SNPLMA Round 16 Conservation Initiative Nomination
U.S. Fish and Wildlife Service, Nominating Agency

Title: Environmental Effects on the Immunity and Health of Desert Bighorn Sheep (*Ovis canadensis nelsoni*) in Southern Nevada

Amount Requested: $ 2,480,638.00

Cooperating Federal Agencies:

- National Park Service, Lake Mead National Recreation Area
- Bureau of Land Management, Southern Nevada District
- U.S. Forest Service, Spring Mountains National Recreation Area
- U.S. Geological Survey

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II. Purpose Statement

The U. S. Fish and Wildlife Service (FWS), U.S. Forest Service Spring Mountains National Recreation Area (SMNRA), the Bureau of Land Management (BLM), and the National Park Service (NPS), as part of the Southern Nevada Agency Partnership (SNAP) Science and Research Team, propose to investigate the pneumonia epidemic that has recently been detected in bighorn sheep populations throughout the Mojave Desert. We hope to identify management actions that can be taken to reduce the impacts of this epidemic on bighorn population in southern Nevada by studying disease incidence, genetic response, and environmental correlates in the Sheep Range, Spring Mountains, River Mountains, Mormon Mountains, and Muddy Mountains, see map in Appendix A. If conditions are such that we cannot successfully sample from one, or more of these populations (e.g., numbers are too low), we will choose an alternate population from a neighboring mountain range (alternate ranges are the McCullough Range, Eldorado Range, and Black Mountains).

The U.S. Geological Survey (USGS) conducted a Round 10 study that attributed recent declines in bighorn sheep populations to this epidemic (Survival and Habitat Requirements of Desert Bighorn Sheep of the Desert NWR, FW-67). Impacted herds in southern Nevada now include those in the Sheep Range, Spring Mountains, River Mountains, Eldorado Mountains, Mormon Mountains and the McCullough Range. Of the 11 herds tested in southern Nevada, only the population in the Muddy Mountains is currently free of infection. Both *Pastuerella species* and *Mycoplasma ovipneumoniae* are important pathogens in the epidemiology of bighorn sheep pneumonia. Infection with *M. ovipneumoniae* predisposes bighorn sheep to pneumonia caused primarily by *M. haemolytica* and *B. trehalosi* often resulting in high rates of mortality across all age classes (Besser et al. 2012). Occasionally, bighorn herds infected with *M. ovipneumoniae* experience little to no clinical disease or resulting impact on population levels or lamb recruitment (NDOW unpublished data, T. Besser personal communication).

At this time, there is no effective vaccine or treatment program, yet populations are routinely monitored to document the overall impact pneumonia has on bighorn sheep herds. The disease typically affects a population for more than a decade, but recovery is variable. Bighorn that survive become carriers, allowing the disease to persist in populations for decades as annual or sporadic pneumonia epidemics (summer pneumonia) in lambs. Recurring years of depressed recruitment due to high rates of pneumonia-induced mortality in juveniles is a major obstacle to population recovery and may explain why population numbers on the Desert NWR remain at approximately 400 animals.

Current health evaluations and diagnostics for desert bighorn provide limited information on the overall health of the animal and almost no information on the condition of its habitat. This lack of diagnostic information makes it difficult to identify specific environmental conditions and environmental stressors potentially linked to the recent increase in disease and population decline for bighorn sheep in Nevada. What do we do when classical diagnostic methods fail to explain mortality events or disease recovery? In such cases we need to use novel techniques to help untangle the conditions leading to excessive mortality. We suggest a holistic system of investigation that acts as a window into the animal's physiology as well as a mirror of the environment by uniting information from multiple scales in spatially-explicit structural equation models (path analysis) and network models (Marshall 1991; Riley 2007; Dubé et al. 2009)

incorporating individual gene expression, physiological health, diet and movement to population-level incidence of infection and sensitivity to environmental variables and future change.. Gene-based techniques have the ability to measure the physiological response of individuals to disease as well as the influence of external factors on the health of the individual. These techniques are used in human medical science, but are only recently used on the American mink, sea otter, polar bear, and the Mojave desert tortoise. Although pneumonia epidemics have been devastating in the past few decades to wild sheep, we still need to understand the effects that genetic and environmental variation have on animal immunity and infections in order to manage these populations through post-disease population recovery. We would like to answer questions such as: Are changes in the environment associated with human land-use (contaminations, non-native plants, human-caused disturbance, wildfire, climate change) making bighorn sheep populations in Nevada more susceptible to pneumonia epidemics? Do these changes affect disease recovery? Are sheep systemically stressed due to changes in food availability and composition and limited access to water? If the probability of disease transmission is higher in habitat with greater sheep density, does *M. ovipneumoniae* infection convert high quality habitat into an ecological trap such that uninfected sheep groups persist in lower quality habitat (Leach et al 2016)? Are there management actions associated with bighorn habitat that might lessen the impact of the disease in infected herds?

This project would use these newly developed diagnostics to link sheep health to ecosystem health, thus helping researchers and managers identify when and why individuals are succumbing to disease and how that relates to environmental conditions. It will improve understanding of the variation in herd response following exposure to important respiratory pathogens and identify factors that contribute to variation in herd responses. To accomplish the above, we propose the following:

1. Conduct genetic analyses and health evaluations for bighorn populations (4 infected and 1 uninfected). Develop a gene transcript panel and genotype major histocompatibility complex class II (hereafter MHC II) genes and associated particulars alleles or genotypes with disease and immunity. Determine patterns for immune function and disease in sheep sampled from the study populations.

2. Monitor habitat and environmental conditions in study populations. Identify specific conditions that potentially link physiological responses to stressors in the environment. Develop spatially explicit, hierarchical structural equation and network models incorporating individual-level correlates and predictors of health and infection with movement patterns, group size, environmental change and population-level incidence of infection.

3. Implement volunteer outreach, education, and training for 1) monitoring of lamb summer pneumonia through use of trail cameras at water sources; 2) spring lamb surveys, conducted on foot, 3) identifying, and reporting adults with signs of disease, 4) assistance with collection of habitat and environmental data.

4. Identify potential habitat management actions that might improve recovery or lessen the impact of the disease.

5. Additionally, sets of transcript values developed for the sampled bighorn sheep populations can be used as baseline data to provide an understanding of the susceptibility of populations at risk from regime shifts, such as may be seen with climate change, and to provide an early-warning indicator for populations at risk from other stressors such as human disturbance.

III. Background and Need

Bacterial pneumonia is one of the primary challenges facing conservation of bighorn sheep populations in North America. During the late 1980s, desert bighorn sheep (*Ovis canadensis nelsoni*) on the Desert National Wildlife Refuge (Desert NWR) experienced a severe population decline. Numbers dropped from 1400 to 400 individuals within a five year period. Since then, sheep numbers on the Desert NWR have remained close to 400 animals. Actual causes of the decline were unknown at the time, but it was hypothesized the initial decline was triggered by drought conditions in 1987. Telemetry data and other observations strongly indicated that mountain lion (*Puma concolor*) predation may also have been a significant cause of mortality. Disease screening was conducted on a small number of individuals (N=5) from the neighboring East Pahranagat Range in 1996, but the screening yielded no evidence of an active disease process that might be influencing population fitness. At that time, however, the population was not tested for presence of the bacteria *Mycoplasma ovipnuemoniae* which can predispose bighorn sheep to fatal pneumonia.

