U.C. Davis Wildlife Health Center
California Carnivore Projects

UC DAVIS VETERINARY MEDICINE
Karen C. Drayer Wildlife Health Center

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A. CALIFORNIA MOUNTAIN LION RESEARCH PROJECT

A.1. Introduction / overview

A.1.1. History of the UC Davis Wildlife Health Center Mountain Lion Study and current focus

This mountain lion (*Puma concolor*; puma, cougar) study is headed by Dr. Winston Vickers of the Wildlife Health Center at UC Davis (WHC). Dr. Fernando Nájera joined the UC Davis Wildlife Health Center as co-Director of Carnivore Projects in March 2023. David Garcelon of the Institute for Wildlife Studies (IWS) is the PI for the northeastern California part of the study, and Dr. Jessica Sanchez of the San Diego Zoo Wildlife Alliance was a key contributor to several study elements in Southern California. Dr.'s Vickers and Nájera prepared this report with contributions from the other project collaborators.

Overall activities and research into mountain lions by the UC Davis WHC have been ongoing since the first capture of a mountain lion in the study in March of 2001. Over the course of this long-term study, cougars were captured, sampled, and GPS collared in Orange, Riverside, San Diego, Modoc, Lassen, and Kern Counties, with some of the most recent captures in the Gabilan Range and Pacheco Pass either in or in close proximity to (Santa Clara and San Benito Counties) (Figure 2).

Figure 2. Geographic scope of our study.

Tissue samples have been obtained in the past from deceased animals in San Bernardino County, but no captures have occurred there. Activities of both types are anticipated in additional counties in the future.

The study in southern California (Figure 3) has incorporated mountainous areas including the Santa Ana Mountains (SAM) portion of the Peninsular Ranges (west of I-15) and the eastern Peninsular Mountain Ranges (ePR) east of I-15 (Figure 3), and has been conducted under the authorization of the California Department of Fish and Wildlife (CDFW) via a number of Memoranda of Understanding and Scientific Collecting Permits.

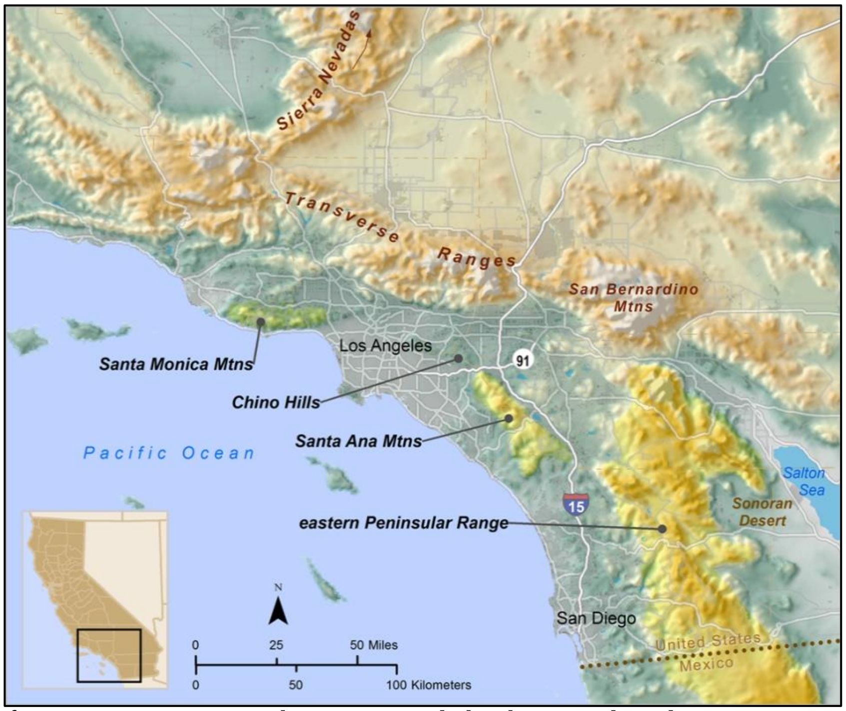


Figure 3. Southern California mountain ranges where WHC study has been conducted since 2001.

In 2016, an amendment to our SCP was approved to allow expansion of the study scope and region to study of cougar ecology and interactions with pronghorn antelope (*Antilocapra americana*) in the northeastern corner of California (Modoc, Lassen, and Siskiyou Counties) (Figure 4). This second part of our study is being conducted in collaboration with, and with the financial support of, Dave Garcelon and the Institute for Wildlife Studies (IWS). The first capture in that study area was in February of 2016.

Figure 4. Northeastern California study area.

A new 3-year Scientific Collecting Permit was approved in early 2022 that allowed further expansion of the study areas to the Tehachapi Mountain Range north of Los Angeles (Figures 5 and 6) and the Gabilan Range (Figure 7) east of the Santa Cruz Mountains and the Pacheco Pass area (Figure 8). The focus in these study areas is on connectivity across barriers, specifically SR58 and I-5 in the Techachapi Range, and US 101 and SR152 as well as other highways in the northern Gabilan and Diablo Ranges and Pacheco Pass areas. The first capture in those study areas was in late November of 2022.

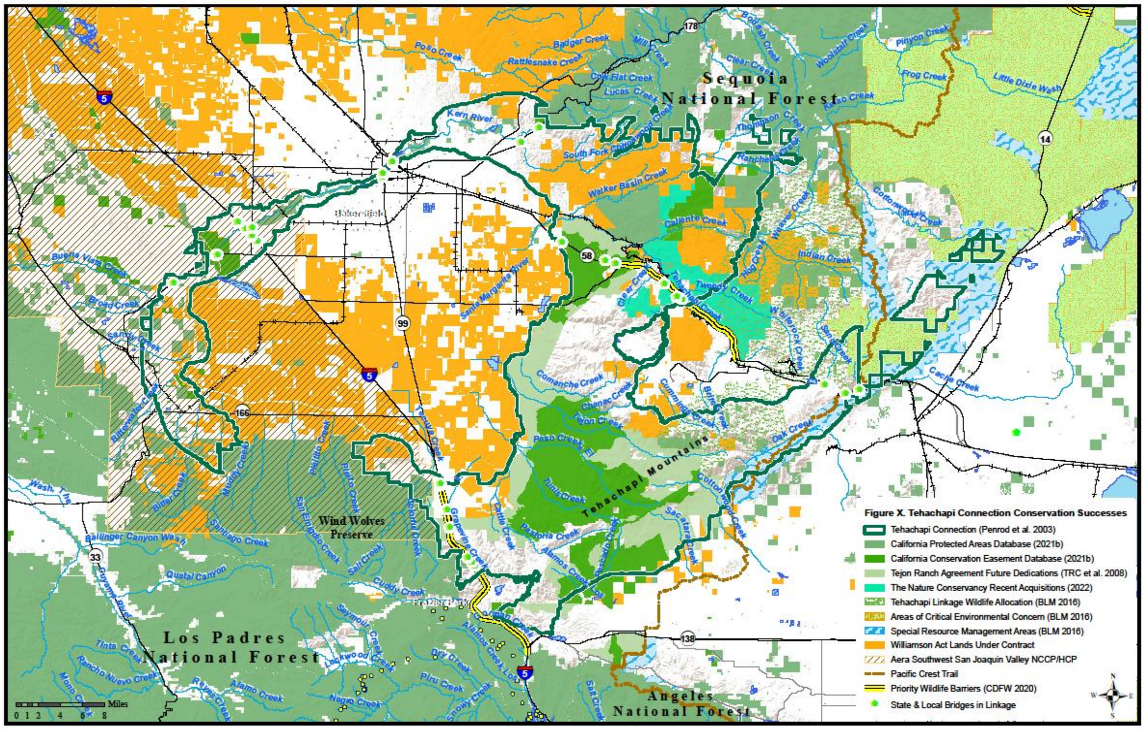


Figure 5. Tehachapi Mountain Range between the Sequoia and Los Padres National Forests, with land status designated.

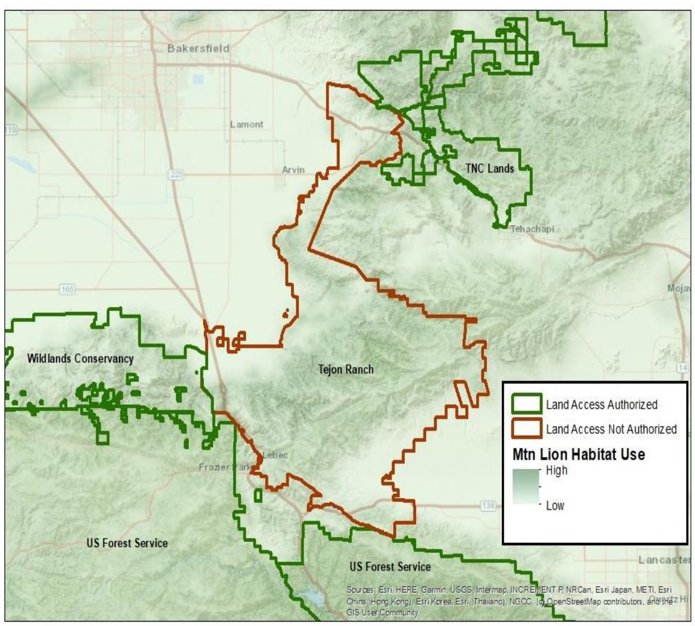


Figure 6. Tehachapi Mountain Range with land access authorizations for WHC research.

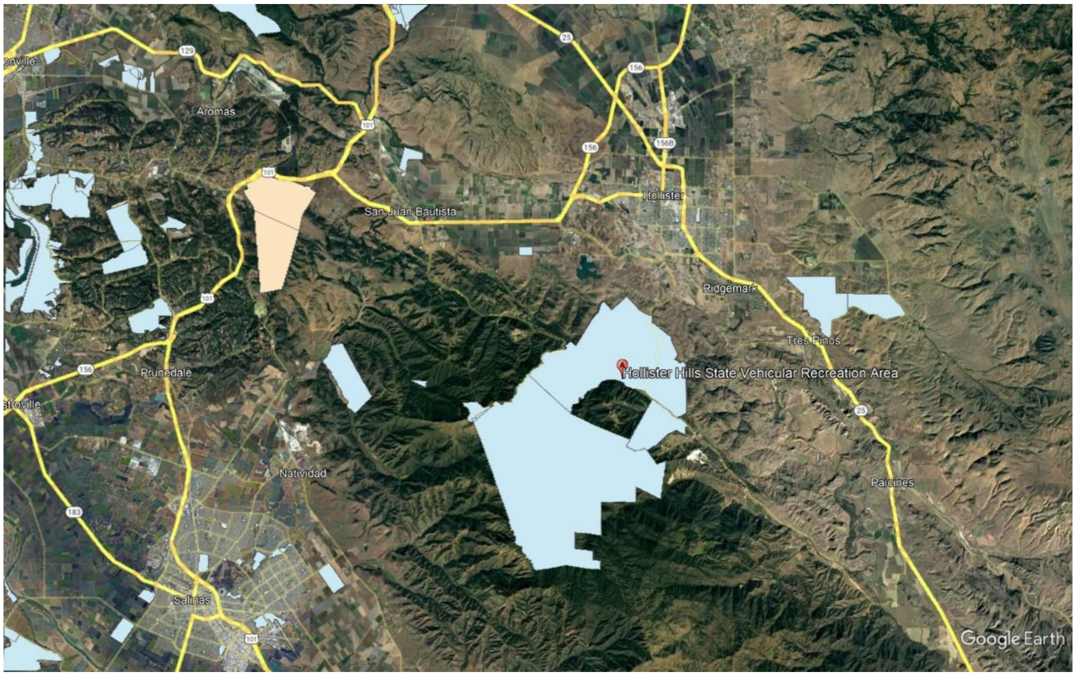


Figure 7. The northern Gabilan Range with conserved lands – Rocks Ranch (tan), Hollister Hills State Vehicular Recreation Area, the adjacent Gabilan Ranch, and other conserved areas (blue) shown.

Figure 8. The Pacheco Pass area with recorded mountain lion vehicle kills and photos noted. Conserved lands in blue.

Broad study goals are to collect data and conduct analyses to inform the understanding of mountain lions in California in a number of areas:

1) Health, infectious disease and toxin exposure patterns and diet, along with relationships to anthropogenic and landscape factors, and sources of mortality;
2) Landscape connectivity as measured by movement data, especially across highways; genetic; camera monitoring of crossing structures; and studying dispersal patterns in more developed versus less developed landscapes.
3) Habitat use and movement patterns in response to anthropogenic influences, fire and fire management, and during various reproductive states and dispersal
4) Assessment of population genetic status and levels of inbreeding depression
5) Development and comparison of non-invasive sampling and population estimation methods, with the special goal of developing best practices and concrete plans for closely monitoring the most at-risk populations such as the SAM population. These methods include opportunistic and systematic scat sampling, hair sampling, camera-based methods, GPS collaring, and others.
6) Methods of reducing conflicts between mountain lions, domestic animals and people, especially in wild areas utilized by people, and rural areas where livestock are often kept in mountain lion habitat. This involves developing and testing deterrent devices and assessing mountain lion interaction patterns with humans and domestic animals in peri-urban or rural areas, and wildland parks. It also involves education and outreach efforts regarding proper protective husbandry for domestic species.
7) Interactions with other wildlife species, especially species of special concern to CDFW such as pronghorn antelope in NE California, and possibly bighorn sheep in southern California.
8) The impacts of anthropogenic influences on mountain lion movement across highways and habitat use in and near other areas that contain levels of human influence.
9) The differences between major habitat areas in all of the areas of focus listed above by collaborating on comparisons between our own study areas as well as the area in Sonoma-Napa Counties where study is overseen by collaborator Dr. Quinton Martins.

As we are now, we will continue to collaborate with other mountain lion researchers and CDFW throughout the state. The results will feed into policy and public outreach programs to promote habitat conservation, persistence of mountain lion populations long term, and improved wildlife and human well-being.

A.2. Status of mountain lions in California and relationship to our study areas

Mountain lions in California have been characterized in multiple studies as being fragmented into multiple subpopulations, with barrier effects of highways and development likely to be the primary contributors (Gustafson et al. 2018, 2022; Figure 9).

