population - is it increasing, decreasing, or stable. Here the results derived from new methods consistently show that the population in Yellowstone is increasing at approximately 4-7% per year since the late 1980s and into the 1990s (Table 2). This demonstrates that if the Recovery Plan monitoring parameters are met or are even close to being met, that the population responds by increasing in numbers at a fairly high rate for a large mammal population. This is strong evidence that the Recovery Plan population monitoring parameters are adequate and sound measures of the status of the population. The new methods are added assurance of the adequacy of the Recovery Plan approach and their results, when added to the Recovery Plan criteria, yield important support to any assessment of a grizzly population.

A REVIEW OF NEW APPROACHES TO POPULATION MONITORING OF GRIZZLY BEAR POPULATIONS DEVELOPED SINCE THE 1993 RECOVERY PLAN WAS COMPLETED

Estimating grizzly bear populations is a difficult challenge. The animals are secretive, occur at low densities, range over vast areas, and often occupy forested habitats in which they are difficult to see. A total census is virtually impossible for most bear populations, therefore requiring that an estimate be obtained of the uncertainty associated with any estimate of population size.

It is desirable to have population estimation procedures that minimize the disturbance and handling of the bears to minimize the risk of adverse influence on the bears. Below is a review of population monitoring methods and data analysis approaches for grizzly bear populations that have been developed and detailed since 1993.

I. CUMULATIVE COUNTS OF FEMALES WITH CUBS

Adult females with cubs of the year (COY) are often uniquely identifiable because the number of cubs varies from 1 to 4, and home ranges are large enough that females with COY are often geographically separated. In addition, unique features such as pelt coloration sometimes can help to identify uniquely adult females with COY.

Observations of females with COY can be accumulated from an area throughout the season, and if sampling intensity is sufficient, most females with COY may be identified.

Such a COY index has been used in Yellowstone National Park as an index of relative abundance (Knight et al. 1995). If data exist on the proportion of the population composed of adult females with COY, the counts may be used to estimate the total bear population (Eberhardt and Knight 1996).

Usually, however, some bears are missed. By plotting the number of unique families observed as a function of the cumulative number of sightings, an asymptotic function can be obtained. Visual inspection of such curves suggests the approach to an

asymptote, which is the total number of adult females with COY. Several methods exist for extrapolating the true number of females with COY (Boyce et al. 1998). Each of these methods assumes population closure, i.e., no births, deaths, emigration or immigration, although the estimates could be modified if radiotelemetry data on movements were available (see Miller et al. 1997).

Maximum Likelihood Estimator

At the beginning of the survey period the probability of any particular bear with COY being observed is $1/N$ where N is the number of adult females with COY in the population. If sightings are accumulated at random throughout the study area, the next sighting will be a different bear with a probability of $1/(N - 1)$. The probability of a bear not being seen after i observations is

$$\theta_i = [(N - 1)/N]^i$$

Conversely the probability of being sighted is $1 - \theta_i$ for each bear. By accumulating the probabilities of sightings in such a fashion, Boyce et al. (1998) derived a maximum likelihood estimator (MLE) for the number of bears with COY in an area. Simulation methods can be used to estimate confidence intervals surrounding estimates of population size.

A simulation study demonstrated that some guidelines are necessary to ensure reliable application of the method. If all bears observed are unique bears, the population estimate would be infinity. Therefore, an estimate of abundance cannot be made if there are no resightings. For the estimator to have good properties it is recommended that the number of counts constitute at least 75% of the estimated population size.

The fundamental assumption is that each individual female with COY has an equal probability of being sighted and that individuals can be uniquely identified. Underestimates of the true population might occur because some adjacent families might be indistinguishable.

Autoregressive Model

An alternative method for estimating the total number of adult females with COY is suggested by the form of the expectation of the summed count of unduplicated families on the i -th bear sighting, S_i , on the results of the $(i-1)$ th observation of female with cubs:

$$\begin{aligned} E[S_i * S_{i-1} = k] &= k(k/N) + (k + 1)(1 - k/N) \\ &= (k + 1) - k/N. \end{aligned}$$

Fitting this model simply entails using the sequential cumulative unduplicated count as the dependent variable, and last year's cumulative unduplicated count as the independent variable and then using nonlinear regression to obtain an estimate of \hat{N} . Estimates are close to those obtained using the MLE, and confidence intervals can be derived using the same simulation procedure as for the MLE (Boyce et al. 1998). The method appears to afford no particular advantage or disadvantage to the MLE. The assumptions are the same as with the MLE.