In 2010, a study (Survival and Habitat Requirements of Desert Bighorn Sheep of the Desert NWR, FW-67) was initiated to assess factors that may have caused the initial decline in the Desert NWR and to understand potential causes of the continued depressed population numbers. The study examined adult survivorship, levels of predation from mountain lions, measures of disease, and the influence of precipitation and population size on growth rates and lamb/ewe ratios. Results found that the strongest evidence for the initial die-off appeared to be presence of disease, although predation and years with low precipitation may have contributed synergistically to the decline. Of the 30 bighorn captured and sampled in the Desert NWR, *Mycoplasma*-species specific antibodies were detected in 21 individuals, undetected in four animals with indeterminate results for the remaining three sheep. One animal was positive for the presence of *M. ovipneumoniae* by polymerase chain reaction (PCR) on a nasal swab. All sheep were also tested for the presence of Pasteurellaceae with 16 positive for *B. trehalosi* and 28 positive for *M. haemolytica.*

Both *Pastuerella species* and *M.ovipneumoniae* are important pathogens in the epidemiology of bighorn sheep pneumonia. Infection with *M. ovipneumoniae* predisposes bighorn sheep to pneumonia caused primarily by *M. haemolytica* and *B. trehalosi* often resulting in high rates of mortality across all age classes (Besser et al. 2012). Die-off survivors may become carriers, allowing the disease to persist in populations for decades as annual or sporadic pneumonia epidemics (summer pneumonia) in lambs. Recurring years of depressed recruitment due to high rates of pneumonia-induced mortality in juveniles is a major obstacle to population recovery and may explain why population numbers on the Desert NWR remain at approximately 400 animals. Occasionally, bighorn herds infected with *M. ovipneumoniae* experience little to no clinical disease or resulting impact on population levels or lamb recruitment (NDOW unpublished data, T. Besser personal communication).

In addition to the Desert NWR, surveillance for *M. ovipneumoniae has* documented infection in bighorn sheep populations throughout the Mojave Desert including Southern Nevada, with variable population level responses. Of the 11 herds tested in southern Nevada, only the population in the Muddy Mountains is currently free of infection with *M. ovipneumoniae*. There is currently no effective preventative vaccine, treatment program or management strategy for bighorn populations infected with *M. ovipneumoniae*. Traditional approaches to understanding bighorn respiratory disease have focused mainly on defining the role that pathogens and environmental resources play in the respiratory disease complex. Health evaluations of herds have been limited to assessing gross physical health and confirming the presence or absence of pathogens known to cause clinical disease in wild sheep. Measuring environmental impacts on sheep health has stressed the evaluation of the habitat resource, specifically, abundance, availability and spatial distribution. We have no ability to determine whether desert bighorn sheep populations are experiencing sub-clinical stressors related to pathogens or environmental factors before they are manifested as clinical disease. This lack of information makes it difficult to identify the specific stressors that could potentially be linked to an individual herd's response to pathogen introduction. Wildlife are essentially a reflection of their environment and its current condition, thus wildlife health becomes an important indicator of ecological health.

Genetic associations with disease resistance have historically focused on two groups of immune genes, those in the major histocompatibility complex (MHC) and non-MHC genes (Acevedo-Whitehouse and Cunningham 2006). Studies have revealed that about half of the genetic variability for pathogen resistance can be attributed to genes in the MHC and half to non-MHC immune function genes (Jepson et al. 1997). However, the functioning of these groups of genes are not mutually exclusive and thus must both be considered when predicting the potential of natural populations in response to pathogens. Immunity or susceptibility should not be regarded as single entities, but as complex cascades of molecular and cellular events. The exact nature of the cascade is dependent on the particular pathogens involved, the host's life stage, as well as a myriad of other factors (Acevedo-Whitehouse and Cunningham 2006). Taking a broad view of immune function will enable us to test hypotheses regarding the possible role of selection in patterns of disease resistance. We have the unique ability to provide a comprehensive immune analysis of bighorn sheep populations currently affected to varying degrees by disease by examining the diversity of MHC class II and microsatellite markers near immune function genes, as well as quantifying MHC and immune function gene transcripts. We will then relate these parameters to population disease status with considerations of environmental inputs.

Immunogenetic diversity and functional immune responses. Preliminary genetic studies undertaken on bighorn sheep in the Mojave National Preserve, by Epps, Jolles, Dolan and Beechler from Oregon State University suggest a strong selective pressure at immune gene loci that confer resistance to certain bacterial infections (C. Epps, pers comm). Additionally, bighorn sheep populations with high *M. ovipneumoniae* prevalence have certain reduced functional, innate immune responses. Further, those populations which are physically (i.e. geographically) connected have higher genetic diversity, and enhanced functional innate immune responses. These preliminary findings set the stage for broader and more in depth studies of the interactions among genetics, immune response, and environmental factors, as they relate to disease-induced bighorn sheep population trajectories. Finally, a method for determining the MHC class II

genotypes of individual animals has been developed and will be used to determine if MHC diversity is related to disease resistance.

The role of gene transcription. Newly emerging laboratory techniques such as gene-based health diagnostics (i.e. gene transcription) provide an innovative opportunity to improve our understanding of health in desert bighorn by evaluating transcript levels for multiple genes that respond to environmental stressors (i.e. pathogens, trauma, contaminants, or environmental disturbances). Gene-based techniques have the ability to measure the physiological response of individuals to disease as well as the influence of external factors on the health of the individual. Gene transcription in particular, uses a quantitative polymerase chain reaction (qPCR) assay to target genes that change in response to bacteria, viral, inflammatory thermal stress, nutritional stress, environmental toxicants, and overall cellular function and metabolic conditions, helping researchers and managers to identify when and why individuals are physiologically responding to their environment. These techniques are used in human medical science, but have only recently been applied to wildlife, including the American mink (Bowen et al. 2007), sea otter (Miles et al. 2012; Bowen et al 2015a.), polar bear (Bowen et al. 2015b), and the Mojave desert tortoise (Bowen et al. 2015c). Gene transcription can be used as a predictive tool with the capability of distinguishing population disease outcomes based on transcript profiles, knowledge of physiologic processes underlying individual outliers (i.e. unique disease states), identification of successful immune strategies associated with select populations, and the relative contribution of environmental variables to the bighorn sheep population declines

Environmental stressors. One of the most important predisposing factors involved in the incidence of pneumonic pasteurellosis in sheep is stress. Both Mannheimia and Pasteurella species are capable of causing infection in animals with compromised pulmonary defense system (Besser et al. 2012). Pneumonia can be triggered by physical or physiological stress created by mycoplasma or other pathogenic organisms, previous infection with respiratory viruses, or stressful environmental conditions including periods of low forage quality and quantity, high predation or harassment, or extreme weather conditions (see review, Mohamed and Abdelsalam 2008). Other stressful situations such as a high load of internal or external parasites can also predispose animals to pneumonia outbreaks (Mohamed and Abdelsalam 2008). The reaction of animals to stress is rather variable, even among individual animals of the same species. Predisposing factors may either act alone or in combination; their significant role in the establishment of infection has repeatedly been demonstrated by experimental means in cattle (Mohhamed and Abdelsalam 2008). Additional factors that may promote suppression of the immune system and predisposition to pneumonia include parasites, such as lungworms (*Protostrongylus* spp.) or mites (*Psoroptes ovis*), inbreeding, or density dependent stress resulting from overcrowding (Risenhoover et al. 1988, Bailey 1990). In addition to the health status of individuals, habitat quality and quantity, movement patterns, environmental factors and human disturbances are all variables worthy of investigation in light of potential stress as it relates to the current pneumonia epidemic.