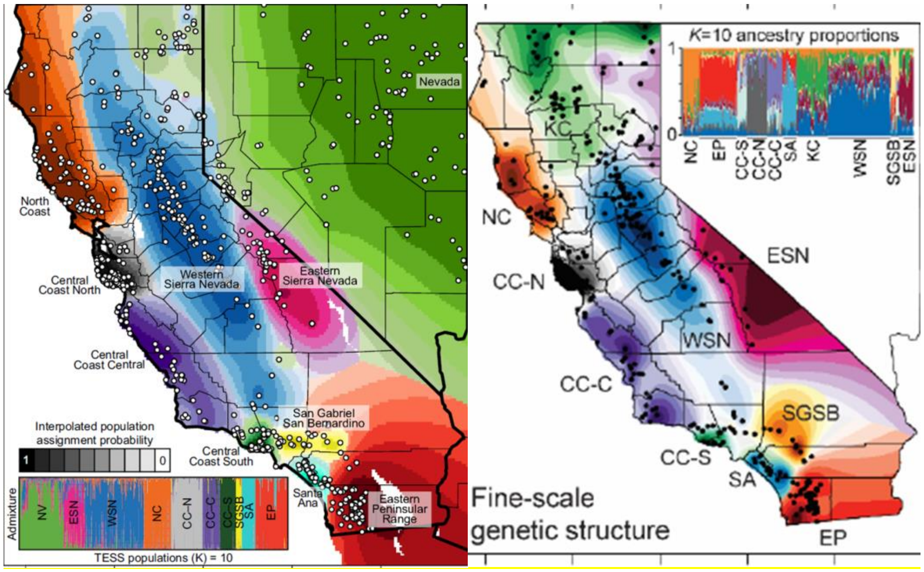


Figure 9. Mountain lion subpopulations in California based on microsatellite (left figure) and Single Nucleotide Polymorphism (right figure) based analyses (Gustafson et al. 2018,2022).

From Gustafson et al. 2022:
"Fragmented populations along the Southern Coast and Central Coast had particularly low genetic diversity and strong linkage disequilibrium, indicating genetic drift and close inbreeding." "Thus, extant variation at the broader scale has potential to restore diversity to local populations if management actions can enhance vital gene flow and recombine locally sequestered genetic diversity."

The two subpopulations in the Santa Ana Mountains (SAM) and Santa Monica Mountains (SMM) are the most seriously genetically restricted subpopulations, with mountain lions in both mountain ranges being second only to the federally endangered Florida panther in their level of inbreeding, and are at risk of extirpation (Benson et al. 2019). A Population Viability Analysis for the SAM population found that there is a 11–21% risk of extirpation in the next 50 years due to low annual survival, and demographic, stochastic, and environmental factors, and a near certain likelihood of extirpation within a median time of 12 years if inbreeding depression should occur. (Benson et al. 2019; Figure 10).

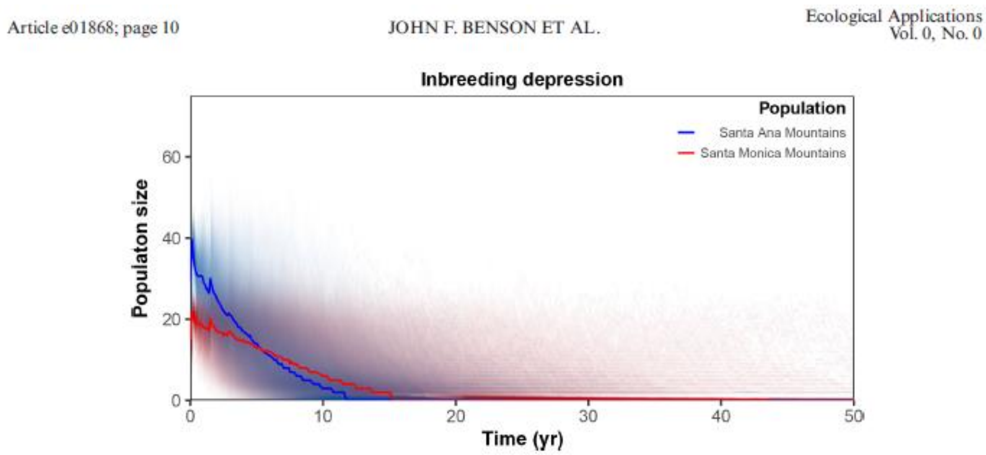


Figure 10. From Benson et al. 2019. Risk of extirpation of the SMM and SAM populations of mountain lions if inbreeding depression occurs in the populations.

The risk to the SAM and SMM subpopulations, as well as the ePR and San Bernardino and San Gabriel (SB/SG) populations over time, has led to a petition being filed to list all four of these subpopulations, plus the Central Coast and Santa Cruz Mountains populations, as threatened under the California Endangered Species Act (CESA). This petition has been reviewed by CDFW and their report relating to that review is currently being peer reviewed by several mountain lion researchers outside the agency.

Long-term viability of mountain lion populations in southern California and in the central coast of California is at-risk due to habitat fragmentation that limits connectivity and gene flow and contributes to deleterious effects of inbreeding (Benson et al. 2019). Though current work to restore connectivity in parts of this geographic area are underway (e.g., in the Santa Monica and Santa Ana Mountains), it is recognized that the success of these current efforts is still dependent on future efforts seeking to further restore connectivity throughout larger geographic area of southern California and the central coast of California (Gustafson et al. 2022).

Notably, the Santa Ana Mountains and the Peninsular Range in southern California must be connected to the Sierra-Nevada Mountains for these populations to remain viable (Dellinger et al. 2020a, Gustafson et al. 2022). This connectivity would be achieved through restoring connectivity that links the Peninsular Range to the Transverse Range and then the Tehachapi Mountains (Figure 11). In addition, the Santa Cruz Mountains, Diablo Range, Gabilan Range, and greater central coast area must be connected to one another, and to the Sierra-Nevada Mountains, for mountain lions to persist throughout the central coast (Dellinger et al. 2020a). There are several major transportation routes that would likely need improved wildlife crossing structures to realize such connectivity. Notably, I-10, I-15, US 101, SR-14, I-5, and SR-58 all need to be assessed with respect to current impacts on connectivity, and recommendations developed for improvement of passageways that allow safe wildlife movement.

Figure 11. Southern California Mountains with priority linkage areas. From South Coast Wildlands and The Nature Conservancy.

The Tehachapi Range is a key linkage between the Sierra Nevadas population of mountain lions and coastal and southern California populations. Highways SR58 and I-5 appear to be partial barriers to mountain lion and other wildlife movement through the Techachapis based on our 2023 research efforts there, and remain a focus of our study.

Though our GPS collaring efforts in the northern Gabilan and Diablo Ranges have resulted in only a three animals generating data so far, those animals have not crossed US 101 or SR 152. Further collaring efforts and data acquisition will better define the role that those highways play in mountain lion connectivity restriction.

Planning for high speed rail construction suggests that barrier effects will be exerted by the rail infrastructure as well as noise associated with train operation. These concerns apply to both the Tehachapi and Gabilan/Pacheco Pass study areas, and helped prompt this current study in order to establish pre-existing connectivity patterns in those area.

Addressing such connectivity issues requires a step-wise approach in implementing and utilizing multi-faceted data collection efforts, and also requires several years of effort to produce meaningful sample sizes and results. Via our suite of projects, we seek to ensure that viable wildlife populations persist throughout southern California and the central coast of California by: 1) understanding current wildlife connectivity and gene flow throughout these areas, and 2) informing efforts to restore connectivity throughout these areas by informing efforts to improve existing crossing structures and/or construct new wildlife crossing structures, as well as conserving key habitat areas, especially in wildlife movement corridors and adjacent to highway crossing sites. These measures require investment of public and private funds, and these investments can only be targeted for the most effect by utilizing accurate data from appropriate wildlife studies. Providing this data is a role that our organization has played for 20+ years in many areas.

A.3. Methods of data acquisition and use

In 2023, mountain lion landscape use and movement areas were detected via scouting / tracking and placement of trail cameras in Modoc and Lassen Counties (joint UCD-IWS project), in the Santa Ana Mountains, in the Tehachapi Range in the vicinity of SR58 and I-5, and in the Gabilan Range and Pacheco Pass west and east of the town of Gilroy respectively. These camera data were used to define areas that were most promising for potential mountain lion capture activities, and to better understand puma patterns of use in the Santa Ana Mountains for use in population estimation efforts and human interactions studies as described below.

As per our SCP, road-killed deer were collected and stored frozen for later use at bait sites. When regular cougar use of an area was detected, road-killed deer were placed at bait sites and checked daily. If cougar feeding was detected at a bait site, cage traps were set and monitored continuously via radio-transmitters affixed to the trap doors. Cameras that could transmit images from the site via cell systems were also used at trap sites whenever possible. Cage traps were used for all mountain lion captures in 2023, however, hounds are a tool that may be used sporadically in the future (see Summary of activities for each of the study sites). Traps were checked as quickly as possible (within half an hour in all cases) after detection of signals indicating a cage door had closed and/or receipt of cell camera pictures indicating a cougar had entered a trap. This assured that no animal was left within a trap unattended for lengthy periods of time, minimizing the potential for self-inflicted injury within the trap.

Anesthesia drugs and dosages listed in our SCP were administered by pole syringe, hand syringe, or dart rifle (in the case of hound captures). Capture methods were approved by the UC Davis Institutional Animal Use and Care Committee (IACUC Permit #22408).

Wildlife veterinarians Dr. Winston Vickers, Dr. Fernando Nájera or Dr. Jessica Sanchez, or Co-PI Dave Garcelon, administered or directly oversaw administration of all anesthesia, conducted and oversaw anesthetic monitoring, conducted all physical exams and sampling, and fitted all GPS-collars. Various students and wildlife biologists had opportunities to participate in captures as data recorders or other roles. We have been complimented on our attention to the well-being of the animals by the UC Davis Institutional Animal Care and Use Committee, and we feel that we have helped to set the standards of "best practices" for mountain lion captures and research.

Each animal captured had blood samples taken for assessment of exposure to infectious disease or toxins, and genetic analysis. Nasal, pharyngeal, and rectal swabs were taken in some cases for additional assessment of disease presence and microbiome analysis. Fecal samples were taken for assessment of intestinal parasites if possible, and a whisker was taken from most animals for other analyses.

Animals were examined, measured, weighed, ear tagged, and tattooed with their individual study ID number in one ear (unless anesthetic considerations dictated not completing one or more of these items). GPS-collars were applied if body weights exceeded 50 pounds. Cougars were released at the site of capture after recovery from anesthesia. All collars were equipped with either timed or on-demand drop-off units, or cotton “spacers” that rot off after a period of time, or some combination of those. This assures that collars drop off the animals after a period of time and are not worn long term.

All data acquired to date from GPS–collared cougars in the southern California, Tehachapis, and Gabilans/Pacheco Pass study areas is, or will be, curated by Brian Cohen, GIS specialist at The Nature Conservancy office in San Diego. Data from cougars collared in northeastern California is being curated by IWS personnel. CDFW regional biologists and certain state staff were notified of all capture attempts prior to their initiation and invited to observe, depending on the area. CDFW biologists or other personnel have had access to our collar GPS data when requested for their conservation and management purposes. All data has also been provided to the San Diego County Management and Monitoring Program (SDMMP), and other entities whose use of the data could help inform conservation action.

Aliquots of sera samples from all live-captured cougars were stored frozen for later forwarding to CDFW veterinarian Dr. Deana Clifford at the CDFW Wildlife Investigations Lab, and remaining blood, serum, etc is now stored at the University of California South Coast Research and Extension station in Irvine, CA, and at the IWS facility in Blue Lake, CA. All deceased cougars recovered in the study areas by the study teams were necropsied at the California Animal Health and Food Safety Lab in San Bernardino and UC Davis if the carcass was not too deteriorated for necropsy. Data on causes of death and locations were added to our overall mortality databases and will be used in analyses that are ongoing. Sample disease and toxin exposure analysis plans are being developed with the collaboration of CDFW and Dr. Jessica Sanchez and others at the San Diego Zoo Wildlife Alliance (see results section for an update on this part of our SoCal research).

During 2023, blood, tissue, and scat samples have been (or are being) forwarded to Dr. Mike Buchalski at the CDFW genetics lab for genotyping. These genotypes from all sources were used in a population estimation analysis completed in 2022 (Vickers and Dellinger 2022; see Appendix A from the 2022 Annual CDFW Report), and will ultimately be utilized in pedigree updating.

In November of 2022, Rogue Detection Dogs was contracted to conduct a range-wide scat collection effort in the SAM. Dr. Dellinger developed a sampling plan based on a grid structure across the range and target areas within each grid based on previous mountain lion GPS data and habitat analyses. Samples collected were also forwarded to Dr. Buchalski at the CDFW genetics lab for DNA isolation. Results have been incorporated in the statewide mountain lion density analysis that is being prepared for peer review.

Analysis for scat for metabarcoding to assess diet and parasite/other microbe presence is ongoing there under the oversight of Dr. Jessica Sanchez (see results section for an update on this part of our SoCal research).

Deer used as bait were monitored for signs of disease in cooperation with CDFW veterinarian Dr. Brandon Munk. A summary table of deer collected and examined for disease by IWS and UCD biologists is submitted via the Mandatory Wildlife Reporting System at CDFW. Samples of lymph nodes were stored frozen for later analysis by Dr. Munk.

Study Area	Number of lymph node samples
Tehachapis	54
Southern California	7
Gabilans Range-Pacheco Pass	17

Table 1. Summary of lymph node samples sent to CDFW.

Capture reports were forwarded to CDFW supervisors Dr. Deana Clifford, regional biologists, and other CDFW personnel specified in our SCP.