Poisson Model

Yet another asymptotic model emerges if one can make the assumption of a Poisson distribution of counts. Suppose that the probability of sighting a particular individual in a small time interval $(t, t + dt)$ is a/dt . Then the number of sightings in time $(0, T)$ will have a Poisson distribution with a mean aT . The probability of not being sighted by time T is therefore $\exp(-aT)$ and the probability of sighting is $[1 - \exp(-aT)]$. Then at time T the expected number of individuals seen for a population size N is

$$E(S_T) = N[1 - \exp(-aT)].$$

If a can be assumed to be a constant, the mean number of sightings per bear is aT . Then the cumulative number of sightings of bears is expected to be $i = NaT$. Substituting into the previous equation gives

$$S_i = N[1 - \exp(-i/N)]$$

For application to bear sightings, the underlying assumption of a constant rate of sightings certainly is not met, because the amount of search effort varies through the season. Nevertheless, the assumption of a constant rate of return while the search is underway may be reasonable, and this might explain the fact that a reasonable fit of data to this model was found by Boyce et al. (1998). Fitting the model again entails use of nonlinear regression to estimate N as \hat{N} . This estimator offers no particular advantage over the MLE but also carries the additional assumption of a Poisson distribution of counts.

Truncated Asymptotic Model

A slightly more general form of the Poisson model is a 2-parameter asymptotic model originally used to describe growth in body size (von Bertalanffy 1938). The advantage of the von Bertalanffy model is that it gives greater flexibility for curve fitting and thus often fits empirical relations more closely. The von Bertalanffy model can be adapted to define z to be a detectability coefficient, i to be the cumulative number of sightings and A to be the asymptote to give

$$S_i = A[1 - \exp(-zi)]$$

Again, nonlinear regression can be used to estimate A and z . This model requires estimation of 2 parameters, z and A , whereas the simpler MLE, autoregressive, and Poisson models only require estimation of N .

When the data on the Yellowstone counts were fit to the von Bertalanffy model, an excellent fit to the model was observed. On inspection, fitted curves of S_i versus i showed that in some years, e.g., 1986, the curve leveled off well toward the asymptote, A . In years with small i , however, the fitted curve did not level off well and appeared to project unrealistically large A . For this reason, to ensure a conservative estimate of \hat{N} equation 8 at an accumulated count of $i = 100$ bears was truncated, i.e., estimated how many unique bear families would be expected given that 100 sightings were accumulated. This method tends to give conservative estimates that vary less than with the previous 3 methods. In certain management applications such minimum estimates may be desirable.

Catch Per Unit Effort

As one gradually removes individuals from a population, we expect that the catch per unit of effort (c/f) will decline to zero, and the cumulative removals at $c/f = 0$ should represent the total population size. If the rate of decline in c/f is constant, N can be estimated even though only a fraction of the population has been removed. The same principle can be used with marked or uniquely identifiable individuals because identifying these individuals can be viewed as essentially removing them from the pool of unsighted animals. Such resighting designs have a variety of applications beyond the usual application of c/f models to fisheries harvests (Bishir and Lancia 1996).

For grizzly bears, the frequency with which new females with COY are sighted (c/f) is expected to decline as the cumulative count of unique bear observations increases. A least-squares linear regression was used to model c/f as a function of cumulative sightings of unique family groups to extrapolate the cumulative sightings of unique bears when c/f goes to zero as an estimate of N (Leslie and Davis 1939). Here each sighting was counted as a unit of effort and new bears were recorded as a catch = 1 and resighted bears as catch = 0 (Boyce et al. 1998). For point estimates, a least-squares regression procedure for catch-effort modeling appears to work about as well as maximum likelihood estimators (Bishir and Lancia 1996).

