

2022 Status of Bighorn Sheep Herds that Utilize Yosemite National Park Report to Yosemite National Park

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This report presents data on Sierra Nevada bighorn sheep (SNBS) herds that use habitat within Yosemite National Park (YNP). SNBS are a unique subspecies of bighorn sheep (*Ovis canadensis sierra*) that are classified as endangered at state and federal levels (U. S. Fish and Wildlife Service 2007). They were first re-established in the YNP region in 1986 via a translocation of native SNBS from the southern Sierra to Lee Vining Canyon to initiate the Mount Warren herd. In the early fall of 1986, three of the ewes released that year and two lambs born to them in Lee Vining Canyon moved south to initiate the Mount Gibbs herd. In the spring of 2015, the Cathedral Range herd was initiated with the release of ten ewes and three rams near Washburn Lake in Yosemite National Park. Those three herd units constitute the current populations within the northern recovery unit (NRU) – one of 4 recovery units identified in the recovery plan for SNBS (U. S. Fish and Wildlife Service 2007). In the spring of 2015 the Mount Gibbs herd was expanded geographically with the release of 5 ewes in the Alger Lakes basin which created a ewe group that has remained a geographically separate unit (deme) within the Mount Gibbs herd, the population dynamics of which have been tracked separately from the rest of the Mount Gibbs herd.

Habitat utilized by females in the Cathedral Range herd lies entirely within YNP, but genetically it is not isolated because some rams from the Mount Gibbs herd migrate to the Cathedral Range during the fall breeding season. The Mount Gibbs and Mount Warren herd units straddle the crest of the Sierra Nevada and occupy a considerable amount of habitat on lands managed by the U.S. Forest Service (Inyo and Toiyabe National Forests) east of the eastern boundary of YNP. Details of the history of these populations can be found in a previous report (Sierra Nevada Bighorn Sheep Foundation 2020). This new report presents the most recent data on the bighorn sheep herds in the NRU and interprets that information relative to recent issues concerning those herds. Included are both demographic and genetic data.

METHODS

Population Data

This work represents a cooperative effort involving the Sierra Nevada Bighorn Sheep Recovery Program in the California Department of Fish and Wildlife (CDFW), YNP, and the Sierra Nevada Bighorn Sheep Foundation (SNBSF). An important component of the CDFW recovery program is the capture and collaring of SNBS with telemetry collars. Those collars have greatly facilitated the development of demographic data for each herd unit. Demographic data have consisted of direct counts with the goal of finding and counting every sheep in each herd unit every summer. The resulting data have been broken into five sex and age categories: adult ewes, yearling ewes, lambs, yearling rams, and adult rams. In some cases rams have been further

classified by age on the basis of horn size and visible horn rings. The count data reported here are derived from field efforts that vary from multiple investigators working simultaneously in collaboration and in radio contact to individual biologists working alone. Collaborative group counts sometimes have failed to find every sheep in the population in question. Follow-up investigations carried out by fewer investigators frequently have been necessary.

All counts have been conducted so as to assure that no double counting of individuals has occurred. The completeness of counts has been evaluated on the basis of collared sheep seen and comparison with results from the previous year, coupled with known mortality events. While technically every count has been a minimum count, there have been concerted efforts to find every sheep in these herd units every year. Over the decades of such counts in the NRU, there have been occasional incomplete counts. Multiple types of data have exposed such undercounts. One has been the genotyping from fecal samples of more different lambs than were counted, implying a nursery group not seen that contained at least the mother of the additional lamb. A second type of information has been counts a year later in which there were more animals in a sex/age class than could be accounted for based on the count the previous year and recruitment into that sex/age class. Third has been the capture in fall of a lactating ewe not known to have lamb early in summer. When undercounts have been detected they frequently have been corrected as reconstructed minimum counts.

Standard VHF telemetry collars deployed on all captured sheep have included mortality sensors which have served importantly as an aid for determining cause-specific mortality. When the collar does not move for 6 hours the pulse rate of its radio signal increased substantially as a mortality signal. Functional GPS collars also identify mortalities by simply not moving for a series of GPS locations. While survivorship rates can be calculated from collar data, the demographic information developed for the Mount Gibbs herd has been so complete that notably better survivorship estimates can be calculated from count data.