In order to provide a temporal insight on the trail use by mountain lions in Orange County Parks, we analyzed all the mountain lion detections captured by our cameras placed at OC Parks human trails. We also differentiated in this analysis Whiting Ranch Wilderness Park, due to the multiple incidences where mountain lions were sighted by park visitors or in a few cases had close encounters, either with some element of aggression including attack or apparent lack of fear on the part of the mountain lions involved. In collaboration with Orange County Parks, we are collecting data on mountain lion occurrence from an array of trail cameras that are deployed by OC Parks, the Irvine Ranch Conservancy, Felidae Fund, our team, and others to detect mountain lions across the landscape (Figure 12). This database will be utilized to enhance long term monitoring of the mountain lion population in the Santa Anas. This effort is funded by OC Parks and individual donors. The main areas where cameras have been deployed include:

- Irvine Ranch Conservancy
- Caspers Wilderness Park
- Audubon Starr Ranch Preserve
- O'Neill Regional Park
- Whiting Ranch Wilderness Park
- Santa Margarita Ecological Reserve
- Rancho Mission Viejo
- Rivers and Lands Conservancy Properties
- Chino Hills State Park
- And others

Camera array results are also being utilized by a Masters degree student at Long Beach State University to investigate mountain lion and human impacts on prey species occurrence in the Santa Ana Range. Photo array data for 2023 will be forwarded to CDFW on request after photo card processing is completed.

Development of a similar database for monitoring of mountain lion occurrence in San Diego County will occur in 2024 with funding from the San Diego County Association of Governments. This database will be developed in collaboration with USGS and other entities with active long term cameras in San Diego County.

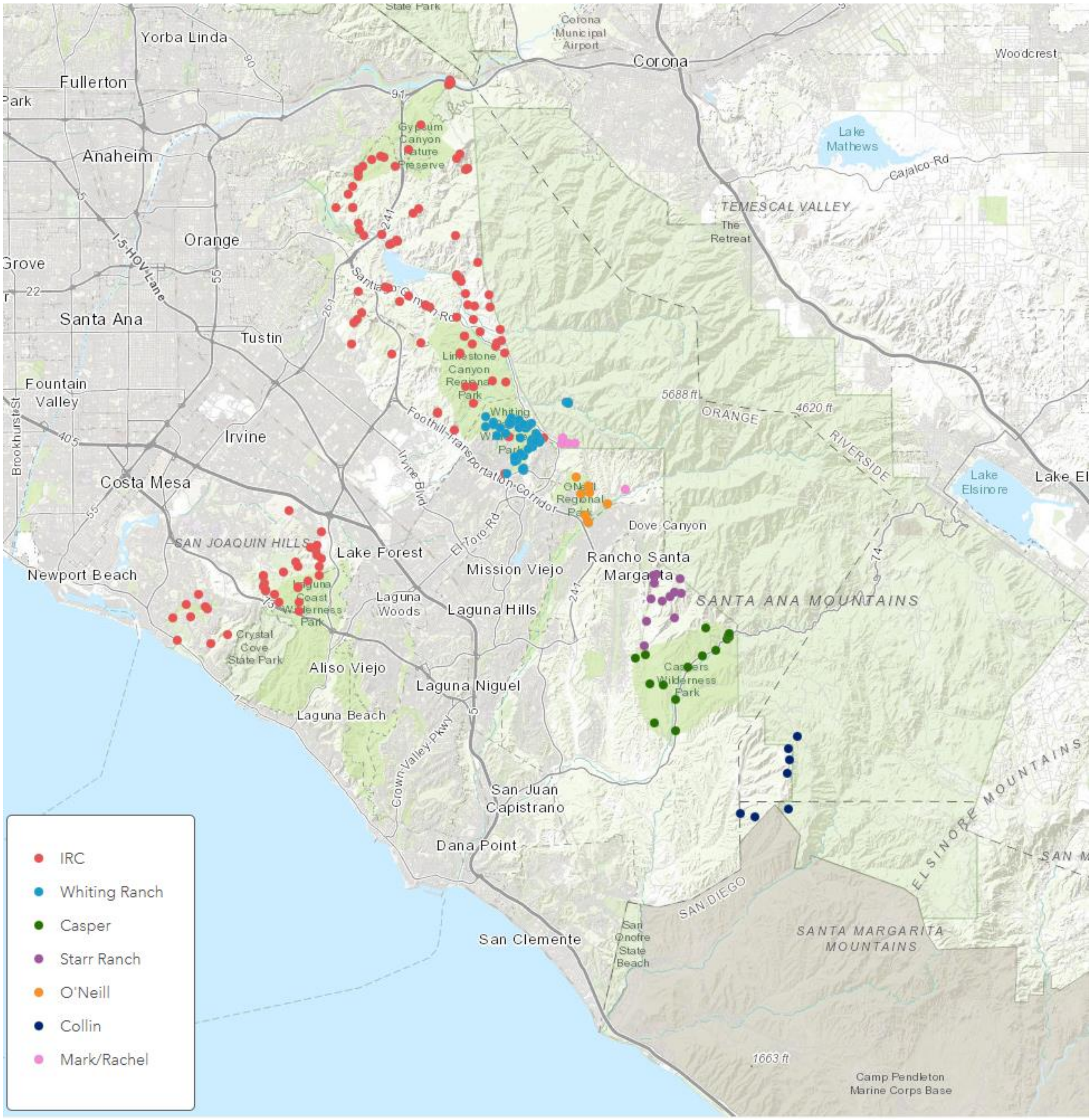


Figure 12. Locations of cameras in our Southern California study area.

In 2024, our team in concert with the UC Davis Road Ecology Center, The Nature Conservancy, and others will be initiating an extensive evaluation via cameras and tracking of use of crossing structures along I-10, the major barrier between the ePR and the San Bernardino/San Gabriel Range. This effort is funded by a new grant from the State of California 30x30 program. It is hoped that this evaluation will help guide future improvements to connectivity for mountain lions between those mountain ranges.

Whiskers from captured and deceased animals have been forwarded to Dr. John Benson's lab at the University of Nebraska, Lincoln, for inclusion in a statewide stable isotope based study of mountain lion diets being led by PhD student Kyle Dougherty. Dr. Benson and Kyle Dougherty have also been funded in 2024 to use our data and that of others to better define the likely trajectories of the different southern

California subpopulations under a variety of connectivity and mortality rate scenarios. That effort is also funded by the State of California 30x30 program.

Our team has also explored the future use of camera arrays as population estimation and monitoring tools. Analytical methods such as time-to-event and space-to-event modeling could potentially be applied effectively to camera data in such an effort. Exploration of a trial effort has occurred with CDFW and other collaborators, and will be explored further.

We provided GPS and camera data for use in a number of other analyses, and to entities such as Caltrans, CDFW, County Governments, and various partner conservation groups.

In order to better understand the effects on mountain lion behavior of electronic deterrent devices or other deterrent measures, we have placed devices at depredation sites, bait sites where collared mountain lions were feeding that were not subject to recapture, and along travel ways. We have also worked with electronics engineers and students to create new devices that could potentially be more effective, and have similarly tested those.

A.4. Results:

A.4.1. Capture and handling results over the entire study period (2001-2023)

The first mountain lion enrolled in the overall study was captured and GPS radiocollared in 2001. As of January 29, 2024, our study team has conducted 294 captures of 217 individual animals (including hand captures of a small number of kittens). The majority of these animals have been GPS collared unless they were too young or below minimum weights for collar placement. Several hundred thousand data points have been collected (Figure 13) and utilized extensively in numerous analyses and studies, and by CDFW and various other agencies and entities for conservation purposes.

Some animals have been recaptured several times with no evidence of ill effects from previous capture or GPS-collar placement (significant weight or condition change) being noted.

Of all the handling events, no animals were recorded as having significant injuries that were capture related except for one mortality that occurred in the early years of the study. Capture-related tooth damage or abrasions were sometimes noted but were rare (Dellinger et al. 2023), but we continue to monitor traps closely when set and sedate and remove animals from traps quickly. With hound captures we also take every precaution possible to prevent injury. We feel that the one mortality was preventable and have taken steps to assure that it does not ever happen again in the future. Despite the one mortality, we feel that our safety record during captures is consistent with or better than most other studies (Dellinger et al. 2023).

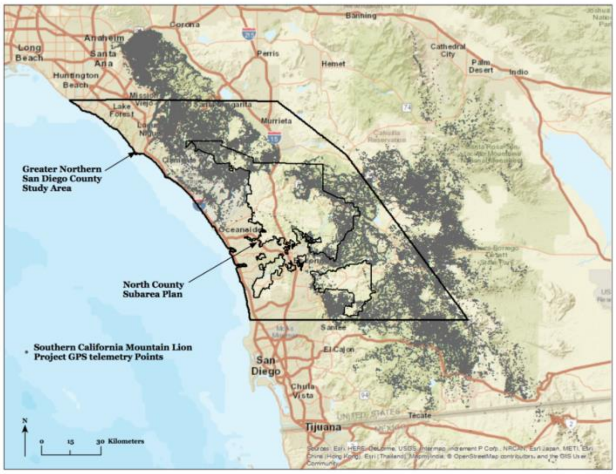


Figure 13. All collared mountain lion data points through 2016. (Vickers et al. 2017)

In addition, we have handled or sampled opportunistically more than 100 animals after mortality for DNA and disease/toxin assessment, and have collected DNA non-invasively via hair or scat from a large number of individuals (exact numbers dependent on pending analysis at the CDFW wildlife genetics lab).

Additionally, some data has been acquired from 3 cougars captured in a USGS study conducted in the northern Santa Ana Mountains, and those data have been incorporated in our analyses.

Over the course of this long-term study (since early 2001), GPS data points collected from cougars in the southern CA study area have indicated that they have utilized lands as far northwest as the 605 Freeway in the Puente Hills region of Riverside County and as far south as the Parque Nacional Constitución de 1857 located approximately 50 miles south of the border in Mexico. They have ranged from the Pacific shore in the west to the San Jacinto and Peninsular mountain ranges in the east (Figure 13). In the Modoc/Lassen Co. study area GPS collared animals have ranged as far north as into Oregon, and east into Nevada. In the Techachapis study area, the collared mountain lions have circulated south of SR 58 and west of I-5 to date, with just two individuals being able to successfully cross I-5, one of which also crossed SR 58, and one other animal being killed attempting to cross I-5. (see Summary of activities for the Tehachapi study area).

The status of collared cougars whose collars dropped off or stopped transmitting prior to recapture, and captured kittens that were never recaptured for GPS-collaring, is generally unknown. Some of those previously captured and/or collared cougars whose status is unknown are also likely deceased given the mortality rates for collared cougars in the study. Trail cameras have captured photos of some of these animals after their collars dropped off, and they were positively identified by their ear tags. Dates of these confirmations of live status are also recorded in our survival database. Mortalities of some of these individuals have been detected due to their ear tags or tattoos being noted in CDFW reports on depredations, vehicle strikes, or other mortality detections.

A.4.2. 2023 and January 2024 Results

A.4.2.1. Summary of activities in the southern California study area (Orange, Riverside, San Diego Counties)

1. In 2023 we continued assessment of multiple different methods of population monitoring. We have been exploring different camera data analysis for this purpose. In this regard, we have been tasked by the San Diego County Association of Governments with developing a long- term monitoring plan for the ePR population. In October 11th, 2023, UC Wildlife Health Center Davis organized the Southern California Mountain Lion Workshop that assisted to reveal what techniques are the most appropriate to perform long-term monitoring for the population of mountain lions in San Diego County. We have completed a literature review (Appendix A) and generated a draft long-term monitoring plan for the San Diego County mountain lion population that is in peer review currently (Appendix B).

2. We captured and GPS-collared three mountain lions in 2023 in the SAM and ePR for studies of human interactions, survival, movement, connectivity, population estimation, deterrent testing, and testing of a lighter weight solar powered collar (Tables 1a,b). One female (F312) was captured two times to replace a prototype solar GPS collar that malfunctioned two weeks after deployment. Samples collected at capture were also incorporated into disease and diet studies via serology, metabarcoding, and stable isotope analysis.

3. We continued to collect all deceased mountain lions (n=4; Table 3) that were known in the study areas when possible to submit for necropsy at the California Animal Health and Food Safety Lab to assess overall health and disease and toxin exposure, survival and sources of mortality, collect DNA, and collect physical, demographic, and other information. Some individual animals found were too deteriorated from age or trauma to have necropsies performed but tissue samples were still collected.

4. In 2023 we continued to monitor crossing structures along I-15 (culverts and the Temecula Creek Bridge) with trail cameras for connectivity assessment, and to help guide planning by The Nature Conservancy and Caltrans for improvements at the Temecula Creek Bridge and possible construction of a new wildlife crossing structure to the south of that location (see report: Interstate 15 Wildlife Crossings: Design Considerations For Focal Wildlife Species Santa Ana-Palomar Mountains Linkage Southern California, attached as Appendix C). We also led a multi-agency group planning for monitoring of crossing structures along I-10, and participated in planning with Caltrans and The Nature Conservancy for improvements to wildlife crossings on SR79, SR 78, SR76, I-8, and other highways in San Diego County, as well as SR's 74 and 241 in Orange County.

5. We are planning to use genetics with our partners at CDFW (M. Buchalski), and others to update previous pedigrees and test the ability of different DNA analysis techniques to detect and characterize close relationships between individuals, as well as their subpopulations of origin.

6. With the collaboration of personnel at the San Diego Zoo Wildlife Alliance (SDZWA) – Drs. Jessica Sanchez, Charlie de la Rosa, Matthias Tobler, and others- we are pursuing opportunities for deterrence testing to reduce depredation and prevent mortality for both pumas and domestic animals. During this time period, we faced some challenges that prevented us from testing deterrent devices at any of our baiting sites.

Historically, most of our deterrent work has focused on our collared male mountain lions that found bait placed for capture of other mountain lions, avoiding taking away access to their own kills. Our males

M316 and M313 naturally gained enough weight to grow out of their collar fit, so we activated the drop-offs and are not currently tracking them. Those two mountain lions were the primary subjects for previous deterrent testing. The male M321 dispersed to Camp Pendleton. We didn't have base access to work with him or monitor his fit, so we decided to drop his collar too. We have had females (F315 and F320) feeding at our baits but, since they were accompanied by kittens, we have not put devices in place and let them consume our artificial bait to avoid negatively influencing the mothers/kittens at all. he has not made kills at locations.
Future directions in this aspect of the project include testing new devices this coming season. One of them consists of a cellular camera with a built-in speakerphone feature to study the live reactions of the mountain lions to our voices. We are also working with volunteers to develop a custom device called an Ora with optional motion and light sensors, programmable sounds, and louder speakers.

