

Application for FY14/15 BGMA funding

Grant Name/Project Title: Characterizing the spread and consequences of respiratory disease for desert bighorn sheep in the eastern Mojave Desert

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Problem Statement: A recently discovered (May 2013) outbreak of epizootic pneumonia caused a substantial die-off of desert bighorn sheep in one of the largest populations in California (Old Dad Peak, Mojave National Preserve [MOJA], Fig. 1). This outbreak was likely caused by pathogens transmitted from domestic sheep or goats and led to the temporary closure of the Old Dad Peak hunt zone. This region of the Mojave Desert supports a large meta-population of desert bighorn sheep, central to the most extensive array of naturally-persisting bighorn herds in North America. Until 2013, pneumonia epizootics in this region were unknown. After the discovery of the die-off, bighorn sheep captured in 9 populations in or near MOJA tested positive for *Mycoplasma ovipneumoniae* (hereafter, *M. ovi.*), one of several pathogens involved in bighorn sheep pneumonia (Besser et al. 2012). Although significant adult mortality has been detected only at Old Dad Peak, persistent high lamb mortality may occur system-wide (Cassirer and Sinclair 2007). Managing other stressors in the system (nearby renewable energy developments, water availability), including managing population connectivity (Creech et al. 2014), requires a clear understanding of the demographic impacts and potential spread of the new disease process.

The proposed study builds on an existing collaborative response to the outbreak involving Oregon State University (Clint Epps, PI), National Park Service, and CDFW. It will use both existing and new data to 1) establish the demographic consequences of the current disease outbreak and environmental variation by evaluating adult survival and lamb recruitment over multiple years across populations where *M. ovi.*, 2) augment a study of seasonal movements of rams and ewes to infer how and when disease is most likely to spread, and 3) support ongoing efforts to recharacterize population genetic structure, genetic diversity, and diversity of immune system genes for bighorn sheep populations in the affected area. Although we take advantage of National Park Service (NPS) funding already secured (Epps, \$157,000) that provides partial support, the proposed project is fully dependent on additional funding. Our findings will clarify the impacts of disease on demography in a highly variable desert ecosystem, use movement analyses and updated gene flow estimates to characterize potential for pathogen spread, and provide management recommendations. This three-year project would be initiated immediately.

Project Description

Background information: Necropsies of affected animals at Old Dad Peak and Marble Mountains confirmed pneumonia and presence of *M. ovipneumoniae* (CDFW, unpublished data). *M. ovipneumoniae* (hereafter, *M.ovi*) has been suggested by Besser et al. (2008, 2013) to be the primary agent causing pneumonia in bighorn sheep, although other pathogens may be involved (Wehausen et al. 2011). The source of this outbreak is unclear, although a domestic goat was removed from the nearby Marl Mountains in fall 2012 (CDFW, unpublished data). Elsewhere in North America, respiratory diseases of bighorn sheep likely resulting from contact with domestic livestock, particularly sheep and goats (but see Wolfe et al. 2010), have resulted in high mortality, low reproductive success, and extirpation of entire populations (Cassirer and Sinclair 2007, McClintock and White 2007, Wehausen et al. 2011).

In response, and with assistance from the CA Chapter of the Wild Sheep Foundation (CAWSF) and the Society for Conservation of Bighorn Sheep, CDFW, NPS, and Oregon State University (OSU) cooperated on capture and radio/ satellite collaring of 73 bighorn sheep in Nov. 2013 in the region (Fig. 1). Subsequent analyses established that *M.ovi* spread across a large area of the eastern Mojave Desert, presumably because of movement of bighorn sheep among some populations (Epps et al. 2005, Epps et al. 2007, Epps et al. 2010). Five hunt zones (Old Dad Peak, South Bristol, & Marble Mtns, Fig. 1; Cady