More attention to alternative removal methods might be useful because a linear regression model does not fit the data well. The general approach appears to have promise, however, because estimates are often close to those obtained from the MLE.

II. MARK-RELEASE-RESIGHT METHODS

If the number of marked bears is known, one can estimate population size from the proportion of a sample of bears that have marks (Seber 1982). The simplest applications use a single resighting period and estimates are usually based on Chapman's (1951) finite-sample modification of the Lincoln-Petersen method:

$$\hat{N} = (m_1 + 1)(n_2 + 1)/(m_2 + 1) - 1$$

here \hat{N} is the estimated population size, m_1 is the known number marked, n_2 is the number of individuals inspected for marks, and m_2 is the number of inspected individuals with marks or previously seen. Corresponding confidence intervals are easily calculated (Chapman 1951). Several different applications of the mark-release-resight (MRR) principle have been attempted on grizzly bears, and additional opportunities exist.

With any of these MRR methods, however, two crucial assumptions need to be considered. First, the population is *closed* meaning no births or death, and no emigration or immigration. Given the insularity of the Yellowstone ecosystem, movements of bears in and out of the area is not a problem, and if the study is conducted over a brief enough time interval, births and deaths might be ignored. If data are sufficient, models for open populations are available (Jolly 1965, Seber 1965), especially if facilitated by survival estimates (DeMaster et al. 1980). Data requirements are too severe for application of open-population estimators to most grizzly bear populations, except when the degree of closure is documented with radiotelemetry (Miller et al. 1997).

Another difficult assumption is that all individuals in the population must have an equal probability of being sampled. Because males usually have larger home ranges, they are often more likely to be sampled than females. If marking alters the behavior of the bears in a way that influences their likelihood of being sampled in the second sampling occasion, estimates of \hat{N} will be biased. This is particularly a problem if bears are captured using bait because their response to bait is likely to be altered by the capture experience. Individual heterogeneity is pronounced in grizzly bears, so careful attention needs to be given to possible consequences for MRR estimates.

Resightings of Females with Cubs

Sightings of uniquely identifiable adult females with COY can be cast as an MRR problem. Eberhardt and Knight (1996) used the distinct families observed on or before 15 July as a sample of marked individuals and then recorded the proportion of these that appeared in the sample of females with COY observed after 15 July for 1990-1994. Because only about one third of the adult females are breeding in any particular year, the estimated number of females with COY was multiplied times 3 to estimate the

number of adult females in the population. This number was divided by 0.274 which is the proportion of the population that is adult females. This yielded an estimate of the number of bears equal to 339.

The estimate of 339 grizzly bears in the Yellowstone ecosystem by Eberhardt and Knight (1996) is remarkably close to an estimate of 342 bears given by the cumulative-counts MLE over the same years. Similar estimates should be expected because one can interpret the MLE as an MRR method. When a female with COY is first seen, it is marked and resightings are recaptures of sample size 1. Samuel (1969) and Darroch (1958) gave the same model as Boyce et al. (1998) MLE in the context of an MRR experiment with individuals sampled one-by-one. Thus, the cumulative-counts problem converges structurally to a generalized MRR problem. Both approaches yield asymmetrical uncertainty surrounding the estimates as illustrated in Eberhardt and Knight (1996:Fig. 1).

Radiotelemetry Marking

When a substantial number of bears are equipped with radiocollars, aerial surveys can be used to obtain MRR estimates of population size. Searches for bears are made from an aircraft and after a bear is sighted, a radioscanner is used to determine whether or not the bear is wearing an active radiotransmitter (Miller et al. 1987, 1997). The method has been used successfully in Alaska (Miller 1990) and works best in open habitats where bears are more easily seen. One advantage of the method is that violations of the population closure assumption can be accommodated rigorously because detailed data regarding movement of animals in and out of the study area is available from radiotelemetry locations (Miller et al. 1997). Unmarked bears were captured and equipped with radios, thus repeated capture-resighting data could be used for a Schnabel-type estimate (White et al. 1982). However, to secure sufficient sample size for reasonable precision, a substantial number of radiotransmitters must be monitored making the method exceedingly expensive.