7. In partnership with Dr. Jessica Sanchez at SDZWA, serum samples were sent to the Cornell Animal Health Diagnostic Lab for disease testing from mountain lions in the Santa Anas (77 samples from 43 individuals), Eastern Peninsular Range (74 samples from 50 individuals), Tehachapi (9 samples from 9 individuals), and Modoc (58 samples from 42 individuals) study sites. They will be tested for feline leukemia virus, feline panleukopenia virus, *Leptospira interrogans*, *Toxoplasma gondii*, and SARS-CoV-2. The results of this testing will be combined with results from other sites in California as part of a study examining how mountain lion habitat use is associated with pathogen exposure, with a special focus on the impact of human development. Once completed, these results will be published in a peer-review journal.

In regards to the dietary reconstruction, CDFW and Rogue Detection Dogs collected mountain lion scat from multiple sites across California from 2018 to 2022. DNA was extracted from these samples by Dr. Michael Buchalski at the CDFW Wildlife Genetics Lab and Dr. Ben Sacks at the UC Davis Mammalian Ecology and Conservation Unit as part of a study estimating the population size of mountain lions in the state. DNA extracts from 762 fecal samples, representing 10 study sites, were then transferred to Drs. Jessica Sanchez and Cynthia Steiner of the SDZWA, who utilized metabarcoding (next generation sequencing) techniques to identify the species of prey items present in the scats. To date, all samples were run using the 12S primer, resulting in 542 samples (70%) being successfully sequenced. Mammals are highly represented as prey items in mountain lion diet (with deer being the overwhelming majority), but birds, reptiles, and fish were also identified. The next step is to sequence the samples using the COIX primer, which will provide a greater taxonomic resolution on the species of prey items that make up the diet of mountain lions in California. Once completed, these results will be published in a peer-review journal.

8. We contributed genetic samples to a statewide mountain lion genome study being led by the University of California, Santa Cruz. This study is expected to yield dramatically new insights into the population genetics dynamics in California pumas.

9. We contributed whisker samples to a stable isotope analysis to determine prey species differences across the state that is being conducted by Kyle Dougherty of Dr. John Benson's lab at the University of Nebraska, Lincoln with the assistance of the UC Davis lab that conducts stable isotope analyses.

10. We collaborated with researchers from a number of other institutions (UCLA, U. of Texas, UC Davis, and the Universidad Complutense Madrid on a study of how mountain lions are affected by light and sound on approach to roadways. An article was published as a result in Philosophical Transactions of the Royal Society B (Barrientos et al., 2023). This information may inform mitigation strategies to reduce the barrier effect of roads on mountain lion movement, and roadkills.

Southern CA study 2023 capture detail:

In the southern CA study, 3 adult mountain lions were captured, sampled, and were GPS-collared in 2023 (Table 2a,b). Only one individual (F312) needed to be recaptured due to an early malfunction of the solar powered GPS collar that we were testing.

UCD ID	Capture Date	Method	Sex	Age (mo.)	Ear tag	Tattoo	Status
M317	2/22/23	Cage Trap	Male	18-24 (estimated)	none (ear torn from last tag)	317	Active
F335	5/22/23	Cage Trap	Female	18 (estimated)	335	335	Active
F312	5/26/23	Cage Trap	Female	46	148	312	Active
F312	9/18/23	Cage Trap	Female	50	148	312	Deceased

Table 2a. Statistics on mountain lions captured during reporting period in the southern CA study area.

UCD ID	General Location	County	UTM Zone	UTM Easting	UTM Northing	Injuries	Mort date	Mort cause	Transmitter frequency
M317	Irvine Regional Park	Orange	11	431512	3739297	Intraspecific wounds	n/a	n/a	160.07
F335	Gypsum Cyn	Orange	11	434814	3745217	None observed	n/a	n/a	160.7
F312	Saddle Creek / Live Oak	Orange	11	443001	3727703	None observed	n/a	n/a	none, solar collar
F312	Saddle Creek / Live Oak	Orange	11	443700	3727673	None observed	01/18/24	Roadkilled	160.91

Table 2b. Locations of captures and animal disposition/Transmitter frequencies.

One of the individuals collared, M317, has included wide areas of the Santa Anas and the Laguna Coast in Orange County, as well as the Chino Hills and Puente Hills in Riverside County since being collared in March of 2022. His 95% kernel density estimate (KDE) is 292.2 km^2; and 50% KDE is 74.7km^2 (Figure 14).

On the other hand, F312, road killed on January 18th 2024, heavily used the Orange County Wilderness Parks (Figure 15). This female was locally nicknamed “Uno” and she was regularly photographed by amateur photographers, and she is also observed by hikers and bikers in these parks. Her last confirmed offspring included four kittens, two of them were road-killed, one died due to disease, and we don’t know the fate of the last one since it hasn’t been recorded by our cameras for the last several months.
Her 95% kernel density estimate was significantly smaller than those recorded for females in the Santa Ana Mountains range previously (52 km^2 versus 193 km^2; Vickers et al., 2017; figure 16).

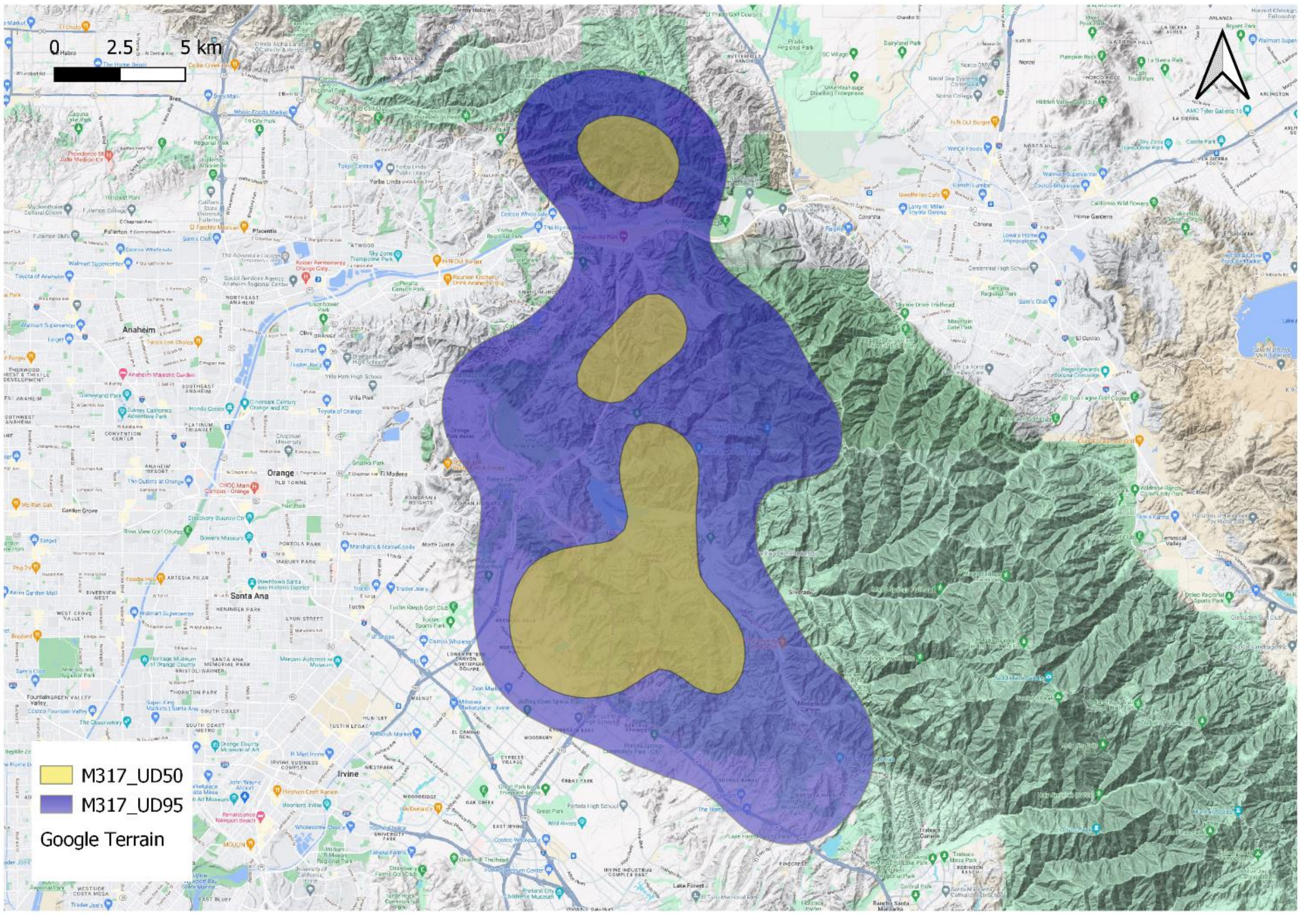


Figure 14. Kernel density estimates and overall land use for M317 obtained during the last 10 months.

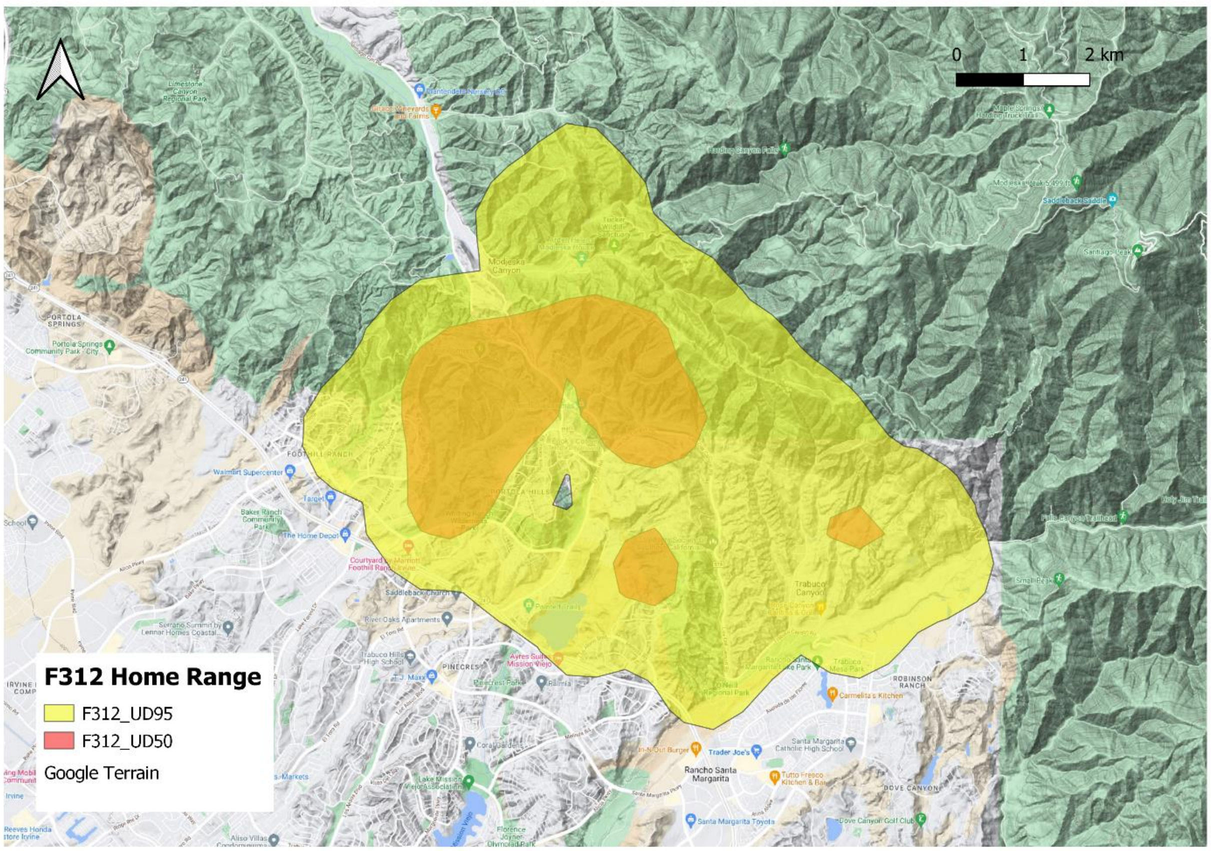


Figure 15. F312's 95% and 50% kernel density estimates: 95% KDE = 52.06 km^2; 50% KDE = 11.14 km^2

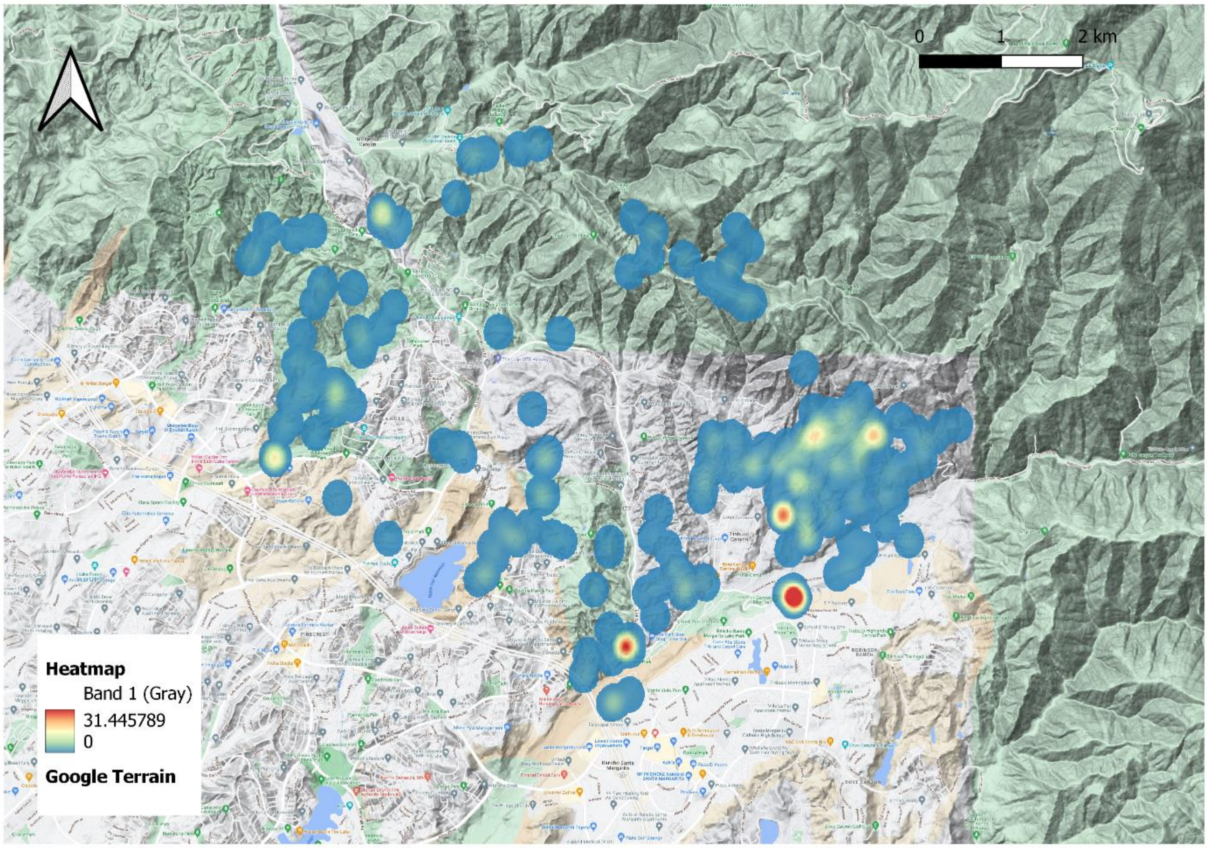


Figure 16. F312's heatmap shows the intensity of land use by this female.