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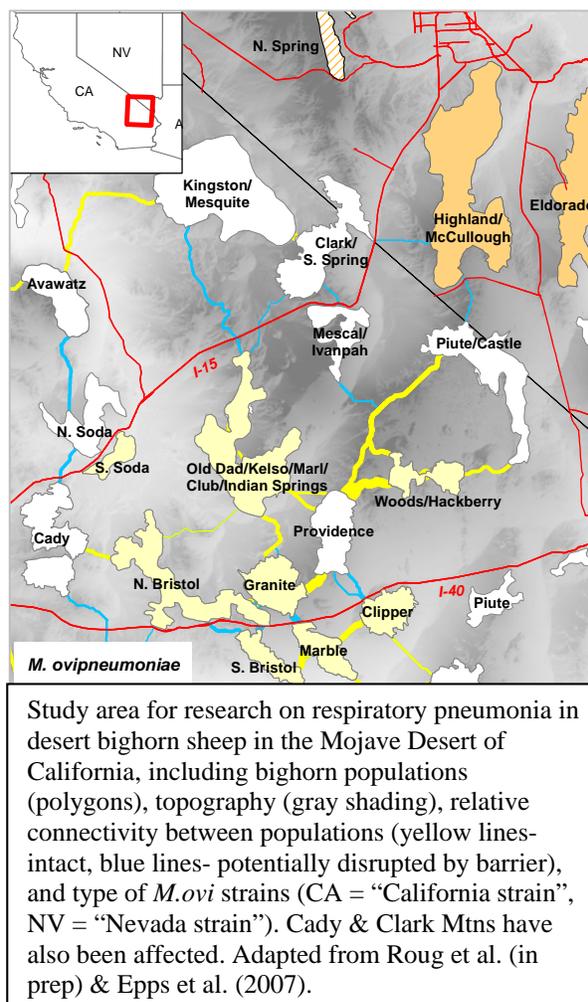
Mtns, CDFW unpub. data; Clark Mountains, suspected) already have been affected and others are at risk. Captures of 75 more animals are planned for October 2014 (Table 1). We propose to capitalize on this opportunity and build on ongoing research to more intensively study the consequences and potential for spread of the disease.

Objectives: We propose to investigate the current extent and impact of the disease on survival and reproduction while accounting for environmental variability (Obj. 1), analyze existing ewe GPS collar data and collar rams with high-resolution GPS collars to establish how seasonal movements may influence disease spread (Obj. 2), and expand genetic analyses from blood and fecal samples to update estimates of genetic structure, gene flow, current connectivity, and genetic diversity of neutral markers and immune system genes (Obj. 3).

Objective 1 Methods—Part A—seasonal adult female survival: Adult female survival will be estimated for seasonal or shorter periods over a 4-year period (2013-2017) using known-fate survival analysis in Program Mark (e.g., Smith et al. 2014). Survival rates will be related to disease presence and environmental variation (forage, precipitation, diet quality- see Objective 1B). We have ongoing mortality and location data from 81 bighorn sheep that were collared during 2005-2009 (n=10) or in November 2013 (n = 71; Table 1) across 8 mountain ranges (Fig. 1), largely with satellite upload GPS collars. In Oct. 2014, CDFW will collar ~75 additional bighorn sheep with satellite upload GPS collars (1 pt/day females, up to 24 pts/day males; Table 1) to increase sample sizes, initiate the ram movement study, and extend the study area. Thus, we expect ~125 animals in 9-15 populations to have satellite upload GPS collars, ideal for known-fate survival analysis, in addition to ~30 animals with other collars. Our goal is collaring >10% of adult females (Arthur and Prugh 2010): 10-15 collars per large population (>75 females, n = 2) and 7-10 collars per small population (<75 females, all others).

Objective 1 Methods—Part B—lamb recruitment: Following adult mortality during initial outbreaks of pneumonia, lamb recruitment may suffer for years after (Cassirer and Sinclair 2007, Cassirer et al. 2013). The persistence and impacts of respiratory disease in desert ecosystems is essentially unknown. However, lamb survival in desert regions is strongly influenced by environmental conditions, particularly positive effects of winter rainfall on forage (Wehausen et al. 1987, Rubin et al. 2000). Even without disease, lamb survival in the Mojave varies greatly with precipitation: e.g., summer lamb:ewe ratios at Old Dad Peak varied from 10-60:100 before the outbreak (Wehausen 2005). Thus, any assessment of disease in lambs must include weather and forage conditions as covariates.