The use of radiotelemetry for MRR estimation could be attempted in the Yellowstone ecosystem. Because a number of bears are observed on moth-foraging sites in some summers, a sufficient sample size might be possible to obtain reasonable estimates. So long as the radiocollaring is done representatively throughout the ecosystem, the fact that the moth-site bears are a subsample of the population should not bias estimates of total population.

Resighting with Cameras

Self-activated cameras can be used to obtain recaptures on marked bears (Mace et al. 1994). Bears 2 yrs on the South Fork of the Flathead River in Montana were captured using snares and equipped with radiocollars, and ear tags with colored streamers. Different color combinations for ear tags permitted individual identification of bears. Bears were then attracted to bait and scent stations where they triggered

automatic cameras. Photographs were used to identify individuals as recaptures. The sample unit was a bear independent of its mother. Standard MRR models were used to estimate population size.

The assumption of population closure was verified in the South Fork study by radiotelemetry. Results indicated that males were most likely to be resighted at the camera stations and females with cubs were least likely to be photographed. The success of the stations at recording bears was dependent on food availability. When food was in scarce supply, bears were more likely to be attracted to bait. In general, capture rates were highest in spring. Mace et al. (1994) recommend reverse sampling where sampling is continued until a desired number of marked bears are sighted. Because of the unequal catchability and sightability of different bears, Mace et al. (1994) suggest using a variety of capture methods and baits to reduce bias.

Biomarkers

Baits containing tetracycline have been used to mark large samples of black bears in the Midwest (Garshelis and Visser 1997). Because tetracycline fluoresces in bone and teeth, individuals eating the bait can be detected in thin sections of teeth or ribs taken from hunter-killed bears. The proportion of bears with fluorescent marks in bones and teeth out of a total sample of bears examined was used for a Lincoln-Petersen estimate of population size.

Baits were distributed widely with 1 bait/23-65 km² to reduce the likelihood that more than one bait would be eaten by an individual bear. The number of bears marked was equated with baits consumed, adjusted for the frequency of marking some individuals in more than one year as indicated by multiple fluorescent annuli in teeth. Accumulating samples over time appeared to reduce bias in population estimates. The technique permits bears to be marked over a large area at relatively little expense.

Application of the biomarker technique to grizzly bears should be possible, except that the sample sizes will not be nearly as large as in the Garshelis and Visser (1997) study. To obtain reasonable confidence intervals surrounding population estimates, Garshelis and Visser (1997) recommend marking at least 200 bears. Returns from hunters will not be available, but because most of the grizzly bears in the Yellowstone ecosystem eventually dies to human-related causes (Boyce 1995), samples of teeth and bones from a substantial fraction of bear deaths still may be possible. The most difficult problem might be distinguishing baits taken by grizzly bears from those taken by black bears (Garshelis pers. commun.). If tetracycline bait stations were set in conjunction with hair-sampling corrals, identification of species could be confirmed. This would also offer a cross reference to population estimates obtained from the analysis of DNA microsatellites from the hair samples.

III. DNA FROM HAIR SAMPLES

Microsatellite DNA can be used to identify individual bears from samples of hair (Paetkau and Strobeck 1994). By making barbed-wire corrals surrounding bait stations, hair samples can be collected for later analysis. The MLE for cumulative counts can be adapted directly for estimating population size based on the accumulation of unique bears at hair traps (Boyce et al. 1998). The method has the advantage that animals do not need to be handled and barbed-wire corrals can be constructed economically. Costs of conducting the DNA analysis have decreased markedly making the procedure a viable alternative for population estimation.

A potential problem is that bears attracted using baits are likely to return to the same site to seek out baits placed at the station at a later date. This would tend to underestimate the true population size. Perhaps hair corrals could be dismantled and moved to different locations once a bear's hair had shown up at a site. This might reduce the possibility of habituation to a site. Yet, individual bears might be prone to attending bait stations.

Each individual in the population is expected to have the same probability of being sampled. This suggests an extensive hair-trapping sampling effort throughout the study area. As for all applications of MRR methods, population closure is a potential problem. One solution to this difficulty is to use hair-trapping corrals as trap webs to estimate density instead of bear population size (Anderson et al. 1983, Parmenter et al. 1989). Trapping webs combine MRR estimates with distance sampling to obtain density estimates.