On the other hand, F335's 95% KDE shows a regular home range size for a southern California mountain lion female (176.02 km^2; Figure 17).
Most of her locations are found east of the SR 241 toll road, with few incursions west of that road (mainly in Irvine Regional Park and Santiago Oaks Regional Park; Figure 18). Despite being considered a young female, her spatial data suggests that she has established a territory.

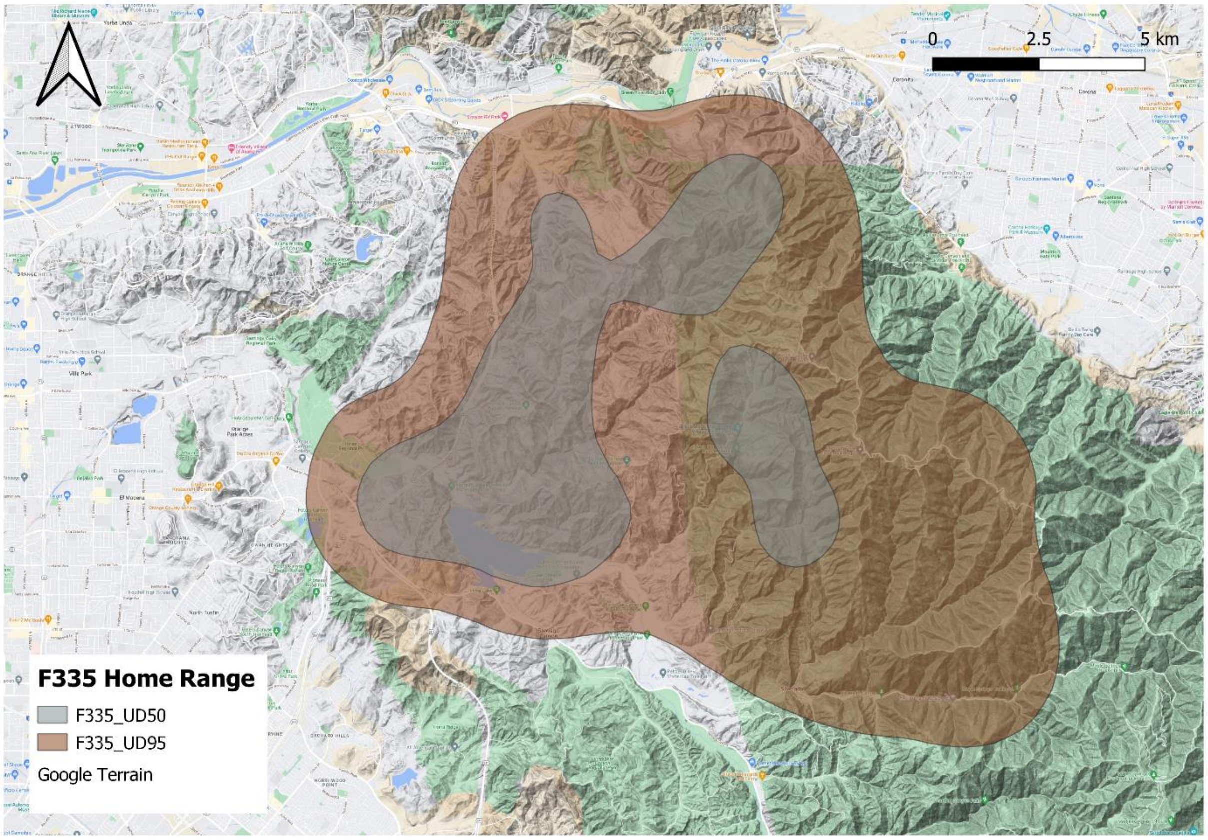


Figure 17. F335's 95% and 50% kernel density estimates: 95% KDE = 176.02 km^2; 50% KDE = 50.69 km^2

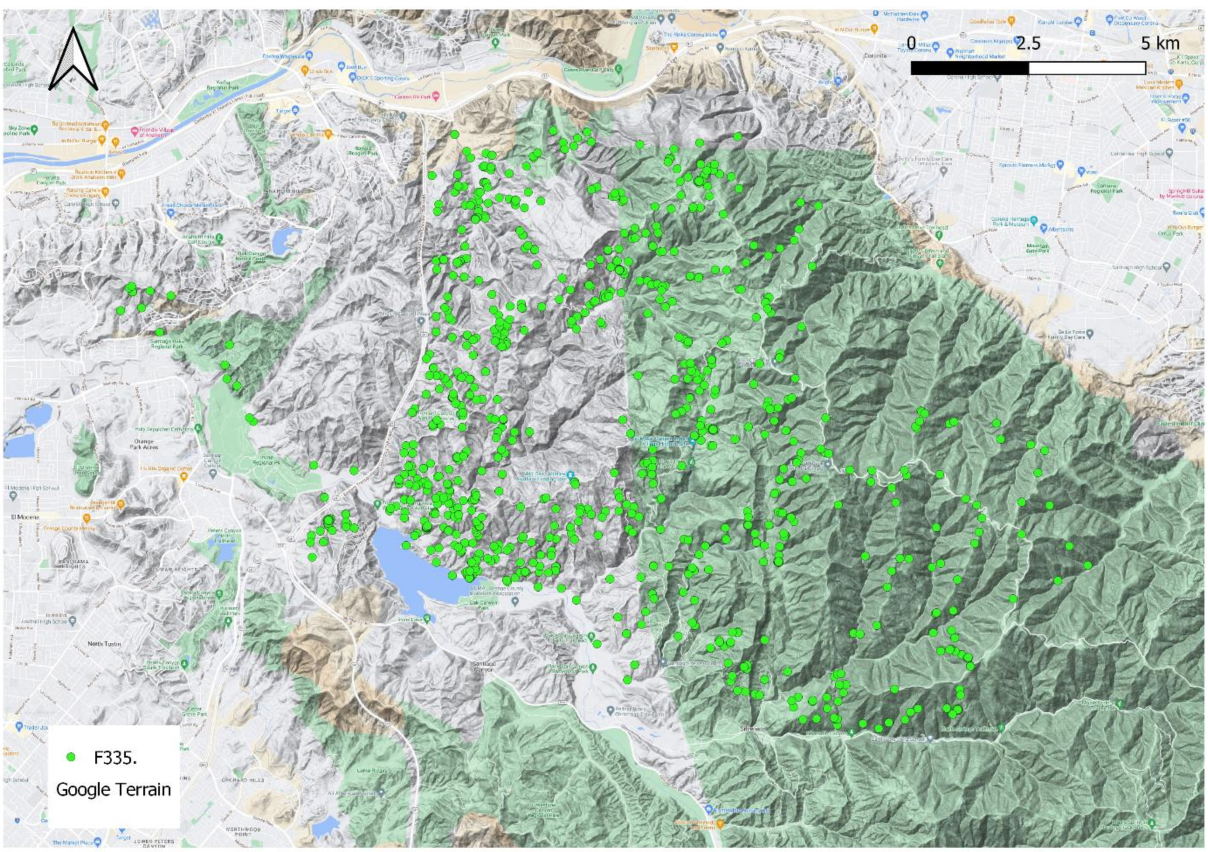


Figure 18. F335's locations during the study period.

Roads as sources of mortality and barriers in the southern California study area:

Except for a portion of SR 241 in Orange County, Los Patrones Parkway in Orange County, portions of SR 76 west of I-15, and along I-15 south of Temecula, no major highways in the region have adequate fencing to prevent cougar intrusion onto roads and funnel them to safe crossing structures.

Our team has studied multiple regional highways at nearly 200 sites where known mountain lion crossings had occurred (based on high frequency GPS data, cameras, and roadkill), least cost path modeling indicating likely crossing points (Zeller et al 2017, 2018), or expert opinion indicated likely crossings. All sites were examined in depth and all findings were documented (Vickers et al. 2020). Findings of that study included that most puma crossing points did not have adequate infrastructure present to allow safe passage, most crossing points had less than 50% of the land within a 500 meter radius conserved, and at most crossing points where adequate infrastructure was present, the land was not conserved at one or both ends of the structure (Vickers et al. 2020).

The lack of conservation at existing adequate structures was of special concern because of the potential for functional loss of some of the adequate structures due to development. Another major concern was that the majority of crossings were occurring at grade where no infrastructure existed, either crossing structures or exclusion fencing, and thus both mountain lions and drivers were being put at risk.

Given that our survival analysis indicated that females were killed as often as males on roads in this region and females are more crucial to the stability of the population, it increases the importance of decreasing this source of mortality as much as possible.

Our mortality data from 2023 and Jan 2024 brings home the relevance of roadways as a source of mortality for mountain lions in southern California. Of the seven known mortalities in the region, 85% (n=6) were from vehicle strikes. F307 was killed while depredating chickens in San Diego County (Table 3).

ID	Date of Mortality	Population	Sex	Cause of Death	Lat	Long	Age	County
M375	1/06/23	ePR	male	Vehicle (I-8)	32.815528	-116.526356	unk	San Diego
X376	1/20/23	SAM	unk	vehicle (Glenn Ranch Rd)	33.67395	-117.64205	~7months	Orange
F336	9/26/23	SAM	female	vehicle (241NB)	33.74297	-117.71865	unk	Orange
F307	10/13/23	ePR	female	shot (chicken coop)	33.18090	-116.97038	36 months	San Diego
X381	11/19/23	SAM	unk	vehicle (Santiago Canyon)	33.75994	-117.70295	unk	Orange
F312	01/18/24	SAM	female	vehicle (Santiago Canyon)	33.685169	-117.620836	54 months	Orange
X388	1/23/24	Chino Hills	unk	Vehicle (SR 60)	34.02520	-117.78537	unk	Riverside

Table 3. Overall mortalities in the southern CA study area.

In 2023 we continued to monitor crossing culverts on I-15, and Temecula Creek as it passes under the freeway south of Temecula, as well as Pechanga Creek east of its junction with Temecula Creek (Figure 19). During monitoring from 2018-2021, we observed only one mountain lion (uncollared) crossing under the Temecula Creek Bridge, and it crossed west to east. Two collared dispersal-age mountain lions crossed

I-15 west to east during 2021-2022. One crossed in Murrieta, and one near Escondido, but both returned to the Santa Anas after relatively short periods of time. In 2022-2023, two additional uncollared mountain lions crossed west to east under the Temecula Creek Bridge. No crossings have been recorded from east to west. Camera monitoring of the Temecula Creek Bridge crossing and I-15 culverts will be continuing in 2024. In addition, our monitoring data is being used in planning processes for improvements to Temecula Creek (vegetation modification, fencing, human presence reduction, etc.) to increase connectivity potential. This monitoring has been funded by the Nature Conservancy and the Wildlife Conservation Board.

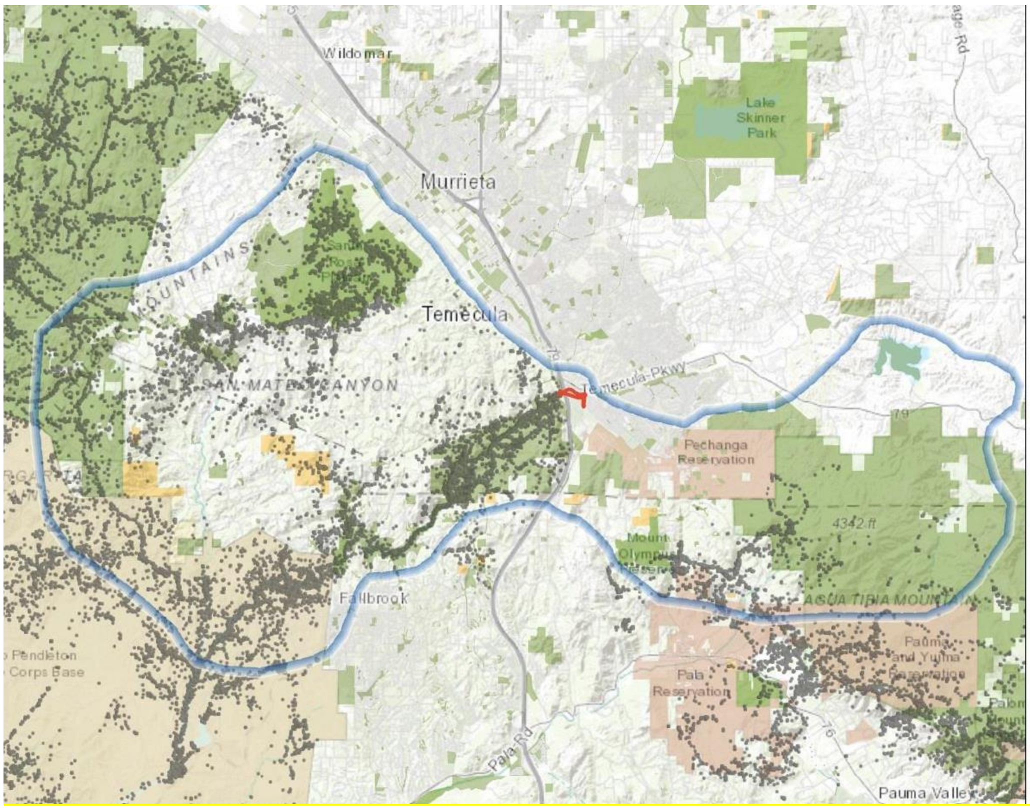


Figure 19. SAM to ePR linkage area with mountain lion data through 2020 depicted. Red line is Temecula Creek at the I-15 bridge area.