Habitat models based on comparisons of observed sheep locations versus available locations in the landscape can provide quantitative predictions about changes in probability of occurrence of sheep relative to a wide variety of environmental variables (Sappington et al. 2007; Longshore et al., 2013). In the proposed research we will determine how variables such as local ruggedness,

slope, aspect, elevation, cover of perennial vegetation, distance to forage areas, distance to escape terrain, and distance to water are related to locations of GPS collared sheep across an environmental gradient. We have conducted a habitat analysis for sheep on the Desert NWR and have included information how bighorn sheep habitat selection is altered by predation risk. Here we extend the analysis to determine how sheep movements contribute to social contacts, generating a social network, and how infection and disease spread within a population is statistically related to the combination of individual responses to environmental variables, including diet and predation risk, movement, and social networks (Riley 2007, Dube et al. 2009, Craft 2015)

Summary. We propose to conduct a comprehensive immune analysis of bighorn sheep populations currently affected to varying degrees by disease by examining the diversity of MHC class II and microsatellite markers near immune function genes, as well as quantify MHC and immune function gene transcripts. This information will allow us to understand how environmental conditions and stressors are linked to the recent disease outbreak and population decline for bighorn sheep in Nevada, and to identify potential management actions associated with bighorn habitat that might lessen the impact of the disease. Completion of this project will provide critical information pertaining to the protection, conservation and management of desert bighorn sheep in the Desert NWR and on federal lands throughout Nevada and the southwest. Use of this approach will provide an invaluable perspective for managing bighorn sheep under current environmental conditions and predicting consequences of future environmental change and probabilities of extinction for local populations.

Promotion of EC values: sustainability, connectivity, community

1. *Restores and protects healthy and resilient landscapes that connect important habitats and protect the integrity of the human and biological communities*;

Wildlife are essentially a reflection of their environment and its current condition, thus wildlife health becomes an important indicator of ecological health. As such, bighorn sheep are an important component of ecosystem health and maintaining viable populations of bighorn sheep is a high priority goal of resource management. Completion of this project will provide information about how environmental conditions and stressors are linked to the recent critical pneumonia outbreak and population decline for bighorn sheep in Nevada, and to identify potential management actions associated with bighorn habitat that may lessen the impact of disease. This project will provide critical information pertaining to the protection, conservation and management of desert bighorn sheep on federal lands throughout Nevada and the southwest.

2. Provide outdoor recreation opportunities that improve the quality of life for the public and encourage interaction with nature;

Bighorn sheep are a charismatic species with high public visibility. Conservation of bighorn sheep populations will ensure this species will have a continued presence on the landscape, enriching the outdoor experience of the public and fostering an appreciation for wildlife in natural areas. This project will provide outdoor educational opportunities for citizen science volunteers who assist with bighorn sheep monitoring at water sources and spring lamb surveys.

3. Incorporate durability, relevancy, and shared support to ensure benefits in the near and long term.

This project will provide vital information and advance the knowledge of desert bighorn sheep biology and disease ecology and will benefit conservation and management of regional bighorn sheep populations that inhabit lands managed by USFWS, NPS, BLM and DOE, as well as local municipalities. At the state level, the Nevada Department of Wildlife, who manages wildlife populations, will benefit through improved knowledge about the interaction of disease and environmental variables for bighorn sheep populations throughout their range, including southern Nevada. Information from this project will also further our understanding of the causes of the depressed population numbers for bighorn in the Desert NWR. This information is necessary to improve the management of federal lands for desert bighorn sheep and other wildlife. Since it will be applicable to a large geographic area, the project will reduce the overall cost in federal land management for bighorn sheep. The results will be exportable and available to disseminate to the scientific community and the public. This information is vital to improving the quality of habitat on federal lands for bighorn sheep and other wildlife species.

IV. Timeframe (years and months)

Project timeframe: 5 years, 0 months

V. Project Location

Populations of desert bighorn sheep will be sampled in all of Southern Nevada Agency Partnership agency lands in southern Nevada, we plan to focus on populations in the Sheep Range, Spring Mountains, River Mountains, Mormon Mountains, and Muddy Mountains, see map, Appendix A. If conditions are such that we cannot successfully sample from one, or more of these populations (e.g., numbers are too low), we will choose an alternate population from a neighboring mountain range (alternate ranges are the McCullough Range, Eldorado Range, and Black Mountains). The Sheep Range Lat long 36°46’00”N 115°26’00”W can be used for reference purposes. The project area is within Congressional districts NV-3 and NV-4.

VI. Project Deliverables

<u>Primary</u>

- Report that includes results of 1) overall health of bighorn sheep populations in the study, 2) results of the gene transcript panel and transcription patterns, 3) patterns for MHC immune function and disease, 4) specific conditions that potentially link physiological responses to stressors in the environment and to the recent disease outbreak and population recovery.
- Report on volunteer outreach and education activities.
- Report identifying potential management actions associated with bighorn habitat that might lessen the impact of the disease.

<u>Anticipated</u>

- Baseline data set of gene transcription patterns that can be used predicting consequences of future environmental change.

- Project results will be published in a peer reviewed journals and provided to other federal and non-federal entities within Nevada and throughout the southwest.
- Volunteers will gain a better understanding and appreciation of their natural resources and will gain field experience working as citizen scientists.

Standard

- Develop scopes of work for university contracts
- Complete Interagency Agreement.

VII. Relevant Performance Measures

SNPLMA Goal 1: Sustain the quality of the outdoor environment by conserving, preserving, and restoring natural and cultural resources detection of disease in

Outcome: **Conserve and restore natural resources -** Maintain or increase the quality of natural resources and protect their ecological integrity and sustainability

Output: Increase or improve the resource base (e.g., habitat), with a focus on landscape-level habitat restoration

Performance Measure *H15- Number of Conservation Actions Implemented for Non-Listed Species* –monitoring and maintenance of the many water sources in each of the 5 study areas. Actions will be taken as identified. This project is designed to identify habitat management actions for bighorn sheep populations exposed to *Mycoplasma ovipneumoniae;* what these actions may be is unknown. Information regarding management actions identified in our results will be provided to all land management agencies.

Output: Promote the value of science and research in increasing the effectiveness of conserving and restoring natural resources, and promoting resource sustainability

Performance Measure *O4 - Number of Scientific / Technical Reports Produced* Minimum of 3 technical reports and 5 scientific reports (scientific journal articles).

Performance Measure *O9 – Number of GIS Databases Generated and/or Map Layers Produced* - Databases:

1) Five collared populations of bighorn sheep consisting of the following map layers:
   a. GPS locations at each time period collected.
   b. Distance moved between each location.
   c. Seasonal, and study period geographic range for each animal within each population: 50% (core use) and 95% (home range) utilization distributions.
2) Habitat Use consisting of the following map layers:
   a. Terrain features: slope, ruggedness, viewshed, elevation, curvature profile, curvature planform, residual slope, residual ruggedness, and residual viewshed.

b. Vegetation type, Normalized Difference Vegetation Index (NDVI).
c. Distance to water sources.