COMPARISONS AND EVALUATION OF MONITORING METHODS

Population estimates based on several of the methods are similar, in part because the principles underlying the methods converge. For example, the cumulative counts model can be recast as an MRR problem. Further confirmation of the estimators is afforded by the similarity in population growth rate estimated by various estimators and those obtained by a demographic analysis of survival and fecundity. Based on demographic data compiled by the IGBST over the period 1983-1994, Eberhardt (1995) estimated a finite growth of $\lambda = 1.053$. For comparison, the geometric mean λ was calculated over the period 1986-1994 using each of the methods reviewed under the section on cumulative counts, finding:

<u>Estimator</u>	<u>λ</u>
Maximum Likelihood Estimate	1.074
Autoregressive model	1.068
Poisson model	1.088
von Bertalanffy model @100 sightings	1.054
Leslie's catch-effort method	1.064

Thus, growth rates from cumulative count estimators compare favorably with those obtained from demographic estimates. In fact, the conservative truncated

asymptotic model gives an estimate of λ within 1/10th of 1 percent.

Rigorous tests of population estimators for grizzly bears are difficult to obtain because we do not know exactly how many bears occur in an area so verification is not possible. Statistical properties based on simulations of the MLE using females with COY appear to be reasonable so long as about 75% or more of the population can be counted. This means that the effort put into counting bears must increase as the population size increases. Reliable estimates using other methods also require a substantial sampling effort. Using multiple methods seems reasonable to provide confirmation. If wildly disparate results are obtained from different methods, further research should be conducted to uncover the reason for such disparity.

It should be noted with caution that attempts to use total counts will almost always underestimate the number of bears. Craighead et al. (1973) reported estimates using Lincoln-Petersen and Schnabel methods that were comparable to their direct counts of bears, but they failed to provide details on their sampling protocol and apparently assumptions were ignored (Mace et al. 1994). Subsequent studies with a variety of methods have repeatedly demonstrated that some form of an estimator is necessary to account for bears missed during censuses. The technology exists to obtain reasonably precise and accurate estimates of bear numbers, but obtaining reliable estimates of the number of grizzly bears is always costly.

SUMMARY OF THE BIOLOGICAL VALIDITY OF THE POPULATION MONITORING METHODS USED IN THE 1993 RECOVERY PLAN

Methods to monitor grizzly bear populations have increased in variety and approach since the Recovery Plan was written in the late 1980s and revised in the early 1990s. The application of newer methods to the Yellowstone data clearly show that the monitoring parameters and target values for these monitoring parameters used in the Recovery Plan are accurate in assessing the status of a grizzly bear population. The Recovery Plan monitoring parameters have been met or are very close to being met since 1992. The newer monitoring methods show that during the same period the population was increasing. The use of the new methods confirms that the Recovery Plan monitoring methods are conservative and provide an accurate and sound way to monitor the status of a grizzly bear population.

Thus, after reconsideration of the population monitoring and recovery criteria in the Recovery Plan, the Service concludes that these criteria are adequate objective and measurable criteria. The selection of the population monitoring and recovery criteria in the Recovery Plan were based on logical interpretations of data and published information. These methods were the best available at the time the Plan was written, and their use was reasonable and biologically sound. The methods continue to be reasonable and biologically sound, and they provide a sound basis to judge the status of a grizzly bear population.