Between 2023 and January 2024, our GPS-collared mountain lions in southern California have crossed busy freeways, toll roads, and state highways 234 times with no mortalities until F312 was killed crossing Santiago Canyon Road in January of 2024. (Table 4). Santiago Canyon Road and Live Oak Canyon Road were not included in Table 4 due to them being less busy County or City Roads versus State or Federal Highways, though F312's death shows that they too can be deadly. M317 has been particularly busy crossing state and federal highways, as well as Santiago Canyon Road, with 156 highway crossings of the 91 Freeway and the 133, 241, and 261 Tollroads due to his wide ranging travels as noted in Table 4. This illustrates how mountain lions in southern California literally find it impossible to have territories in this landscape without crossing busy roads and highways.

ID	Roadway					
	74	**91**	**133**	**241**	**261**	**Total**
F312				51		51
F315	19					19
F320	2					2
F335				6		6
M317		28	1	115	12	156
Total	21	28	1	172	12	234

Table 4. Numbers of crossings of busy freeways, toll roads, and major highways in 2022 by 8 GPS-collared mountain lions.

Extensive tall and buried wildlife fencing with barbed wire outriggers (Figure 20) and safe crossing structures (bridges and large culverts; Figure 21), are present in the area where the majority of crossings of the SR241 and SR261 toll roads by collared mountain lions occurred. Since the construction of this fencing project, no mountain lion, bobcat, or deer have been killed in the fenced section (approximately 6 miles), and only a small number of coyotes. This is a dramatic reduction from the period before the fencing was built.

Figure 20. SR241 Wildlife Protection Fence on SR241.

Figure 21. SR241 Oak Canyon Wildlife Undercrossing.

Genetics, Disease, Mortality, and Toxins:

We continued to collect tissue samples with CDFW of deceased cougars in the study area, and record the locations and causes of death. We also have had every deceased mountain lion from the region necropsied at the California Health and Food Safety Lab if the carcasses were not too deteriorated. This will assist in assessment of health, disease, toxin exposure, and genetics of live and deceased cougars in the study area, as well as mortality patterns.

We had previously contributed serum and whole blood samples to a study of feline leukemia in pumas and domestic cats. That study was published (Petch et al. 2022) and determined that that the source of the virus in pumas is usually domestic cats but puma to puma transmission can occur, and pro-viral loads were higher in populations that were more genetically restricted. Because epizootics of feline leukemia have affected puma populations in certain areas, and puma to puma transmission has been demonstrated, this raises the level of concern about this virus's ability to affect the small genetically restricted population in the SAM. The proximity of pumas to domestic cats in the SAM makes introduction of the virus to this population a possibility.

Our study's previous findings (Vickers et al. 2015) and those of a statewide survival study published in 2023 (Benson et al. 2023) confirm that annual survival rates of collared pumas in our southern CA study area are low enough to be of significant concern for these populations. Both studies emphasize the need for long-term monitoring of the at-risk Santa Anas population, and the adjacent ePR population, major goals of our current work in southern California.

Deaths due to vehicle collisions and secondary to depredation permits are the first and second most common causes of death in our southern California study area. In 2023, vehicle collisions continued to be the most common cause of death that we detected. All mortalities except F307 and F312 were uncollared animals. We are unlikely to detect the deaths of uncollared mountain lions unless the cause of death is a a vehicle strike or depredation kill.

During this time period, we registered one mortality due to a depredation. Mountain lion F307 was shot while depredating on chickens. She was nursing at the time, which prompted a search for the kittens in the denning area showed by F307's collar data. Personnel from UC Davis Wildlife Health Center, CDFW, and SDZWA rescued 3 kittens during one week of searching (Figures 22, 23).

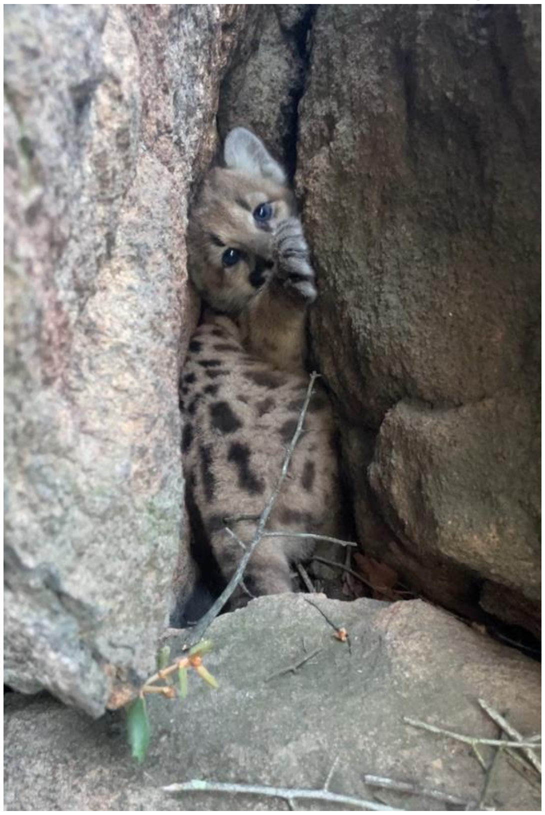


Figure 22. Mountain lion kitten in the den site.

Figure 23. Kitten being handled during the rescue operations.

Education and depredation prevention:

In 2023, we continued to educate and inform the general public, livestock and pet owners, agency personnel and managers, and others about mountain lions and how to most successfully coexist with them in the southern California landscape via prevention of depredation events.

In 2023, our team continued to assess the effectiveness of different hazing and deterrent devices on cougars and is collaborating with other mountain lion researchers and CDFW in that effort. We have had conversations with CDFW state personnel and those in southern California and in the Tehachapi Range to collaborate in collaring efforts for conflict cougars as well as assisting with deterrence device testing and placement if livestock predation by cougars occurs in our study sites. Although we have not had the chance to implement deterrence devices at depredation sites in the southern California or Tehachapi study areas during 2023, we had the opportunity to collaborate with CDFW in the Gabilan Range, where we collared a dispersing male that had depredated alpacas and goats. We assisted CDFW in educating that owner and neighbors regarding depredation prevention and husbandry practices.

Developing and comparing population monitoring methods for the Santa Ana Mountains.

In 2021, we also began collaborating with USGS, Panthera, IWS, and the Sonoma/Napa County Living with Lions project to test the use of photos to potentially identify individual mountain lions for use in spatial capture-recapture modeling. Several thousand photos of known mountain lions from several study areas were incorporated in the library of images that initially were being analyzed by Dr. Jeff Tracey of USGS. In 2023 Dr. Tracey had to forego his work on that project due to other commitments and our team is currently looking of other partners to continue those efforts.

Another monitoring method, estimation of the population density based on DNA from scat collected systematically by scat detection dogs across the Santa Ana Range, was tested by conducting collections in this way in November 2022 (see 4th Quarter 2022 report to CDFW, Appendix B). Rogue Detection Dogs was contracted to do the scat collections as they had in multiple other parts of the state previously for CDFW. Isolation of the DNA from the scat samples and genotyping was conducted by the CDFW Wildlife Genetics Lab. Individual ID's were assigned to each scat that had DNA isolated that could be assigned an ID. All collared individuals in the range except one were detected by the scat dogs, as well as two individuals that were collared previous to our current efforts. Results of those analyses were included in a statewide population estimate being prepared for peer review by Dr. Dellinger and coauthors, including Dr. Vickers. Results are being utilized to compare to density estimates derived from hair, scat, and other collections and reported in Vickers et al. (2022; Appendix A). Results of population estimates for the SAM were obtained during 2023, yielding a total of 41 individuals (20 males, 15 females, and six unknown). Age distribution of the majority of those is not known, so this count does not reflect adults only.

The density calculations that resulted when the Santa Anas results were included in the statewide analyses were (dependent on model), 1.84 individuals/100 sq km (telemetry prior model) or 1.5 individuals/100 sq km (no telemetry prior model). Calculations were based on scat dog efforts in the main body of the Santa Anas. Scat dogs were not utilized in the Chino Hills due to overall lesser habitat quality there and minimal evidence of regular mountain lion use, though M317 does utilize a small part of that area as part of his territory. Abundance calculations based on the approximate habitat value in the SAM of 2,000 sq km results in median values of from 30 – 37 individuals in the range, less than the minimum count in 2022, but generally consistent with the abundance estimates derived from the earlier study using hair, scat, and tissue-blood DNA combined. Density estimates for the ePR in the statewide study were lower at from 1.3 – 1.57 individuals/100 sq km.

Analysis of population viability in the Santa Ana Mountains:

A population viability analysis published in 2019 (Benson et al.) suggests that increased connectivity and movement of mountain lions from the adjacent areas into the Santa Anas appears to be key to the viability of mountain lions in the Santa Anas in terms of reducing probability of extinction due to both demographic and genetic factors.

With immigration at a low level consistent with what has been documented during the last 15 years (3 males; Gustafson et al. 2017), the likelihood of extirpation of the population over the next 50 years is uncomfortably high (1 in 5 or 6 chance). At the current or lower levels of immigration, genetic diversity (heterozygosity) is predicted to decline substantially in the next 50 years raising concerns about inbreeding depression (Benson et al. 2019). If inbreeding depression develops then extirpation in a much shorter time frame (<15 yrs) becomes very likely without intervention (Benson et al. 2019).

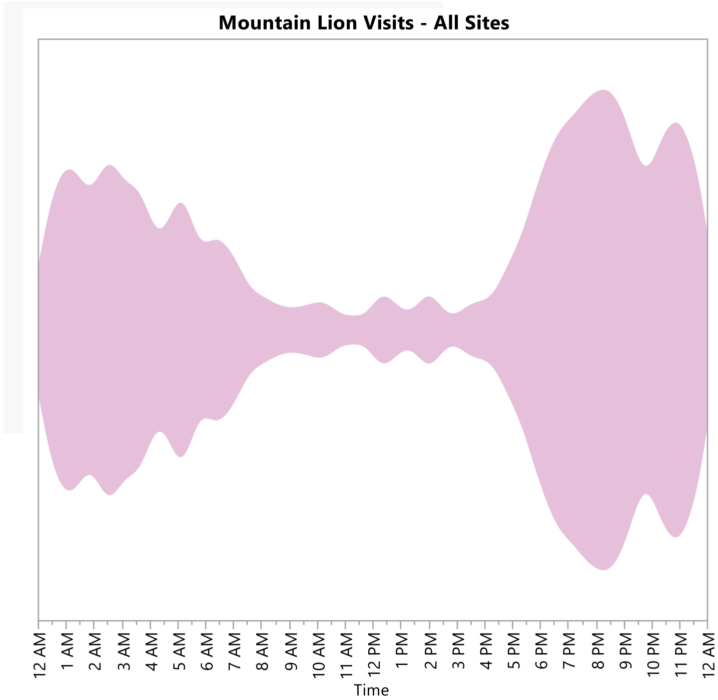
Initial results of semen quality analysis in 2020 from two male mountain lions in the study area that died (one from the Santa Anas and one from the eastern Peninsula Range population) teratospermia was present (high percentages of abnormal sperm; Huffmeyer et al. 2021). Poor semen quality was also documented in the SMM populations and new observations of kinked tails were noted there. These findings of signs of inbreeding depression amplify concerns relating to future decline of both of those relatively isolated populations.

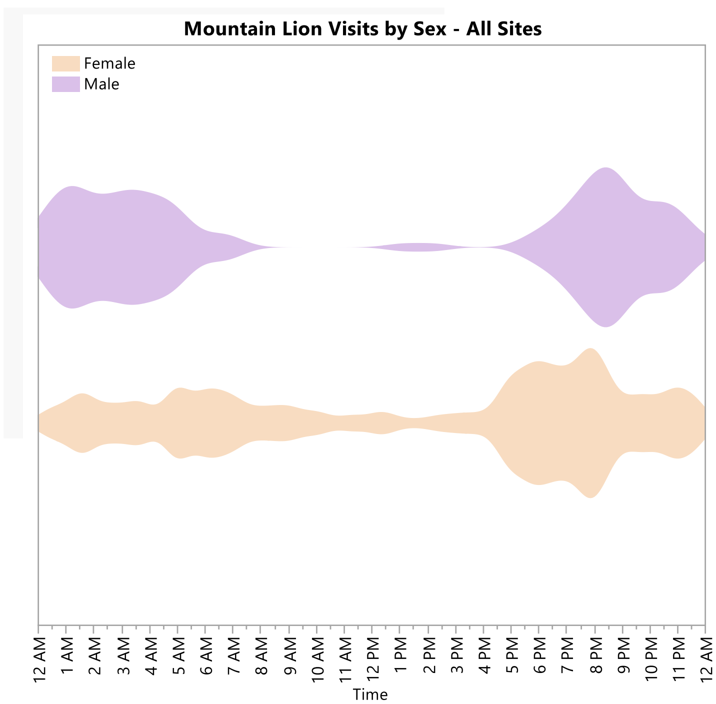
Subsequently, an analysis of semen from an additional deceased male from the SAM yielded inadequate sperm numbers due to the young age of the male (17 months). Further analyses will be done as opportunities for sampling arise. Similar assessments are ongoing in the Santa Monica Mountains population and our collaborators at the SDZWA reproductive lab are assisting in these evaluations.