3) Habitat Quality consisting of the following datasets, analyses, and maps:
a. Set of candidate models with biological support/hypotheses for each model.
b. AIC model selection process details
c. Analyses, performance, and predictor variable strength values and definitions for the most probable (highest-ranked model).
d. Probability of occurrence model(s) and maps.

4) Relating gene transcription to habitat use:
a. Combined dataset of habitat use and transcription parameters.
b. Analyses, performance, and predictor variable strength values and definitions.

Totals:
- 5 population databases.
- Approx. 120 layers per population.
- Approx. 200 candidate models (10 for each population).
- Approx. 15-25 (seasonal) habitat use models.
- Approx 15-25 (seasonal) habitat quality maps.

Outcome: **Promote Education** - Provide opportunities to improve the public's connection with natural and cultural resources through education

Output: Use Federal, local, State, and regional government lands as learning laboratories to help develop the next generation of environmental understanding and advocacy

Performance Measure *O5 - Number of Outreach Contacts Made* – Interagency presentations years 3, 4, and 5. Public presentations (minimum 1/ year) starting in year 2. Interagency workshop for potential management actions during year 5.

Performance Measure O6 - *Number of New Interpretive or Educational Publications/Signs/Kiosks/Displays/etc* –We will produce a minimum of 3 educational publications and displays

Performance Measure *O7 - Number of Interpretive or Education Presentations Given and/or Community Events Participated In or Hosted* – 10-12 classroom presentations/year (public schools) about bighorn sheep and their importance in the ecosystem. Minimum of 2-3 public presentations/year.

Performance Measure *O10 – Number of Volunteers Used* – We will start with a minimum of 15 volunteers and will increase numbers throughout the project as needed.

SNPLMA Goal 2: Improve the quality of life for all publics in urban and rural communities by enhancing recreational opportunities that connect people with the outdoor environment.

Outcome: **Enhance Recreational Opportunities** - Provide recreational opportunities and improve access to those opportunities on Federal, local, and regional government lands, increasing the availability and quality of public recreation

Output: Protect or improve the integrity of environmental, educational, community, recreational, and open space resources to enhance the quality of the human experience.

Performance Measure *H15- Number of Conservation Actions Implemented for Non-Listed Species* – 1) Health assessments for 20 individual sheep in 5 different mountain ranges, 2) Monitoring of water use by bighorn sheep and other species (including humans).

Performance Measure *O4 - Number of Scientific / Technical Reports Produced* Minimum of 3 technical reports and 5 scientific reports (scientific journal articles).

Performance Measure *O9 – Number of GIS Databases Generated and/or Map Layers Produced* - Totals:
- 5 population databases.
- Approx. 120 layers per population.
- Approx. 200 candidate models (10 for each population).
- Approx. 15-25 (seasonal) habitat use models.
- Approx 15-25 (seasonal) habitat quality maps.

Outcome: **Promote Education** - Provide opportunities to improve the public's connection with natural and cultural resources through education

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Performance Measure *O10 – Number of Volunteers Used* – We will start with a minimum of 15 volunteers and will increase numbers throughout the project as needed.

VIII. Project Implementation Process

This project will be a collaboration between the following agencies; USFWS, NPS, BLM, USFS, USGS, Nevada Department of Wildlife (NDOW), and some universities (to be determined). Details regarding responsibilities of the partners are discussed in more detail below.

Task 1: *Establish project partnerships.*

Through established interagency partnerships, USFWS will develop necessary agreements, and establish cooperative work statements for participating agencies (USGS, NDOW) and some universities. The USGS will bring together the stated partners, to establish the project partnerships. Detailed project statements will be written and funding exchange agreements will be established

Task 2: *Capture, collect biological samples and deploy GPS collars on bighorn sheep from populations in selected mountain ranges.* Partners in this effort will include NDOW, USGS, DNWR and the universities. Task oversight will be by NDOW. Telemetry collars will provide location data for use in:

- Development of habitat models
- Determining causes of mortality of telemetry-collared bighorn sheep.
- Documenting movement patterns within the study range and between adjoining mountain ranges.

Our goals are to capture 20 desert bighorn, from each study population: Sheep Range, Spring Range, River Mountains, Mormon Mountains, and the Muddy Mountains. The first four populations were chosen because they represent different responses to disease exposure. The Muddy Mountain population was chosen because it is currently, the only uninfected population in southern Nevada. Bighorn sheep will be captured using a helicopter and net-gun. Collect biological samples and deploy GPS collars. Bighorn sheep will be fitted with GPS collars with satellite uplink capability. Each collar will be programmed to record locations a minimum of 6 times daily and will be equipped with an automatic breakaway release and mortality sensor. GPS locations for bighorn sheep will be uploaded via satellite every two days. These data will then be evaluated for lack of movement/evidence of mortality. Any evidence of mortality will be investigated immediately.

Task 3: *Assess the overall health of individual from each bighorn sheep population.* Dr. Peregrine Wolff, NDOW, will oversee this part of the study. Collaborators will include NDOW, DNWR, USGS and the universities.

Bighorn sheep will be tested for the following diseases:

- Infectious Bovine Rhinotracheitis (IBR),
- Bovine Respiratory Syncytial Virus (BRSV)
- Parainfluenza 3 (PI-3)
- Leptospirosis
- Epizootic Hemorrhagic Disease (EHD)
- Bluetongue (BTV)
- Anaplasmosis

- Mycoplasma ovipneumoniae (Movi)
- Malignant Catarrhal fever (MCF),
- *Bibersteinia trehalosi,*
- *Mannheimia haemolytica*

Our objective will be to collect baseline data for disease titers, parasites, trace minerals, and standardized body condition. Once captured, the animal will be blindfolded and immobilized with leather straps (or hobbles). During handling, we will monitor the animal's heart rate, temperature, and respiration. Immobilized bighorn sheep will be sexed, aged, weighed, and morphological measurements taken. Body condition will be assessed with a portable ultrasound. We will also collect blood, nasopharyngeal and parasite samples from each individual for genetic analyses, disease pathogens, and trace mineral analyses.

Task 4: *Gene transcription: 1) development of the gene transcript panel, and 2) determine gene transcription patterns for sheep sampled from the study populations.* USGS will complete this task.

Part 1. Development of the gene transcription panel: Development of a gene transcription panel (Bowen et al. 2007) specific for desert bighorn sheep that includes genes representative of multiple physiological pathways (i.e. immune function, nutritional, detoxification). Panel development is a first step in facilitating an understanding of how environmental conditions and stressors are linked to the recent disease outbreak and population decline across southern Nevada. Once developed, we can apply the gene transcription panel to bighorn sheep populations throughout their range.