Genetic Data

The collection of genetic data on these herds began in 1998 with the Mount Gibbs herd. In that year all six sheep in that herd were genotyped for a set of microsatellite loci using DNA extracted from fecal pellets. All sheep in that herd were similarly genotyped the following year. Thereafter, a concerted effort was made to genotype every lamb in the population every year using fecal DNA. For occasional years when the number of different lamb genotypes obtained did not reach the number of lambs counted, yearlings were similarly genotyped the following year to find missing genotypes. Similar efforts began in 2000 for the Mount Warren herd with annual lamb genotyping beginning in 2002.

We developed our own laboratory method of fecal DNA extraction for this study (Wehausen et al. 2004), which for many years has replaced use of the Qiagen Stool Extraction Kit with the AquaGenomics DNA extraction method. When bighorn sheep have been captured for collaring or for releases in these herds, blood samples have been used as sources of DNA, using Qiagen blood and tissue DNA extraction kits. Those captured sheep also were aged to the extent possible based on tooth replacement and horn rings, providing an estimated year of birth. DNA also has been extracted using tissue from dead sheep where appropriate.

Over time a standard set of 18 microsatellite loci was developed to genotype bighorn sheep, of which 17 are variable, and thus informative, for SNBS (Table 1). Following the optimization of fecal DNA methods (Wehausen et al. 2004), two independent polymerase chain reactions (PCRs) were run for each sample, including DNA from blood samples. When there was a question about the correct genotype for a sample, another two replicates have been run in attempts to assure correct genotypes. Additional replicates have been run for occasional difficult samples, sometimes including a second DNA extraction.

Forward primers have included fluorescent dye labels and microsatellite PCR products were run on an ABI 377 DNA sequencer using tamra 350 size standards (Applied Biosystems). The full set of loci could be run in two lanes with different loci in adjacent lanes such that one lane could not influence the results of loci in adjacent lanes. For some loci, lengths of alleles were increased by adding tails to reverse primers to optimize spacing between loci.

Genes come in multiple forms called alleles, which arise from mutations. A locus is some segment of DNA that is sampled and can provide potentially useful information if it includes variation. Microsatellites are clearly defined loci. A locus is heterozygous for a sample if it has two different alleles and homozygous if it has two copies of the same allele. Data for numerous loci can be compiled as the proportion of the loci that are heterozygous for a DNA sample. This is a measure of genetic diversity we use that can be compared across individuals and among populations. In this study heterozygosity of lambs has been tracked over time and compared between populations. Focusing on lambs has the advantage that they are the forefront of change in genetic population structure.

Sierra bighorn barely survived what were presumably repeated die-offs from respiratory disease epizootics. These would have been initiated by microbial spillover events involving widespread intensive domestic sheep grazing in the Sierra Nevada beginning in the second half of the 19th century. Sierra bighorn survived in only 3 herd units in the Owens Valley region, but not without genetic scars documented as reduced genetic diversity (U.S Fish and Wildlife Service 2007) and a heterozygosity-fitness correlation (Johnson et al. 2008). This makes them more vulnerable to genetic issues. In combination with small population concerns, this elevates inbreeding as threat to be avoided. Currently this is an issue in the Mount Warren herd. To the standard 17 microsatellite loci discussed above, another 32 have been run for the Mount Warren herd to determine parents of lambs born mostly in 2021 resulting from breeding the previous fall. That was the first breeding season following the augmentation of that population with 6 ewes translocated from the Wheeler Ridge herd. These additional loci were run in 4 lanes from 5 multiplex PCRs.