We propose to assess summer lamb:adult ewe and yearling:adult ewe ratios for 4 years (including summer 2014, see below), using remote cameras at waterholes and supplemented by ground observations, within a subset of populations. These methods are well-established in this system (Wehausen 2005), and the extremely remote, rugged terrain it very



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difficult and costly to collar lambs for direct estimates of survival. In 2014, in collaboration with longtime bighorn sheep researcher John Wehausen, we are collecting preliminary camera and ground observation data in the Marble Mtns, Old Dad Peak, South Bristol Mtns, Hackberry Mtns, Clipper Mtns, and the Wood Mtns (Fig. 1). Lambs, ewes, and yearlings can be clearly distinguished. Cameras are placed in populations where there are a small number of point water sources allowing essentially all females and associated lambs to be sampled repeatedly. Lamb: and yearling:adult ewe ratios will thereby be determined in each population over short time periods during the hot season when bighorn sheep routinely use water (~May-September).

We will use mixed-effect models to model lamb: and yearling:adult ewe ratios in *M. ovi.*-positive populations as a function of elevation, forage quality, precipitation, and other variables. To describe forage quality, we will use a) fecal nitrogen (FN), which has been estimated monthly in two populations (Marble and Old Dad) since 1984 and is a well-established metric in this system (Wehausen 2005), and b) an index of forage quality based on NDVI (Crech et al. in prep). Maximum elevation predicts both persistence (Epps et al. 2004) and genetic diversity (Epps et al. 2006) of populations. Lamb survival increases with winter-spring diet quality except in very wet years (Wehausen 2005). In 3 populations (Old Dad Peak, Marble, South Bristol), lamb:ewe ratios prior to the outbreak were assessed by helicopter surveys (CDFW, annual before 2010), ground counts, and remote cameras (J. Wehausen, unpub. data). Using those data, we will contrast pre- and post-outbreak lamb:ewe ratios across multiple years using a) mixed-effect models including environmental factors described above, and b) comparison of means and 95% confidence intervals for all years or years with similar forage conditions.

Objective 2 Methods—ram and

ewe movement: During the initial 2013 outbreak at Old Dad Peak, strategies considered for containing the spread of the disease were hampered by lack of knowledge on movement. Most radiotelemetry data in the system have been collected sporadically and from ewes, with the exception of a radiotelemetry study at Old Dad Peak 1981-1990 that found males and females aggregated during the rut and males were willing to move farther from water and steep terrain (Bleich et al. 1997). Until now, no high-resolution spatial data have been available to analyze daily movements

Table 1. Current and proposed sample sizes for collared bighorn sheep.

Population	New collars (anticipated)		Purpose	Anticipated total incl. 2013 collars
	Male	Female		
N. Bristol		5	Survival	11
S. Bristol	5		Male movement	18
Clipper			2013-survival	4
Granite			2013-survival	5
Hackberry		5	Survival	11
Old Dad Peak/ Indian Spring			2013-survival	19
Marble			2013-survival	15
Soda			2013-survival	4
Newberry		10	Survival/surveillance	10
Old Woman		10	Survival/surveillance	10
Cady		10	Survival/surveillance	10
Black Mtns		10	Surveillance/survival	10
Eagle Mtns		10	Surveillance/survival	10
Orocopia Mtns		10	Surveillance/survival	10

We will analyze movement of males and females as a function of forage quality, season, age, and mating condition. We propose to supplement planned bighorn sheep captures by CDFW (focused on ewes) by adding 15 rams (mix of young and mature) fitted with GPS collars capable of producing high resolution data (e.g., 1 location/hour), plus 2 rams fitted with GPS collars in 2013. In fall 2014, CDFW will collar 10 rams in the South Bristol and possibly Marble Mountains, using GPS collars purchased by OSU from NPS funds. Here we propose funds for 5 more such collars in 2015: 17 collars will provide an adequate sample to capture seasonal movement patterns (Cushman et al. 2010). GPS collar data from ~145 ewes, 1-6 points per day, will allow assessing female movements. We will analyze movement and habitat selection as influenced by water, terrain, forage conditions (NDVI) during short and long-distance movements using path-based analyses to assess differences between available versus used movement