Panel development consists of a series of steps that include:

a. Collection of blood for use in development
    a. RNA extraction from blood
    b. cDNA synthesis
b. Extraction of relevant and similar gene sequences from Genbank
    a. Find common and conserved regions among sequences for each gene of interest
    b. Develop universal primers for amplification of genes of interest
c. Apply universal primers to blood from step 1
    a. Obtain bighorn sheep sequences from amplification
    b. Design bighorn-specific primers from amplified sequences
d. Multiple bighorn-specific primer pairs become the gene transcription panel

Part 2. Apply gene transcription panel to desert bighorn sheep populations including, Sheep Range, Muddy Mountains, Mormon Mountains, River Mountains, and Spring Range.

Once developed, we can apply the gene transcription panel to bighorn sheep populations throughout their range. We will: 1) provide a comprehensive analysis of transcript panels at individual and population levels; 2) associate transcript profiles with disease outcomes through use of non-metric multidimensional scaling (NMDS); 3) identify outliers in each population and as a whole and associate these with individual health assessments; and 4) associate transcript profiles with specific environmental conditions.

Task 5. Determine patterns for immune function and disease in sheep sampled from each study population. Partners in this effort will include a university and the USGS. Task oversight will be by the USGS. See Appendix A for a detailed description of Methods.
Objectives for this task are to:

- Determine major histocompatibility complex class II (MHC II) genotypes in all animals
- Determine genetic and immunogenetic diversity of individuals and populations by measuring known microsatellites
- Measure functional innate and adaptive immune responses
- Relate all the genetic and phenotypic changes to *M. ovipneumonia* infection status in selected bighorn sheep populations

Part 1. Determine MHC II diversity in individual animals. MHC class II genes are among the most polymorphic genes in vertebrate animals and such genetic diversity at the population level is essential to ensure some individuals will be capable of efficiently activating the adaptive immune responses in response to particular pathogens. Studies in wild animals tend to limit the exploration of MHC class II diversity to one gene, DRB, and disregard other MHC class II gene products, such as DRA, DQA, and DQB which also contribute diversity to the pool of antigens which can be recognized by T lymphocytes. The genomes of domesticated bovid species, such as cow and sheep, have revealed multiple copies of some of these genes, such as DQB and DQA. By restricting analysis of MHC class II to a single gene, researchers may not have adequately captured the true diversity of MHC class II alleles in a population. We will therefore determine the allelic type and diversity of each individual animal at all relevant MHC II genes.

Part 2. Determining genetic and immunogenetic diversity using microsatellites. Genetic diversity in a population is necessary for species preservation particularly at adaptive genetic loci. Genetic diversity can be measured using microsatellites, which are known stretches of non-coding DNA which are often inserted as repeated sequences that are located in close proximity to functional genes. Microsatellites can be classified as neutral if they are located distally from functional genes or adaptive if they are located close to genes which are likely to provide evolutionary advantages to the individual. We have previously identified numerous microsatellites in desert bighorn sheep which are both neutral and adaptive, particularly because they associate with genes of known immune function. Additionally, we have found that populations which have a larger diversity of immune-linked microsatellites have lower incidences of *M. ovipneumonia* infections. We will therefore measure both neutral and immune-linked microsatellite diversity in captured sheep and incorporate these data into our overall findings.

Part 3. Measure the functional innate and adaptive immune responses of individual animals. In addition to measuring genetic diversity of genes related to immune function, we will also measure functional immune responses in a variety of assays to determine if either underlying genetics, nutrition, or other conditions can alter the actual immune response and what impact this may have on disease susceptibility and outcomes.

Part 4. Relating immune parameters to disease status. Once these steps are complete, we will use non-metric multidimensional scaling (NMDS), to associate transcript profiles, MHC

genotypes, innate bacterial responses, lymphocyte activation, cytokine and acute phase protein profiles, and other immune parameters, as well as genotypes at linked microsatellite loci and genetic diversity of neutral and linked microsatellite loci at population and individual levels, with disease outcomes. The estimated time to completion for this segment of the project is 3 years.

Task 6: *Volunteer outreach, education, and training: monitoring presence of summer lamb pneumonia and signs of pneumonia in adults.* Partners in this effort will include the USGS, USFWS. Task oversight will be by the USGS.

Objectives for this task are to conduct outreach, and training for volunteers to:

- Monitor study populations for summer pneumonia in through observations and trail cameras at water sources for summer lamb/ewes counts
- Assist in spring lamb surveys
- Assist in identifying, and reporting adults with signs of disease
- Assisting with collection of habitat and environmental data
- Conduct outreach to educate students and teachers in Clark County Schools about the ecology and behavior of bighorn sheep and their place in the desert ecosystem

This task will provide additional information on the health and recovery characteristics of each of the study populations. A volunteer coordinator will conduct outreach through public meetings to contact potential volunteers, and train volunteers who choose to assist with one or more of the activities listed above. Volunteer activities are available for a wide range of age groups and physical abilities. We will also provide outreach to Clark County schools by conducting educational programs designed to provide an understanding of the ecology and behavior of bighorn sheep and their place in the ecosystem.

Task 7: *Identify specific conditions that potentially link physiological responses to stressors in the environment*. Partners in this effort will include USGS, and a university. Task oversight will be by the USGS.

Objectives are to:

- Assess habitat quality and quantity for each of the study populations
- Determine seasonal food habits for each population
- Assess diet quality
- Assess quality of important forage species in the diet
- Quantify movement of bighorn sheep in each study population to estimate social encounters, frequency of encounter with infected individuals, and exposure to potential biotic stressors such as human disturbance and predators.
- Weather variables (precipitation, temperature, etc)
- Use Path analysis with logistic regression to relate environmental variables and stressors (e.g., human disturbance, predation risk) to disease physiology and disease state of sheep in all populations.

Task 8: *Combine quantitative information from Tasks 3-7 to develop structural equation and network models that relate environmental variables and stressors such as human disturbance and predation, to sheep movement, group size and social interaction networks, disease transmission, to individual infection, physiology, and gene expression.* Partners in this effort will include the universities and the USGS. Task oversight will be by the USGS.

Objectives for this Task are to:

1. Construct spatially-explicit structural equation/path models and network models incorporating variables characterizing environments, sheep populations, social groups, and individuals to determine the interplay of influences related to spread of infection and co-infection from each pneumonia related pathogen.

2. Evaluate habitat selection, incidence of disease, and existence of ecological traps for sheep. Using quantitative variables and results from analysis of habitat selection and effects of social contacts on disease incidence it will be possible to determine if the probability of disease transmission is higher in habitat with greater sheep density and or larger group sizes of ewes. We will also evaluate the role of low quality habitat in sustaining uninfected groups of sheep, a role that may be critical for long-term recovery from disease.

Task 9: Identify potential habitat management actions that might improve recovery or lessen the impact of the disease. Partners in this effort will include the USGS, USFWS, and the universities. Task oversight will be by the USGS.

Management actions will be identified based on results of the above Implementation Tasks. This information will be disseminated through presentations at interagency meetings, written report(s) and publication(s).

IX. Level of Readiness for Project Implementation

USFWS staff are available to manage this project and provide environmental compliance (NEPA etc), which is expected to be minimal. The SNAP Science and Research Team supports this project and is ready to provide inter-agency coordination. The Nevada Department of Wildlife (NDOW) considers this project essential to the management of desert bighorn sheep in southern Nevada, see their attached letter of support.