In this report we present findings of analyses of gender and parentage of recent lambs in the Mount Warren herd. Gender was determined via the amelogenin gene using the primers SE47 and SE48 (Enis and Gallagher 1994). Parentage was determined using the software GIMLET (Valière 2002)

RESULTS

Population Data

Mount Warren Herd

In 2019 this herd had declined to only 4 sheep: 1 ewe and 3 rams. In March of 2020 this herd was augmented with the release of 4 adult and 2 yearling ewes from the Wheeler Ridge herd. In the summer of 2020 there were 7 ewes, 5 lambs and 3 rams. Four of those lambs arrived *in utero* with the 6 translocated ewes, while the fifth lamb belonged to the one surviving ewe in this herd. All adult bighorn in this herd survived to the summer of 2021 as did the 5 lambs, all of which were female. Thus, largely as a result of the 2020 translocation, in a little more than a year the reproductive base of the Mount Warren herd grew from 1 to 12 ewes.

In the summer of 2021 the 7 adult ewes were accompanied by 7 lambs, putting the total number of sheep in this herd potentially at 22 if all 3 adult rams were still alive. In late October of 2021 an adult ewe died in Lundy Canyon in an unusual early season snow avalanche and a mountain lion killed one of the yearling ewes in Lundy canyon early in 2022, thus decreasing the number of ewes to 10. Data developed in late June and early July of 2022 documented that all of the remaining sheep had survived. There were 4 2-year old ewes, 7 yearlings (4 male, 3 female), and 6 older adult ewes, but they were accompanied by only 2 lambs, each belonging to older, collared ewes. This was the second year in a row in which all lambs survived to become yearlings. With the addition of 3 yearling ewes the reproductive base grew to 13 ewes (Table 1). During a capture episode in late October this year two of the 2-year old ewes were captured. One of those was lactating, indicating the existence of at least one additional, late-born lamb.

In the summer of 2022 these sheep were accompanied by the youngest of the 3 adult rams last documented in 2020, a 4-year old ram that was the last lamb born in the population prior to augmentation in 2020. It is very unusual for an adult ram to accompany ewes in summer. This behavior may reflect the loss of the 2 older rams. This further highlights a concern for a potential situation of inbreeding in this little herd, given the large distance between it and the closest neighboring herd at Mount Gibbs.

Table 1. Summer 2022 population data for the bighorn sheep herds in the NRU. Sheep categories are adult female (AdF), yearling female (YF), lamb (L), adult male (AdM), and yearling male (YM).

Herd	Deme	Total	AdF	YF	L	AdM	YM	Other
Warren		20	10	3	2	1	4	
Cathedral		12	5		5	1	1	
Gibbs	Gibbs/Lewis	23	11	2	6		4	
Gibbs	Alger	11	6	1	3	1		
Gibbs	All ¹	53	17	3	9	19 ²	5 ³	
NRU Totals		85	32	6	16	21	10	

¹ includes all rams for the herd unit, including 1 associated with Alger ewes, but an incomplete ram count.

² includes 2 collared rams not seen.

³ includes one yearling ram associated with adult rams.

Mount Gibbs Herd

Mount Gibbs/Mount Lewis Deme

This year was particularly challenging for counting this herd because of behavioral changes. The ewes that utilize Mount Gibbs and Mount Lewis typically split into 2 separate nursery groups for the first couple of months in summer, one of which lives on Mount Lewis and one on Mount Gibbs and part of Mount Dana. Knowledge of their preferred habitat patches has made it possible to develop reliable early summer counts in past years with enough field effort. For numerous years the nursery group from Mount Lewis has been documented to join those on Mount Gibbs in the last 10 days of August, providing another good opportunity for making reliable population counts.

In 2022 the Mount Lewis ewes used their usual habitat and were readily found in early summer, in part because of a couple of functional telemetry collars on adult ewes. That group was classified as 7 adult ewes, 1 yearling ewe, 5 lambs, and 2 yearling rams, but yearling ewe classification was uncertain. In contrast, the Mount Gibbs ewe group had no surviving collared sheep and did not utilize their usual favored habitat patches. Those patches were visited multiple times in summer. In early July there was a notable lack of evidence of use. Later in July there were fecal pellets from perhaps 1-3 sheep that had visited a favorite patch once. It was not until August 27 that this group was observed. However, its size and composition relative to that of the 2021 Mount Gibbs nursery group, coupled with other information, indicated that it very likely included a couple of uncollared ewes and 1-2 lambs that recently had moved there from Mount Lewis.