These findings highlight the importance of continuing to monitor the SMM and SAM populations closely, and slow the rate of loss of genetic diversity to avoid the potential for compromised demographic performance due to inbreeding depression. Dr. Benson’s results suggest that movement of individuals into the Santa Ana’s needs to occur at a higher rate than we have documented to date in order to avoid the projected further loss of genetic diversity. This again highlights the importance of maintaining and increasing linkages that allow mountain lions to move from adjacent areas into the Santa Anas.

Temporal use of human trails by mountain lions in Orange County Parks.

In order to inform Orange County Parks personnel of the activity peaks in human trails of those mountain lions that heavily use these Parks, we analyzed camera trap data from a large array of cameras maintained by Parks personnel and our team to determine what times of the day mountain lions have been detected on trails. Our data indicates that activity on trails starts to escalate from 5:00 to 6:00 pm with a peak of activity between 8:00 and 9:00 pm. Detection rates begin to decrease after this time frame although very gradually. The lesser number of detections occur from 9:00 am to 4:00 pm. Between sexes, it seems that males decrease their activity more on those trails during the central times of the day than females (Figures 24, 25, 26). We also analyzed data pertaining just to Whiting Ranch Wilderness Park since this park is heavily visited by hikers, mountain bikers, and trail runners, with a level of visitation estimated to be over 97,000 visitors per year just prior to the SARs CoV2 pandemic (Monz et al. 2019). Whiting Ranch data showed a similar temporal pattern to all data combined (Figures 27, 28, 29).





Figures 24 and 25. Diagrams showing temporal activity of mountain lions in Orange County Parks trails.

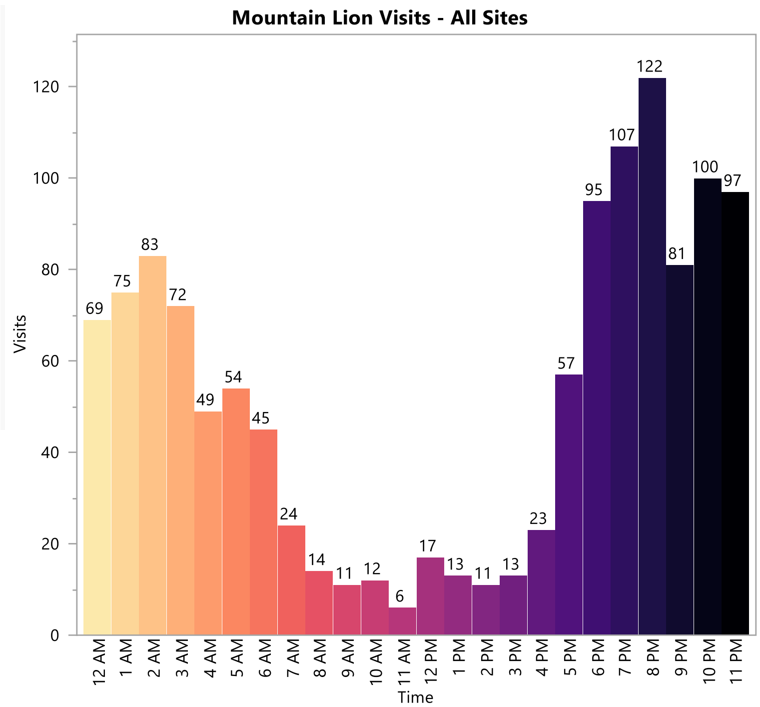


Figure 26. Photographic rates by time of the day in Orange County Parks trails.

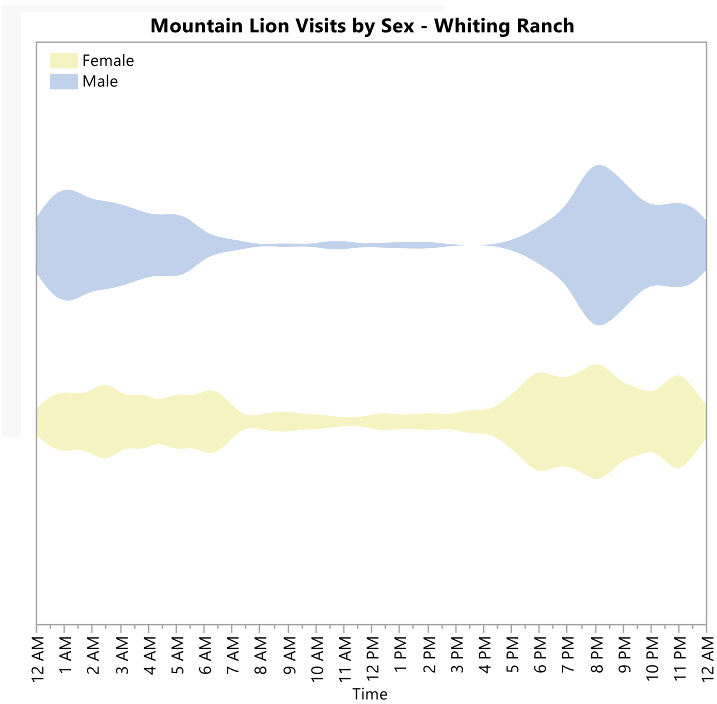


Figure 27. Temporal activity by sex in Whiting Ranch.

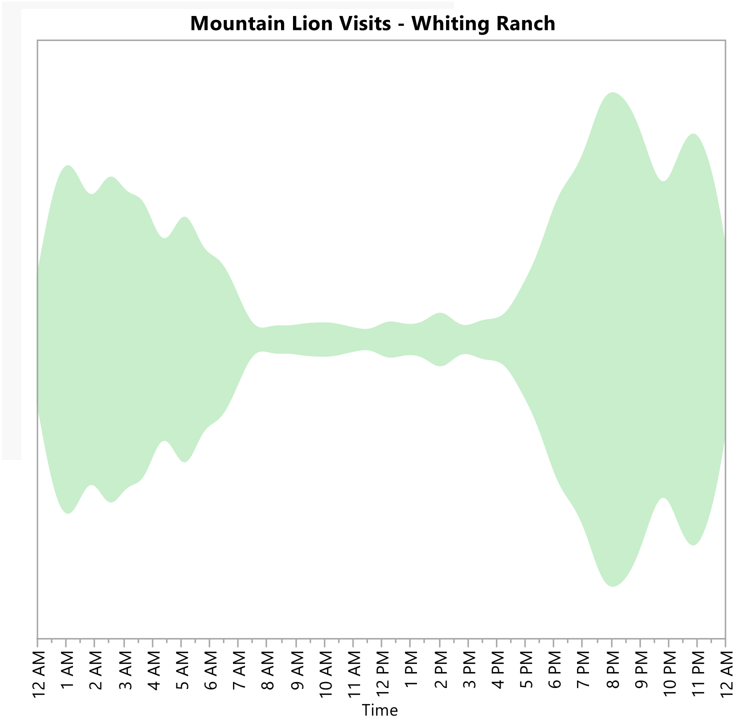


Figure 28. Overall temporal activity in Whiting Ranch trails.

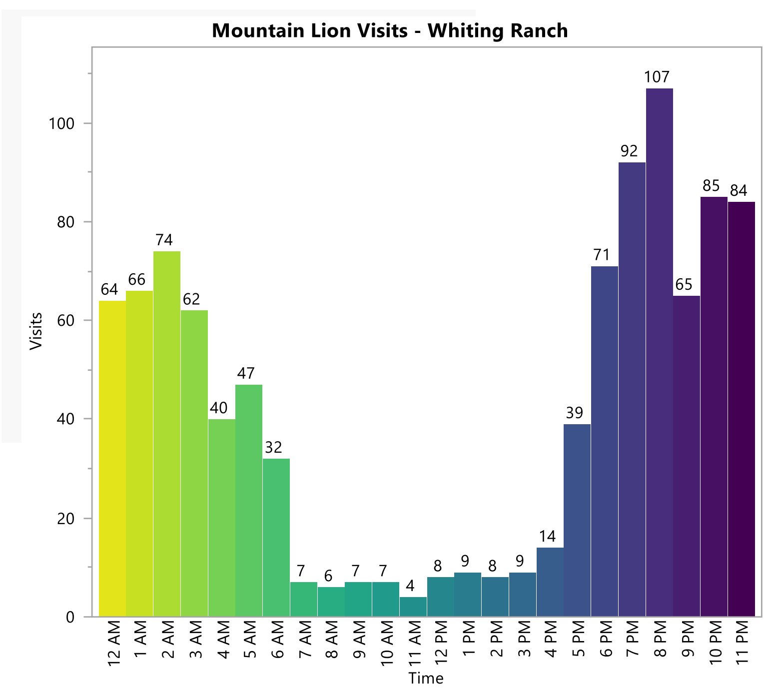


Figure 29. Photographic rates by time of the day in Whiting Ranch trails.

We are working with Orange County Parks staff to increase training of Rangers and education of Park visitors about mountain lions in order to minimize the likelihood of negative outcomes of encounters. We are also working with faculty and a Masters degree student at Long Beach State University to develop a graduate level analysis of the impacts of people and mountain lions on other wildlife in the Park and surrounding area.

A.4.2.2. Summary of activities in the northeastern California study area

NE CA study capture detail:

From 1 January through 31 December 2023, six mountain lions were handled on the NE CA project (Table 4a,b). Five were adults (1 male, 4 females), and one was a juvenile male. The five adults were all GPS collared or re-collared, four of which were recaptures. Of the four adult lions that were recaptures: M216, a 55-month-old male whose previous collar had failed on 9/26/20 was recaptured in April; F204, a 63-month-old female whose collar had also failed was recaptured in December. All captures went without incident.

ID	Date	Method	Sex	Age	Ear Tag	Tattoo
F340*	2/5/2023	Cage Trap	F	120+	340-R-Org	N/A
F199*	3/17/2023	Cage Trap	F	77 mo.	199-R-Org	N/A
M342	6/18/2023	Dogs	M	11 mo.	342-L-Org	342-R-Blk
F343	6/21/2023	Dogs	F	25 mo.	343-R-Org	343-L-Blk
F272*	6/23/2023	Dogs	F	85 mo.	272-R-Org	N/A
M166*	7/19/2023	Cage Trap	M	133 mo.	Missing	N/A

Table 4a. Statistics on mountain lions captured during reporting period in Modoc and Lassen counties, California. *Indicates a recaptured mountain lion.

ID	Gen'l locale	County	UTM Zone	Easting	Northing	Injuries	Mort Date	Mort Cause	Trans. Freq.
F204*	Jess Valley	Modoc	10	727629	4568718	None	2/28/2023	Shot: self-defense	173.021
F340	North of Big Sage Res.	Modoc	10	692170	4609968	None	2/6/2023	Pulmonary hemorrhages	173.330
F199	Mud Lake2	Modoc	10	715447	4565048	None	N/A	N/A	173.330
M342	Mosquito Creek	Lassen	10	735068	4560570	None	N/A	N/A	160.560
F343	Devil's Garden, NW Tramp Springs	Modoc	10	677216	4636425	None	N/A	N/A	173.350
F272	South Warner Mountains	Lassen	10	733828	4557350	None	N/A	N/A	173.165
M166	Timbered Mountain	Modoc	10	687069	4622435	None	N/A	N/A	173.375
M216*	W. of Hyw 395; Modoc Co. Road	Lassen	10	708069	4558287	None	3/22/2023	Highly Pathogenic Avian Influenzia	173.675
M283*	Parker Creek	Modoc	10	721686	4592496	None	2/19/2023	Unknown; carcass not recovered	173.625
* Captured Prior to 2023									

Table 4b. Locations of captures and animal disposition/Transmitter frequencies

We had three mortalities of collared mountain lions during the reporting period. An older adult female mountain lion (F340) was captured for recollaring on 2/5/23 and the work-up went without incident. While F340 walked away looking fine after she recovered, she was later found lying in the snow and while responsive would not move upon our approach. After leaving and consulting with Dr. Winston Vickers, we returned in the morning to check on F340 and she ran away after we approached closely. A mortality message was received later in the day on 2/6/23 and the carcass was recovered on 2/7/23. Necropsy results found severe acute generalized pulmonary hemorrhages, vascular bacterial emboli in several organs and other preexisting conditions. We received a mortality notification on 2/20/23 from a collar on an adult male mountain lion (M283). Unfortunately, no staff were available to investigate the notification until 3/6/23. M283 was located on a hillside above a creek and was lying in the open in the snow. There were no signs of injuries and he appeared to be in good flesh. Due to the weather and terrain conditions we were unable to recover the carcass for necropsy. On 2/28/23 an adult female (F204) was shot by a ranch hand when he said he was surprised after she jumped up from behind a dead horse that she was feeding on. CDFW law enforcement investigated the incident. On 3/22/23 we received a mortality notification from the collar worn by an adult male mountain lion (M216). When investigated on 3/24/23 the M216 was found lying in the snow under a tree with no obvious injuries and in apparent good flesh. Biologist John Randolf then proceeded to examine the cluster of GPS points that M216 had been at since 3/16/23. There he found two dead juvenile mountain lions that had been fed upon and cached. John recovered the carcass of M216 and the following day he returned with a CDFW warden who investigated the scene after which the two juvenile lions were recovered. All three mountain lions were later transported to the WHL for necropsy and testing. All three tested positive for highly pathogenic avian influenza (HPAI), with the virus being the confirmed cause of death for M216 and one of the juveniles. Exact cause of death could not be ascertained with certainty for the second juvenile, but due to the presence of the virus in the carcass it was presumed to be contributory.

NE CA Project Research Detail:

All six of the mountain lions captured in 2023 tested negative for FIV and FeLV using the Witness Antibody Test Kits (Zoetis Inc.).

We are continuing to investigate dispersal of juvenile mountain lions from the NE California study area to determine more about dispersal corridors used as well as survival during the dispersal process.

As part of our efforts to learn more about denning behavior and kitten survival we will be continuing to visit den sites of GPS collars females to mark kittens and collect genetic samples.