X. Relationship to Previous Phases and Anticipated Future Phases

This proposal builds on information obtained from the Round 10 study (Survival and Habitat Requirements of Desert Bighorn Sheep of the Desert NWR, FW-67), which attributed recent declines in bighorn sheep populations to the pneumonia epidemic. This project will allow for an in-depth investigation of how pneumonia may be causing depressed population numbers in the Sheep Range and by examining the link between disease, physiology and environmental stressors for the Desert NWR and other populations on Federal lands. This information will contribute to ongoing projects that include assessments of demographics, health, habitat use and availability, population structure (genetic diversity and connectivity), and threats (loss of habitat, increased mortality) within bighorn sheep populations in southern Nevada; critical factors necessary for conservation planning for these regional populations.

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XI. Proposed Project Budget

Best-Value Option

Use of the CESU to establish cooperative agreements with two universities; 1) to conduct MHC II analyses, and 2) to conduct path analyses and ecological modeling, will provide a best-value option because universities involve students as workers, allow them to gain research experience, learn new methods, and provides student employment. In addition, CESU administrative costs are relatively low.

Use of volunteers to monitor for signs of summer pneumonia in lambs will provide best value because volunteers are a willing workforce who are interested in our natural resources and want contribute to their conservation. Hiring people to do this work instead of using volunteers would be very expensive.

Use of USGS is a best value option because 1) they have the experience and expertise to oversee a large collaborative project such as this one, 2) have expertise in gene transcription (USGS scientists instrumental in the development of gene transcription methodology), 3) USGS has scientists who are experts in bighorn sheep biology and ecology, and 4) USGS scientists have extensive experience conducting research on bighorn sheep in Clark County. USGS scientists would also contribute salary, which would save money on the project.

XII. Conservation Initiatives Ranking Criteria

I. Conservation Initiatives Ranking Criteria (applicable priorities **bolded**)

Ranking Criteria: Priorities identified in the Strategic Plan or Implementation Agreement or developed by the Subgroup	**TOTAL POINTS POSSIBLE**
1. **Project supports one of three priority types of projects**: *(Points should be awarded on a scale of 0-10.)* **Habitat enhancement –** *This project will provide information on actions to enhance bighorn sheep habitat that can improve disease outcomes for bighorn sheep, critical for the protection, conservation and management of desert bighorn sheep on federal lands throughout Nevada and the southwest. This information is vital to improving the quality of habitat on federal lands for bighorn sheep and other wildlife species.* a. Cultural Resources b. Safety and Public Health- *Citizen science volunteers who assist scientists will learn how to safely experience natural environments.*	10
2. **Project includes outreach/education as a component** - examples could include, but are not limited to: **Volunteer/Partnership Coordination**, **Youth Initiatives - Teach the Teachers**, Mobile Models, Make the Classroom/Lessons Accessible, Off Highway Vehicle Impacts, Water Conservation, projects that facilitate getting youth into the environment - i.e., Hands on the Land, or projects that incorporate modern outreach efforts and techniques using social media platforms. *This project will provide outdoor educational opportunities for citizen science volunteers who assist with bighorn sheep monitoring at water sources and spring lamb surveys. Information from this project can be used in the development of educational displays, and interpretive products such as brochures and kiosks, students and the general public can learn about bighorn sheep, and their place in the local ecosystem. The cooperative agreements with the universities will also provide 'hands-on' educational opportunities for students. Results from this study will be presented at public meetings and reports will be made available to the public.* *(Points should be awarded on a scale of 0-5.)*	5
3. **Project supports 1 of the 20 priority sub-types of projects**: *(Factors a-b are mutually exclusive. If the project supports more than one priority sub-type, award points based on the primary priority sub-type. This list is not all inclusive, but gives priority to projects that leave a legacy on the landscape and those that are important to program partners.)* a. Project supports any of the priority sub-types – 10 pts 1. Cultural – Protection/Site Stewards 2. Habitat Enhancement – Endangered Species – 3. Safety and Public Health – AML Reclamation with habitat restoration	10

component *4.* **Habitat Enhancement – Proactive Steps to Prevent Listing** *bighorn sheep are an important component of ecosystem health and maintaining viable populations of bighorn sheep is a high priority goal of resource management. Completion of this project will provide information about how environmental conditions and stressors are linked to the recent critical pneumonia outbreak and population decline for bighorn sheep in Nevada, and to identify potential management actions associated with bighorn habitat that may lessen the impact of disease* **Habitat Enhancement – Invasive Species Treatment and/or Control (Plant or Animal)** 5. Habitat Enhancement – Restoration of Habitat for Sensitive Species at the watershed or landscape level -- 6. Cultural – Restoration/Stabilization – 7. Safety and Public Health – Litter/Dumping Clean-up 8. Cultural – *9.* **Habitat Enhancement – project addresses climate change –** *the data set of transcript values developed for the sampled bighorn sheep populations can be used as baseline data to provide an understanding of the susceptibility of populations at risk from regime shifts, such as may be seen with climate change, and to provide an early-warning indicator for populations at risk.* 10. Habitat Enhancement – Water Quality and Quantity Monitoring – *Monitoring of water sources will occur as we monitor sheep use at water sources.* 11. Habitat Enhancement – Cave Management 12. Habitat Enhancement – Restoration of Springs/Streams/Rivers – 13. Habitat Enhancement – Road decommissioning and rehabilitation 14. Habitat Enhancement – Reintroduction of Extirpated Species to restore overall ecosystem b. Project supports any of the priority sub-types – 5 pts 15. Cultural – Surveys – 16. Cultural – National Register Nominations 17. Safety and Public Health – Information Kiosks and Signs 18. **Safety and Public Health – project addresses and mitigates adverse impacts to resources caused by the volume of people using the resource –** 19. **Safety and Public Health** –	
4. **Project enhances partnerships in promotion of cooperation and collaboration** – 5 pts *(Points should be awarded between 0-5 based on the following factors:)* a. **Involves individual citizen groups or organizations in the development and accomplishment of resource management goals and other activities during project implementation** – 2 pts *citizen science volunteers who assist with bighorn sheep monitoring at water sources and*	5

spring lamb surveys. We will conduct outreach for volunteers from a number of organizations, including the Audubon Society & Fraternity for Bighorn Sheep and local university and colleges *b.* **Addresses the needs of more than one Federal agency** – 1 pt *This project includes bighorn populations on lands in Clark County that are managed by all four agencies.* c. **Involves non-Federal public partners** – 2 pts *The project involves Nevada Department of Wildlife and 2 Universities.*	
5. Project has identified committed non-SNPLMA sources of funding or in-kind contributions in the development and/or implementation of the project – 3 pts *a.* Volunteer Labor – *Volunteers will contribute a substantial in-kind contribution in donated salary and travel (vehicle) expenses.* b. Salaried employees – actual hourly rate plus the value of any fringe benefits received c. Actual costs for material, equipment and supplies should be used *Overhead costs may not be included in determining in-kind contributions. In-kind	3
Ranking Criteria: Executive Committee Values	
6. **Project promotes sustainability** by providing benefits in the near and long term by emphasizing healthy and resilient landscapes as well as durability, relevancy, and shared support – 10 pts *(Points should be awarded on a scale of 1-10 based on the following factors. Projects can be given a score of 0 to 2 for each factor, depending on how well they address the factor.)* a. **Conserves resources to ensure availability to future generations** – 2 pts - *Monitoring bighorn sheep at water sources provides land managers accurate data to take appropriate actions to maintain springs and other water sources.* b. **Restores or maintains natural processes as demonstrated by implementation monitoring within the project timelines** – 2 pts-. c. **Will remain relevant and continue to provide a benefit beyond the existence of SNPLMA** – 2 pts - *The use of trained volunteers will allow for program sustainability and monitoring to continue to help track disease in bighorn sheep populations.* d. **Includes a stewardship component to broaden support and share responsibility for operating or maintaining the project** – 2 pts – e. **Reduces long-term costs and minimizes the human impact on the environment** – 2 pts - *The volunteer citizen scientists will reduce costs both initially and over time as the number of volunteers increase.*	10
7. **Project promotes connectivity** by linking people to nature and recreational opportunities, and by uniting important places across the landscape – 5 pts *(Points should be awarded on a scale of 0-5 based on how well the project supports the following factors:)* **a. Encourages people to meaningfully connect with their natural environment and helps them appreciate and care for the environment**	5