On September 9 a count was made on the south side of Mount Gibbs that clearly included more of the Mount Lewis ewes (including the 2 collared ewes). That count logged 13 total ewes, of which 2 were classified as yearlings under difficult classification conditions that included smoke, heat distortion, wind and distance. With them were 6 lambs, which was 1 more than recorded on Mount Lewis in early July. However, that count lacked 3 of 4 yearling rams seen later in July with some of the Mount Lewis ewes. Those 4 yearling rams were seen many times in summer and fall, but always with adult ewes. This suggests the possibility that the count on September 9 may not have included all ewes. Considerable later field work never recorded 2 yearling ewes, but also never again found all of the ewes seen on September 9.

Two 2-year old ewes and a 4-year old ewe captured for collaring in late October had horn characteristics that could cause them to be misclassified at yearlings, and one of the 2-year old ewes also had a shorter face typically associated with yearlings. Intensive field work in early fall twice documented 19 sheep consisting of 9 total ewes, 6 lambs, and 4 yearling rams. Based on the September 9 count, another group of ewes clearly existed; but, similar to early summer for the Mount Gibbs ewes, they could not be found despite considerable field effort. There appears to have been a significant change in habitat use patterns by ewes that previously were centered on Mount Gibbs in early summer. Future reliable population counts may require discovering the new area that those ewes are utilizing.

There is sufficient remaining uncertainty about the total size and composition of the Mount Gibbs deme to preclude using the data to calculate reliable ewe and lamb survivorship rates that have been possible in most past years. A very reliable count in 2021 logged 16 total ewes (9

adults and 7 yearlings). Two adult ewes died in the fall of 2021 constituting 12.5% mortality. One was due to an early snow avalanche and the other was from mountain lion predation. Those losses left 14 ewes in this deme. If all of the ewes observed on September 9 were actually adults and constituted all of the ewes, the total annual loss would be 3 (18.75%). If an additional ewe accompanied the 3 yearling rams not seen on September, the total loss would be only 12.5%. However, if one or both of the ewes classified as yearlings on September 9 were actually yearlings and those sheep constituted all surviving ewes, the ewe survivorship could be as low as 68.75% which would be essentially the same as the low survivorship the previous year and similar to low recorded survivorships for 2018 and 2019. In short, ewe survivorship potentially lies between 68.75% and 87.5%, but it is not clear where it is in that range. This is unfortunate because it would be useful to know if the recent pattern of low ewe survivorship in this deme is continuing or abating.

Survivorship of the 7 lambs from 2021 is similarly affected by yearling classification questions. If there were 2 yearling ewes in 2022, then 6 lambs survived constituting 86.7% survivorship. That drops to 57.1% if there are no yearling ewes. This situation highlights the importance of good classification data for this deme and the need for accurate yearling classification to be able to calculate ewe and lamb survivorship – something that is possible for small populations of bighorn sheep with complete counts. In contrast, the alternative of using collared sheep for survivorship calculations yields mostly crude results in small populations because the number of collars is small unless a high proportion of the ewes are collared. The Mount Lewis deme lost 50% of its collared ewes between the summers of 2021 and 2022 summers, but the actual survivorship was not that low.

Alger Lakes Deme

Data developed for the Alger Lakes ewe group also suggested possible behavior that may have been influencing the ability to develop complete population data for this group. In most years this group is counted only once. In 2022 this group was found and counted three times. The first time was on August 31 when they contained 5 adult ewes and associated sheep. In a count 9 days later that had increased to 6 ewes, which was verified in the third count about a month later. The count of this group in 2021 included 6 total ewes, of which 2 were yearlings and 3 were collared. One of those collared ewes died during lamb birth this spring. Thus, the finding of 6 ewes in this population is not consistent with the data from 2021. Perhaps this group includes a ewe with an unusual behavior of sometimes living separate from the rest of the group. The same behavior may have led to an undercount in 2021 as it did in the first count in 2022. Alternatively, this group has gained an immigrant. Fecal pellet samples were collected this fall from adult sheep in this group to further explore these alternative explanations using genetic data.