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paths (Cushman et al. 2010). We will contrast daily movement rates (Cushman et al. 2005) and movement among ewe groups in different seasons. Any inter-population movements will be contrasted with gene flow models (Obj. 3; see Epps et al. 2007). We predict that rams will make long-distance movements more frequently before or during the rut, inter-population movements will show exploratory movements unlikely to result in gene flow, and young rams and ewes will make more long-distance movements.

Objective 3 Methods—population genetic structure and genetic diversity: Previous analyses of genetic structure and gene flow in this system offered unparalleled understanding of movements among populations (Epps et al. 2005, Epps et al. 2007, Epps et al. 2010), but must be linked to actual movement data: individuals may move but not breed, transmitting disease but not genes. However, gene flow analyses inform relative risk of spread. Since the previous analyses, newly colonized and expanded populations within the study area may have caused dramatic shifts in genetic structure. Therefore, we will use genetic samples from captures and ongoing fecal DNA collections to reevaluate gene flow among populations in the study area. Epps/OSU has already initiated collection and analysis of updated genetic data but more samples are needed. We also will evaluate whether strong variation in neutral genetic diversity observed across the study system (Epps et al. 2006) is reflected in diversity of immune system genes. In Nov. 2013, Epps and collaborators brought 4 technicians to the capture to collect DNA and data on immune system function from blood samples. Funding in this proposal will allow a similar effort during capture in Nov 2014 and will partially support follow-up lab analyses.

Expected Benefits: This project will enhance understanding of population dynamics, habitat selection, and disease and will provide improved population survey data. We anticipate 1) scientific publications regarding the effects of pneumonia in this unique desert ecosystem, and 2) management recommendations regarding maintaining or enhancing population connectivity and water developments (as proposed in the draft Desert Bighorn Management Plan) based on our description of likely routes, timing, and patterns of disease spread and the demographic effects of the disease.

Itemized Budget: CAWSF will subcontract with Oregon State University (OSU). CAWSF charges no grant administration fees; however, budget includes indirect costs levied by OSU on subcontracted funds.

Expense	Year 1	Year 2	Year 3	Total
Personnel (salary and benefits) (Total)	\$28,399	\$29,411	\$30,748	\$88,558
PI Salary- Epps- 2 weeks/year	\$5,823	\$5,998	\$5,998	\$17,818
Graduate student (Ph.D., 6 months/year)	\$18,090	\$18,927	\$18,585	\$55,602
Lab tech (2 months total)	\$4,486	\$4,486	\$0	\$8,972
Undergraduate field/lab assistant (3 months total)			\$6,166	\$6,166
Operating expense (Total)	\$44,822	\$25,149	\$25,130	\$95,101
Telemetry receivers, antenna, ram collars	\$14,000			\$14,000
4WD vehicle lease and mileage	\$1,590	\$1,590	\$7,950	\$11,130
Travel to field sites and meetings for project personnel	\$4,100	\$4,800	\$1,500	\$10,400
Field supplies (food, GPS, optics, software)	\$4,690	\$1,690	\$2,890	\$9,270
Lab work/supplies (genetic, immuno, nitrogen work)	\$3,600	\$3,600	\$1,600	\$8,800
Housing during field work- Granite Mtn Reserve	\$600	\$600	\$600	\$1,800
Subcontractor-J. Wehausen-analyze past demographic data	\$2,500	\$2,500	\$0	\$5,000
Publication charges	\$0	\$400	\$400	\$800
OSU indirect- CaWSF allowed rate (25%)	\$13,742	\$9,969	\$10,190	\$33,901
Grant Administration (Total)	\$0	\$0	\$0	\$0
Total cost	\$73,221	\$54,559	\$55,878	\$183,658

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