LITERATURE CITED

- Anderson, D. R., K. P. Burnham, G. C. White, and D. L. Otis. 1983. Density estimation of small-mammal populations using a trapping web and distance sampling methods. *Ecology* 64:674-680.
- Boyce, M.S. 1995. Population viability for grizzly bears: A critical review. A report to the Interagency Grizzly Bear Committee, Missoula, Montana. 79 pp.
- Boyce, M. S., B. F. J. Manly, S. Cherry, R. R. Knight, and C. W. Servheen. 1998. Cumulative counts of unique individuals for estimating population size. *J. Wildl. Manage.* 62:
- Bishir, J., and R. A. Lancia. 1996. On catch-effort methods of estimating animal abundance. *Biometrics* 52:1457-1466.
- Craighead, J. J., J. R. Varney, and F. C. Craighead. 1973. Computer analysis of the Yellowstone grizzly bear population. *Mont. Coop. Wildl. Res. Unit, U. Mont., Missoula.* 146pp.
- Darroch, J. N. 1958. The multi-recapture census. I. Estimation of a close population. *Biometrika* 45:343-359.
- DeMaster, D. P., M. C. S. Kingsley, and I. Stirling. 1980. A multiple mark and recapture estimate applied to polar bears. *Can. J. Zool.* 5:633-638.
- Eberhardt, L. L. 1995. Population trend estimates from reproductive and survival data. Pages 13-19 *in* R. R. Knight and B. M. Blanchard. *Yellowstone grizzly bear investigations, 1994.* National Biological Service, Bozeman, Mont.
- Eberhardt, L. L., and R. R. Knight. 1996. How many grizzlies in Yellowstone? *J. Wildl. Manage.* 60:416-421.
- Eberhardt, L. L., R. R. Knight, and B. M. Blanchard. 1986. Monitoring grizzly bear population trends. *J. Wildl. Manage.* 50:613-618.
- Eberhardt, L. L., B. M. Blanchard, and R. R. Knight. 1994. Population trend of the Yellowstone grizzly bear as estimated from reproductive and survival rates. *Can. J. Zool.* 72:360-363.
- Garshelis, D. L., and L. G. Visser. 1997. Enumerating megapopulations of wild bears with an ingested biomarker. *J. Wildl. Manage.* 61:466-480.
- Harris, R. B. 1986a. Grizzly bear population monitoring: current options and considerations. *Mont. For. Conserv. Exp. Sta., Univ. Montana, Missoula, Misc. Publ.* 45. 84pp.
- Harris, R. B. 1986b. Reliability of trend lines as obtained from variable counts. *J. Wildl. Manage.* 50:165-171.
- Harting, A. L. 1987. Methods for estimation population size and trends. Pages 103-109 *in* C. W. Servheen, ed. *Grizzly bear compendium.* National Wildlife Federation, Washington, D.C.
- Hovey, F. W., and B. N. McLellan. 1996. Estimating growth of grizzly bears from the Flathead River drainage using computer simulations of reproduction and survival rates. *Can. J. Zool.* 74: 1409-1416.
- Jolly, G. M. 1965. Explicit estimates from capture-recapture data with both death and

- immigration-stochastic models. *Biometrika* 52:225-247.
- Klein, D. R. 1959. Track differentiation for censusing bear populations. *J. Wildl. Manage.* 23:361-363.
- Knight, R. R., and L. E. Eberhardt. 1985. Population dynamics of Yellowstone grizzly bears. *Ecology* 66:323-334.
- Knight, R.R., B. M. Blanchard, and L. L. Eberhardt. 1995. Appraising status of the Yellowstone grizzly bear population by counting females with cubs-of-the-year. *Wildl. Soc. Bull.* 23:245-248.
- Mace, R. D., S. Minta, T. Manley, and K. Aune. 1994. Estimating grizzly bear population size using camera sightings. *Wildl. Soc. Bull.* 22:74-83.
- McCullough, D. R. 1983. The Craighead's data on Yellowstone grizzly bear populations: relevance to current research and management. *Int. Conf. Bear Res. And Manage.* 6: 21-32.
- Miller, S. D. 1990. Detection of differences in brown bear density and population composition caused by hunting. *Int. Conf. Bear Res. and Manage.* 8:393-404.
- Miller, S. D., E. F. Becker, and W. B. Ballard. 1987. Black and brown bear density estimates using modified capture-recapture techniques in Alaska. *Int. Conf. Bear Res. Manage.* 7:23-35.
- Miller, S. D., G. C. White, R. A. Sellers, H. V. Reynolds, J. W. Schoen, K. Titus, V. G. Barnes, Jr., R. B. Smith, R. R. Nelson, W. B. Ballard, and C. C. Schwartz. 1997. Brown and black bear density estimation in Alaska using radiotelemetry and replicated mark-resight techniques. *Wildl. Monogr.* 133. 55pp.
- Parmenter, R. R., J. A. MacMahon, and D. R. Anderson. 1989. Animal density estimation using a trapping web design: field validation experiments. *Ecology* 70:169-179.
- Paetkau, D., and C. Strobeck. 1994. Microsatellite analysis of genetic variation in black bear populations. *Mol. Ecol.* 3:489-495.
- Roth, H. U. 1983. Diel activity of a remnant population of European brown bears. *Int. Conf. Bear Res. and Manage.* 5:223-229.
- Samuel, E. 1969. Comparison of sequential rules for estimation of the size of a population. *Biometrics* 25:517-527.
- Seber, G. A. F. 1965. A note on the multiple-recapture census. *Biometrika* 52:249-259.
- Seber, G. A. F. 1982. *The estimation of animal abundance.* Second ed. Charles Griffin Co., Ltd., London, U.K. 654pp.
- Valkenburg, P. 1976. A study of the brown bear (*Ursus arctos*) in the proposed northeastern addition to Mount McKinley National Park. M. S. Thesis, Univ. Alaska, Fairbanks. 86pp.
- White, G. C., D. R. Anderson, K. P. Burnham, and D. L. Otis. 1982. Capture-recapture and removal methods for sampling closed populations. Los Alamos National Laboratory, LA-8787-NERP, Los Alamos, NM.