The 2021 total bighorn summer minimum count for the Mount Gibbs herd came to 53 sheep (Table 1), which is 1 fewer than 2021. However, the actual total for 2022 almost certainly is higher given an incomplete count of rams and potential undercount in the Mount Gibbs ewe deme.

Cathedral Range Herd

Telemetry collars were successfully deployed in the Cathedral Range herd this spring. This greatly facilitated a good count this summer when 5 ewes, 5 lambs, a yearling ram and a young adult ram were logged (Table 1). The increasing trend for this herd is clearly continuing.

Genetic Analyses

Genetic Diversity

Long term patterns of genetic diversity based on DNA samples from lambs were presented in the 2021 annual report. It was noted, however, that the data for 2020 and 2021 were incomplete in that all lamb genotypes had not yet been obtained. Since then more samples have been run until all genotypes emerged. Figures 1 and 2 present those completed data sets which involve only small changes.

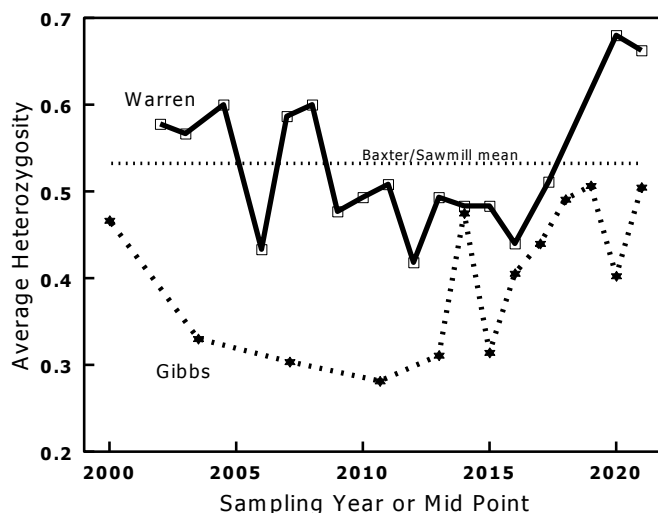


Figure 1. Average heterozygosity of lambs in the Mount Warren herd and the Mount Gibbs/Lewis deme of the Mount Gibbs herd by year or longer sampling periods based on 15 variable microsatellite loci. Longer sampling periods were used only where numbers of lambs in individual years were very low.

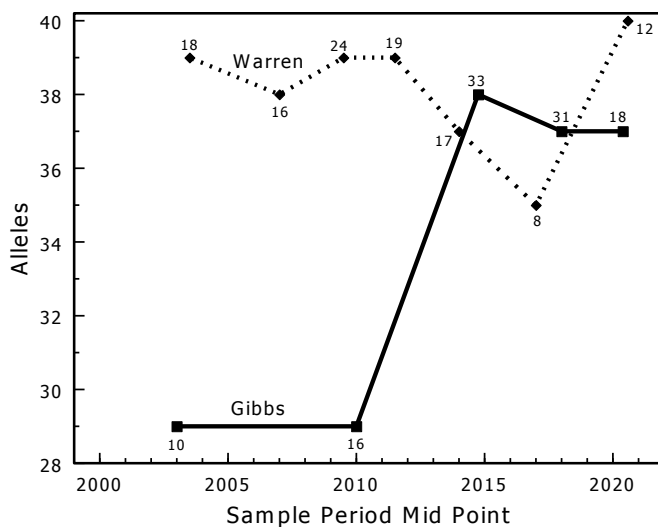


Figure 2. Numbers of alleles for 15 microsatellite loci (Table 1) represented in pooled lamb samples from 1999 to 2021 for the Mount Gibbs and Mount Lewis deme and 2002 to 2021 for the Mount Warren herd. Each pooled sample was plotted at the weighted midpoint and sample sizes (different lamb genotypes) are listed next to points.