– training volunteers to collect data will create a sense of ownership and appreciation of natural resources AND/OR b. Connects habitats, migratory corridors, or protected areas	
8. **Project promotes community** by improving the quality of life for the human community and protecting the integrity of biological communities – 15 pts *(Points should be awarded on a scale of 0-15 based on the following factors. Projects can be given a score of 0-5 for each factor depending on how well they address each factor.)* a. Conserves or restores the functionality, resiliency and integrity of biological communities – 5 pts – *b.* Encourages partnerships and helps build a sense of community – 5 pts – *Nonprofit partners and volunteers involved directly builds interest in the program to monitor bighorn from the grassroots community level.*	15
TOTAL POINTS AWARDED *(63 pts possible)*	

Schedule B						
CONSERVATION INITIATIVE						
ESTIMATED NECESSARY EXPENSES						
Project Name:	Environmental Effects on the Immunity and Health of Desert Bighorn Sheep in Southern Nevada					
Project #:		**Priority #:**		**Agencies:**	FWS	
Prepared by:	Laurie Simmons					
Phone:	702-515-5466				**Initial**	x
Date:	4/26/2016				**Updated**	

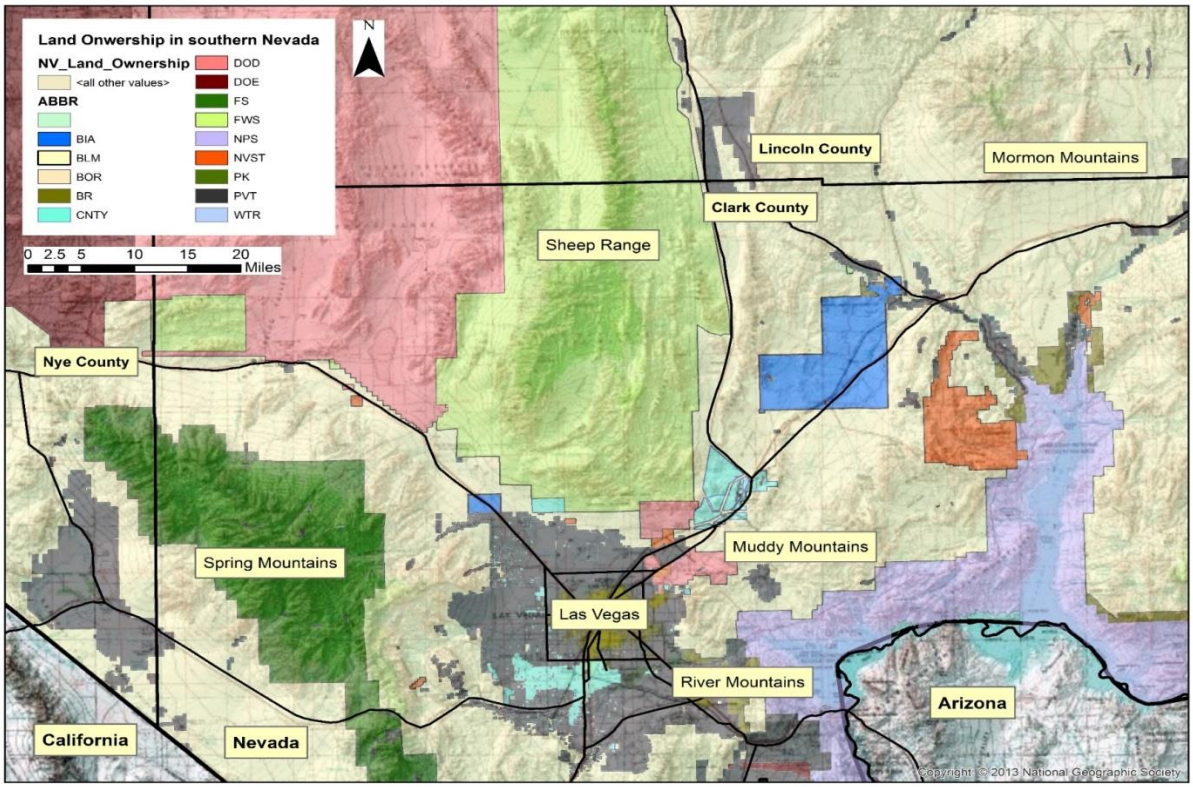
1. Planning & Environmental Documentation		$ -	0%
(Surveys/ reports for cultural, natural, biological, archaeological resources, NEPA documentation, etc)			
2. FWS Consultation - Endangered Species Act		$ -	0%
(direct expenses for FWS if consultation is required)			
3. Direct Federal Labor to Implement Project (Payroll)		$ 47,705.00	2%
(Federal labor costs for completing the project)			
4. Project Equipment and/or Supplies/ Materials		$ -	0%
(include specialized equipment, supplies and materials not included in contracts/ agreements)			
5. Travel & Per Diem for Implementation		$ -	0%
6. Official Vehicle Use		$ -	0%
(Based on agencies procedures for use, fuel, equipment, and mileage charges)			
7. Contracts/Grants/Agreements to complete the project		$ 2,385,228.00	96%
8. Required Training to Implement Project		$ -	0%
(includes initial and annual training for LEOs and training necessary to implement the project)			
9. Other Necessary Expenses - See Expanded Budget		$ 47,705.00	2%
	TOTAL	$ 2,480,638.00	100%

COMMENTS

Detailed Cost Estimate Other Necessary Expense Worksheet

Project Title: Environmental Effects on the Immunity and Health of Desert Bighorn Sheep in Southern Nevada			
DETAILED COST ESTIMATE			
1. Planning and Environmental Assessment Costs			
	Subtotal	$	-
2. FWS Consultation - Endangered Species Act	**Subtotal**	$	-
3. Direct Labor/Payroll to Perform the Project (use fully loaded labor rate)			
Refuge Complex Biologist - GS-13		$	47,705.00
	Subtotal	$	47,705.00
4. Project Equipment			
	Subtotal	$	-
5. Project Materials and Supplies			
	Subtotal	$	-
6. Travel (airfare, car rental, per diem, etc)			
	Subtotal	$	-
7. Official Vehicle Use			
	Subtotal	$	-
8. Required Training for Project Implementation (list purpose)			
	Subtotal	$	-
9. Cost of Contracts and/or Agreements to Perform Project			
USGS		$	2,150,892.00
Cooperative Agreement 1		$	118,495.00
Cooperative Agreement 2		$	115,841.00
	Detailed Cost Estimate Subtotal	$	2,385,228.00
OTHER NECESSARY EXPENSES (APPENDIX B-11)			
10. Examples of Other Necessary Expenses (providing a breakdown of these costs is optional, however a			
ADMINISTRATION COSTS			
Budget Tracking/Accounting and Execution		$	47,705.00
	Other Necessary Expenses Subtotal	$	47,705.00
GRAND TOTAL		**$**	**2,480,638.00**
CASH/ IN-KIND CONTRIBUTIONS			
Contributor 1		$	-
Contributor 2		$	-
	Total	**$**	**-**

Appendix A. Map showing location of mountain ranges with bighorn sheep study populations (Sheep Range, Spring Mountains, Mormon Mountains, River Mountains, and Muddy Mountains).