FURTHER INFORMATION ON ADEQUATE REGULATORY MECHANISMS

One of the five criteria for delisting or removal from protected status under the Endangered Species Act requires the Fish and Wildlife Service to determine that existing regulatory mechanisms, including state laws, provide adequate regulatory protection so that a species can maintain recovered population levels. The Fish and Wildlife Service has to determine that regulatory mechanisms, in existence at the time of delisting, are adequate for the Service to have reasonable assurance that a recovered population and its habitat or range will be maintained after delisting. This determination should be based on the sufficiency of the mechanisms to adequately deal with future threats to habitat or range, and to a recovered population.

PROPOSED SUPPLEMENT TO THE GRIZZLY BEAR RECOVERY PLAN CONCERNING ADEQUATE REGULATORY MECHANISMS

Part 3, Recovery (begins on p. 33)

Replace (2) on p. 34 with the following recovery criteria:

(2) Demonstrate the existence of adequate regulatory mechanisms for population and habitat management.

(a) For the YGBE, regulatory mechanisms of the FWS, NPS, USFS, BLM and the States of Wyoming, Montana, and Idaho must be adequate for FWS to be reasonably certain that the circumstances exist for it to determine that the delisting criteria at 50 CFR 424.11 (c)(1),(2),(3), and (5) will be maintained after the population is delisted.

(b) For the NCDE, regulatory mechanisms of the FWS, NPS, USFS, BLM and the State of Montana must be adequate for FWS to be reasonably certain that the circumstances exist for it to determine that the delisting criteria at 50 CFR 424.11 (c)(1),(2),(3), and (5) will be maintained after the population is delisted.

(c) For the Cabinet-Yaak, regulatory mechanisms of the FWS, USFS, and the States of Montana and Idaho must be adequate for FWS to be reasonably certain that the circumstances exist for it to determine that the delisting criteria at 50 CFR 424.11 (c)(1),(2),(3), and (5) will be maintained after the population is delisted.

(d) For the Selkirks, regulatory mechanisms of the FWS, USFS, and the States of Idaho and Washington must be adequate for FWS to be reasonably certain that the circumstances exist for it to determine that the delisting criteria at 50 CFR

424.11 (c)(1),(2),(3), and (5) will be maintained after the population is delisted.

(e) For the North Cascades, regulatory mechanisms of the FWS, NPS, USFS, BLM and the State of Washington must be adequate for FWS to be reasonably certain that the circumstances exist for it to determine that the delisting criteria at 50 CFR 424.11 (c)(1),(2),(3), and (5) will be maintained after the population is delisted.

(f) For the Bitterroot, regulatory mechanisms of the FWS, USFS, and the States of Idaho and Montana must be adequate for FWS to be reasonably certain that the circumstances which exist for it to determine that the delisting criteria at 50 CFR 424.11 (c)(1),(2),(3), and (5) will be maintained after the population is delisted.