Mount Warren Herd

Three rams were documented in the Mount Warren herd in 2019 and 2020 when fecal samples were collected from each of them. The youngest of those rams was born in 2018 and was first sampled that year as a lamb. The other two rams were notably older with one older than the other based on horn size. All 5 of the lambs in 2020 were female of which one belonged to the single surviving ewe prior to augmentation of this herd. That mother was identified genetically for that sample and the father was identified as the largest of the 3 rams, which is consistent with that ram having been observed with that ewe early in the 2019 breeding season. For that ram there is a question about his genotype for 1 of the 49 loci used for parentage analysis. When that locus was removed in the parentage analysis, that ram continued to be identified with high confidence as the father of that lamb. Both parents of 3 of the 7 lambs from 2021 were similarly identified with high confidence (no incompatibilities in the data). The largest ram was the father of 1 of those lambs and the youngest ram born in 2018 was the father of the other 2.

Mothers were readily assigned for 3 of the remaining 4 lambs from 2021 with no data incompatibilities, but fathers were not. For one lamb the analysis had to be relaxed to allow 3 incompatibilities in the data to assign a local ram as its father. The loci involved in those incompatibilities were identified and the data examined carefully. In every case the data appeared to be very reliable. This suggests that a ram from a different herd was the father. Of the remaining 3 lambs from 2021, the mother could be assigned with no data incompatibilities for 2, but no father could be assigned without allowing 1 data incompatibility. The sources of those incompatibilities also were identified, and again the data appeared very reliable. For the fourth lamb, neither a mother nor a father could be assigned for one locus; yet, the data for that locus again appeared reliable.

All loci involved in parentage assignment incompatibilities will be rerun for the samples in question, but currently it appears likely that at least some of those incompatibilities may simply reflect 1 or more outside rams involved in the breeding. That this can come down to just 1 incompatibility may reflect the influence of greatly reduced genetic diversity in SNBS in its influence on resolution in assignment of parents to lambs; the 48 variable loci used barely have enough resolution to distinguish parents for this herd that has particularly high genetic diversity for SNBS (Figures 1, 2).

Inbreeding will degrade the current high genetic diversity in the Mount Warren herd. These analyses were made in an attempt to identify the potential for inbreeding to occur in the form of father-daughter matings. Part of that effort was to determine the sexes of the 2021 lambs genetically. The result was 4 males and 3 females, which matches the sexes of 7 yearlings identified in the field this summer (Table 1). Of the 3 females, the only clear father assignment is for one whose father was the youngest of the 3 local rams – the 4-year old ram that has lived with ewes this summer and fall, and last seen in late October. For him the question is whether he might breed the yearling ewe that is his daughter in the breeding season that is currently in progress. In SNBS, about half of ewes first breed as yearlings; so the potential for a father-daughter mating this breeding season involving that ram is about 50% if he is the only ram present. The finding that at least 1 outside ram appears to have been involved in breeding ewes in this herd in 2020 lowers that percentage considerably. If the largest local ram last seen in 2020 is still alive, there is the potential for a father-daughter mating involving his 2-year old daughter. It

is not clear what the inbreeding potential is for the remaining 2 yearling ewes, given uncertainty whether their fathers are local or immigrant rams.

Probably the most important finding here is that there appears to be a source of rams from outside of this small herd involved in breeding. In the absence of an unknown nearby deme of SNBS, those rams most likely came from the Mount Gibbs herd. In recent years that herd has had a sex ratio biased to males, which may result in more rams dispersing in the fall looking for other female groups. The potential for inbreeding in the Mount Warren herd will to be pursued further via genotyping and parentage analysis for lambs born in 2022. We currently have fecal samples for the 2 lambs known in early summer. As discussed above, there exists at least one additional late-born lamb that needs to be sampled. If the single mature ram documented in this herd in 2022 is found to be dominating the breeding, future inbreeding can be avoided simply by catching that ram and translocating him to a herd further south in the Sierra Nevada. Currently, that would leave 4 young rams in this herd from different lineages (Mount Warren and Wheeler Ridge herds) to compete for breeding opportunities.

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