Forest Service

Humboldt-Toiyabe National Forest

Spring Mountains NRA
4701 N. Torrey Pines Drive
Las Vegas, NV 89130

File Code:
Route To:

Date: April 25, 2016

Subject: Support for *Environmental Effects on the Immunity and Health of Desert Bighorn Sheep (Ovis canadensis nelsoni) in Southern Nevada* Nomination for SNPLMA Funding

To: Christy Smith, Project Leader
Desert National Wildlife Refuge Complex
U.S. Fish and Wildlife Service
4701 North Torrey Pines Drive
Las Vegas, Nevada 89130

Dear Ms. Smith,

We support the Southern Nevada Agency Partnership (SNAP) nomination for Southern Nevada Public Lands Management Act (SNPLMA) funding to study the recent pneumonia disease epidemic in desert bighorn sheep and environmental effects that may contribute to its outcomes on Federal lands in southern Nevada. We hope this study will identify habitat management actions that can be taken to ameliorate the adverse effects of this disease on desert bighorn populations to aid us in our goal to sustain healthy populations of this desert icon.

We appreciate your efforts to pursue healthy landscapes in southern Nevada.

Sincerely,

Donn Christiansen
Area Manager
Humboldt-Toiyabe National Forest Service
Spring Mountains National Recreation Area
4701 N. Torrey Pines Dr.
Las Vegas, Nv. 89130
702-515-5448

United States Department of the Interior

BUREAU OF LAND MANAGEMENT
Southern Nevada District Office
4701 N. Torrey Pines Drive
Las Vegas, Nevada 89130
http://www.blm.gov/nv/st/en.html

In Reply Refer To:
6840 (NVS00000)

Christy Smith, Project Leader
Desert National Wildlife Refuge Complex
U.S. Fish and Wildlife Service
4701 North Torrey Pines Drive
Las Vegas, Nevada 89130

Dear Ms. Smith:

The Bureau of Land Management's Southern Nevada District Office supports Southern Nevada Agency Partnership (SNAP) nomination for Southern Nevada Public Lands Management Act (SNPLMA) Round 16 proposal "*Environmental Effects on the Immunity and Health of Desert Bighorn Sheep (Ovis canadensis nelsoni) in Southern Nevada.*" We hope this study will identify habitat management actions that can be taken to ameliorate the adverse effects of this disease on desert bighorn populations to aid us in our goal to sustain healthy populations of this desert icon.

We appreciate your efforts and hard work in developing this proposal. We believe that the completion of the stated goals will be invaluable in managing public land in southern Nevada.

Sincerely,

for Erick Kurkowski
Acting District Manager
BLM-Southern Nevada District Office

United States Department of the Interior

NATIONAL PARK SERVICE

LAKE MEAD NATIONAL RECREATION AREA
601 NEVADA HIGHWAY
BOULDER CITY, NEVADA 89005

April 25, 2016

Christy Smith, Project Leader
Desert National Wildlife Refuge Complex
U.S. Fish and Wildlife Service
4701 North Torrey Pines Drive
Las Vegas, Nevada 89130

Subject: Support for *Environmental Effects on the Immunity and Health of Desert Bighorn Sheep (Ovis canadensis nelsoni) in Southern Nevada* Nomination for Southern Nevada Public Lands Management Act (SNPLMA) Funding

Dear Ms. Smith:

I am pleased to provide this letter of support for the Southern Nevada Agency Partnership Round 16 Nomination for SNPLMA funding to study the recent pneumonia disease epidemic in desert bighorn sheep and environmental effects that may contribute to its outcomes on Federal lands in southern Nevada.

The pneumonia epidemic has significantly affected herds of bighorn sheep in and around Lake Mead National Recreation Area, and, at this time, there is no effective vaccine or treatment program. Additionally, current health evaluations and diagnostics for bighorn sheep being used in the area provide limited information on the overall health of the animals and almost no information on the condition of the animals' habitat.

This project will use newly developed diagnostic techniques to link sheep health to ecosystem health across southern Nevada, thus helping researchers and managers identify when and why individuals are succumbing to disease and how disease state relates to environmental conditions. Improving our understanding of this variation in disease response and identifying environmental factors that contribute to the variation in response will allow managers to better implement habitat management actions that can ameliorate the adverse effects of this disease on desert bighorn populations to aid us in our goal of sustaining healthy populations of this desert icon.

We appreciate your efforts to pursue healthy landscapes in southern Nevada.

Sincerely,

for Lizette Richardson
Superintendent

STATE OF NEVADA
DEPARTMENT OF WILDLIFE
6980 Sierra Center Parkway
Suite 120
Reno, Nevada 89511
Phone (775) 688-1500 • Fax (775) 688-1495

BRIAN SANDOVAL
Governor

TONY WASLEY
Director

LIZ O'BRIEN
Deputy Director

JACK ROBB
Deputy Director

May 4, 2016

Christy Smith, Project Leader
Desert National Wildlife Refuge Complex
U.S. Fish and Wildlife Service
4701 North Torrey Pines Drive
Las Vegas, Nevada 89130

Dear Ms. Smith:

I want to express the support of the Nevada Department of Wildlife for the desert bighorn sheep proposal "Environmental Effects on the Immunity and Health of Desert Bighorn Sheep (*Ovis canadensis nelsoni*) in Southern Nevada," which is being submitted for funding under Round 16 Conservation Initiatives of the Southern Nevada Public Lands Management Act.

The goal of this project is to use newly developed gene-based techniques to link sheep health to ecosystem health, thus helping researchers and managers identify when and why individuals are succumbing to disease and how that relates to environmental conditions. Information obtained from this project will allow us to understand how environmental conditions and stressors are linked to the recent disease outbreak and population decline for bighorn sheep in southern Nevada, and to identify potential management actions associated with bighorn habitat that might lessen the impact of the disease. Completion of this project will provide critical information pertaining to the protection, conservation and management of desert bighorn sheep on federal lands throughout southern Nevada and the southwest.

We believe our collaborative efforts are important in providing long-term protection for bighorn sheep populations in southern Nevada and that these strategies may also benefit sheep populations throughout the state. Thank you for your consideration of this project.

Please feel free to contact me should you have any questions or require additional information.

Sincerely,

Brian Wakeling
Game Division Administrator