We are involved in a study being conducted on feral horses in the Devil's Garden region of Modoc National Forest in collaboration with the US Forest Service, US Geological Survey and the Utah State University. The objectives of this study include determining horse population size, distribution, and movements, along with examining the impact of mountain lion predation on population trajectory. We will be continuing to collar mountain lions in the Devil's Garden and will investigate their kills to determine the number and age of horses taken by mountain lions.

We are discussing a collaborative study with CDFW and UC Davis on impacts of wolves and mountain lions on elk and deer where the species overlap in the NE California study area.

How Data from the NE CA study is being collected and used for mountain lion conservation throughout California:

-Contributed to paper on habitat selection of mountain lions in California (Dellinger et al. 2020; J. Wildl. Manage.)

-Contributed data to a paper on statewide mountain lion survival in California (J. Benson et al. 2023; PNAS)

-Contributed to a paper on mountain lion capture procedures (Dellinger et al. 2023; Wildl. Soc. Bull.)

-Transferred whiskers collected from mountain lions captured in NE California to Dr. John Benson at University of Nebraska who is collaborating with CDFW and other statewide researchers on a project using stable isotopes to examine food habits across California

-Collaborating on a project to compare disease exposure of mountain lions across California in relation to human population density (with Dr. Jessica Sanchez, San Diego Zoo Wildlife Alliance)

-Completed a draft manuscript for submission from a master's thesis at Humboldt State University (IWS supported this graduate student research) examining microhabitat kill site selection by mountain lions in Modoc and Lassen counties

-Supporting a graduate school project (Utah State University) to examine dispersal behavior of mountain lions from NE California and Nevada (Randolph et al. manuscript in prep)

-Supported field work by a graduate student at University of Nebraska working on a thesis involving the influence of anthropogenic features on kill site selection by mountain lions in the North Bay

-Currently working on a paper examining denning behavior of mountain lions in Modoc and Lassen counties (Brinkman et al. in prep)

-Currently working on a project to examine familial/pedigree analysis of mountain lions in northeastern California using genetic samples (Analyses pending).

A. 4.2.3. Tehachapi Mountains Project

Current efforts are underway to improve habitat connectivity among isolated mountain ranges in southern California to support sustainable wildlife populations, including the Santa Monica, Santa Ana Mountains, Tehachapi Mountains and the Transverse Range. However, data show that the success of these current efforts is ultimately contingent upon restoring connectivity between these isolated mountain ranges and the Sierra Nevada Mountains (Figures 30, 31). Thus, to successfully ensure viable wildlife populations in all portions of southern California, and along the central coast of California, there is a great need to better understand current wildlife connectivity and gene flow across existing major transportation routes.

To that end, in July 2022, we initiated our study in the Tehachapi Range with funding from the Wildlife Conservation Network (WCN) and The Nature Conservancy (in-kind). The Tehachapi Mountains have substantial prime mountain lion habitat, and we feel it is critical to understand as best we can the movement of mountain lions through the Range.

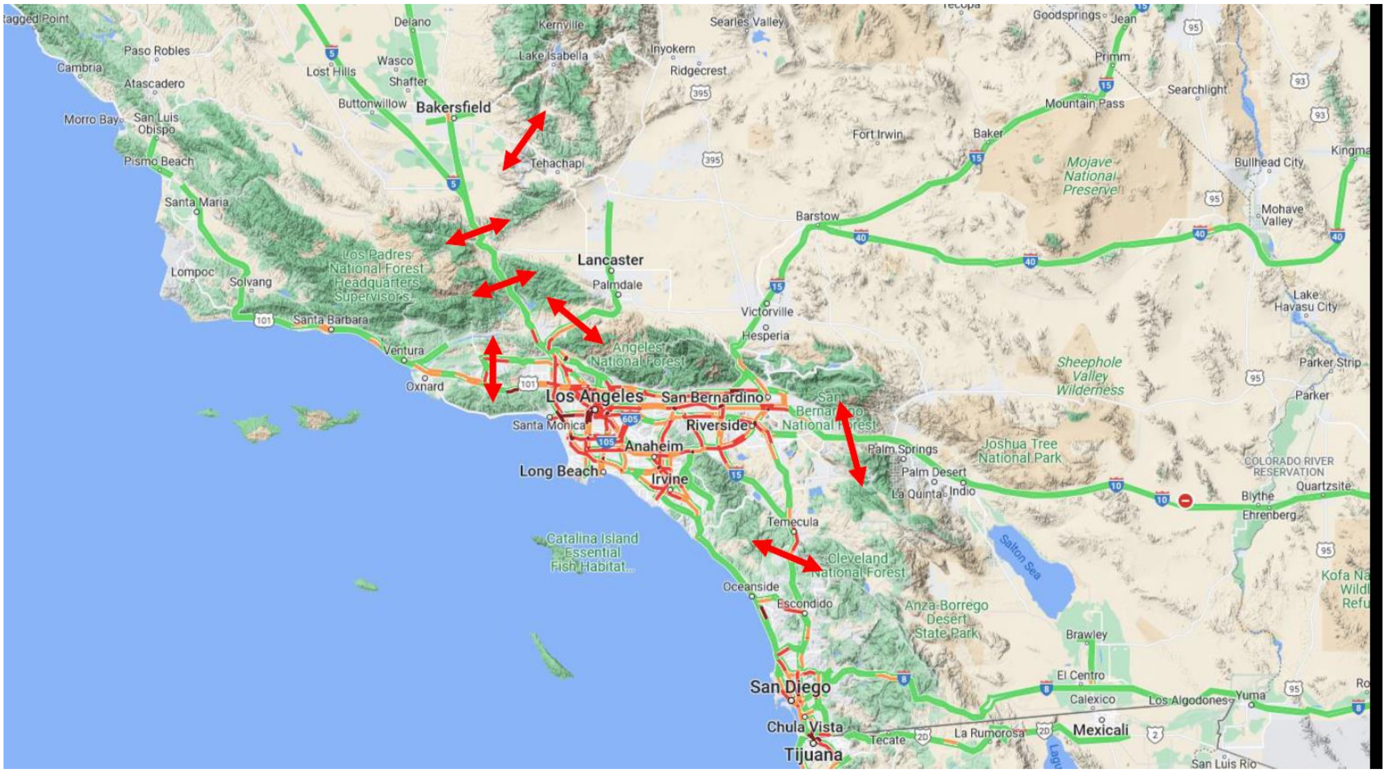


Figure 30. Connectivity areas of focus (red arrows) and major highways in the Tehachapis and southern California region.

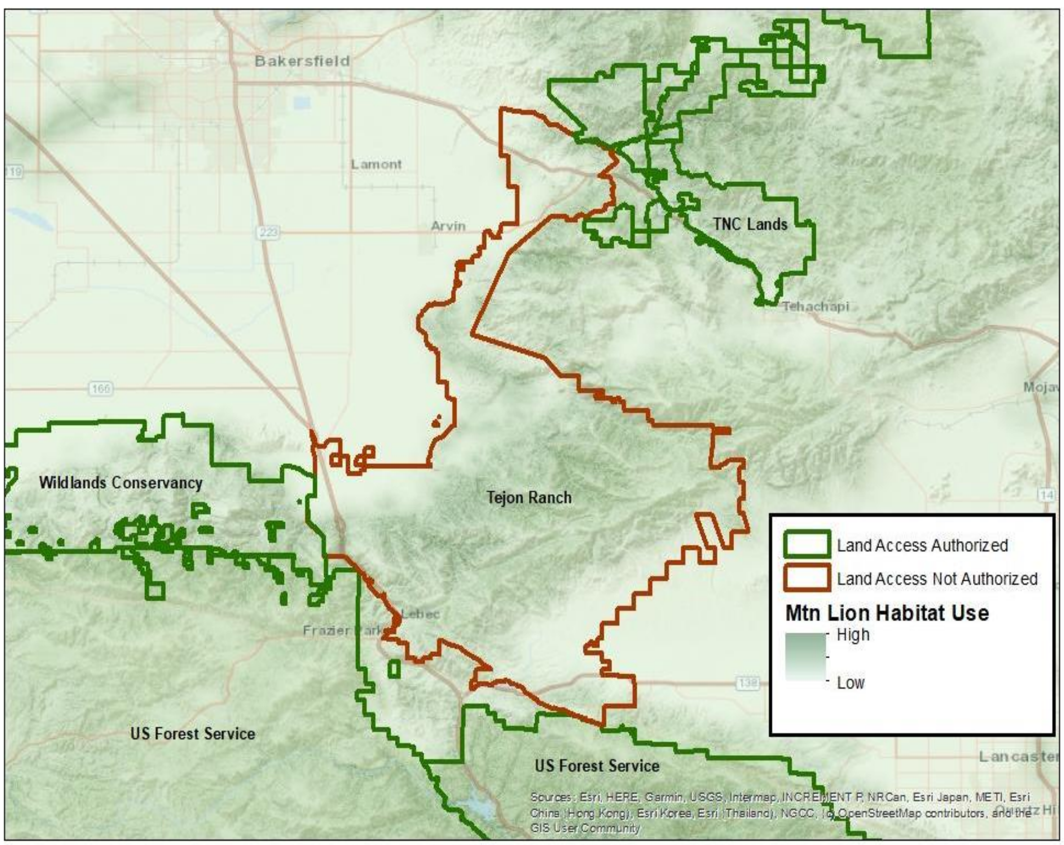


Figure 31. Mountain lion habitat in the Tehachapi Range with I-5 and SR 58 shown, and lands adjacent to those highways where proposed camera and mountain lion capture work would focus.

Tasks and Approach

Task 1. Monitor use of existing crossing structures by multiple wildlife species using remote cameras (Ford et al. 2009): Caltrans and USFS, as well as CDFW, are collaborators with UCD in placing and monitoring cameras at crossings along SR58 and I-5 in the study area (Figures 32, 33). The UC Davis Road Ecology Center (F. Shilling) is assisting this effort via photo processing assistance and database development and management.

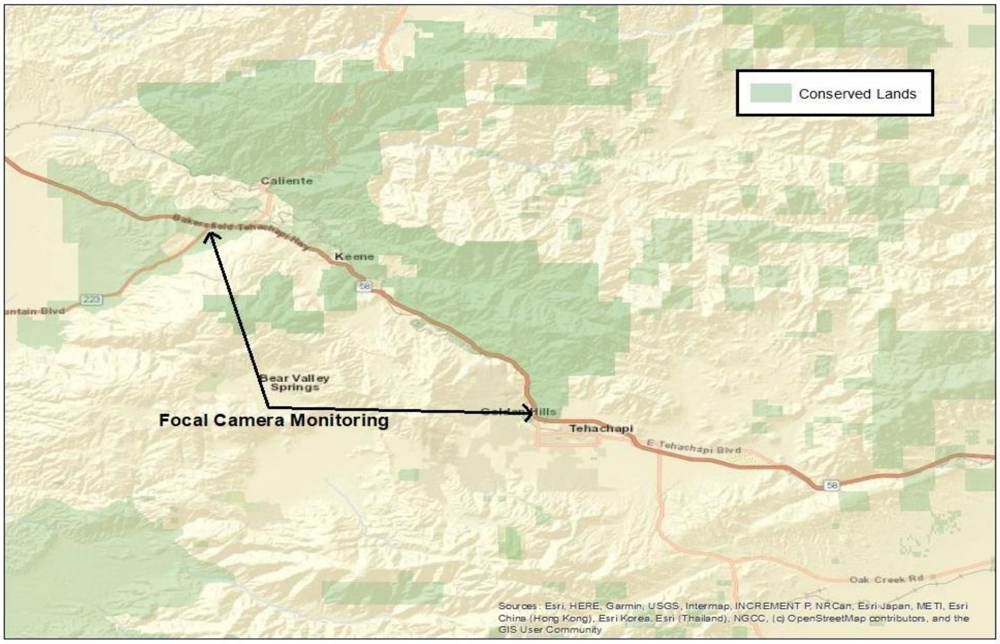


Figure 32. Focal area of planned camera monitoring of crossing structures along SR 58 in the Tehachapi Mountains with conserved lands shown.

Figure 33. Focal area of planned camera monitoring of crossing structures along Interstate 15 in the Tehachapi Mountains with conserved lands shown.

Task 2. Determine if and where additional crossing structures might be needed by capturing and deploying GPS radio-collars on mountain lions (Zeller et al. 2017): Take samples for genetics studies and other purposes. Collars will be remotely programmed if possible to increase location fix rate to get finer scale road crossing data.

Task 3. Understand gene flow of mountain lions and other mammals by opportunistically gathering genetic samples e.g., from captures, roadkill, scat, and hair; Gustafson et al. 2017): Genetic samples can help document the current level of gene flow in relation to major roadways with existing crossing structures. Though mountain lions are being targeted, this can be done opportunistically with multiple species. CDFW's Wildlife Genetics Lab has committed to storing and analyzing all mountain lion genetic samples and would take high quality genetic samples (e.g., tissue) from other species (Mike Buchalski – CDFW Wildlife Geneticist; michael.buchalski@wildlife.ca.gov). If connectivity is improved, additional genetic samples can be used to monitor changes in gene flow in response to mitigation measures.

Task 4. Compile results for informing efforts to improve/maintain connectivity given existing and future transportation infrastructure: After processing photos, occupancy and detection analyses will be used to analyze remote camera data (Dellinger et al. 2019). Resource and step selection function models will be used to analyze radio-collar location data (Dellinger et al. 2020b). Genetic samples will be analyzed using a single nucleotide polymorphism (SNP) assay to determine individual ID's and contribute to understanding of genetic connectivity (Buchalski et al. 2022).

Activities by Task

Task 1.

We have coordinated with CDFW and Caltrans biologists on combined monitoring of highway crossing structures along SR 58. Cameras were already in place at some crossings and being monitored by biologists from those agencies, but gaps remained. Beginning in August 2022, we placed cameras at locations that were lacking monitoring and were agreed among the group to be high priority. Camera placement at crossings along I-5 began in the latter half of 2023. We deployed 17 trail cameras among ten different culverts/crossings on Highway 58 (Figure 34). These cameras were deployed with the objective of increasing the wildlife monitoring effort already in place by Caltrans and CDFW. Through this collaborative effort, a total of eighteen different crossings were monitored within our study area along Highway 58 from August 2022 to August 2023. (Table 5). Currently, seven culverts of SR58 are being monitored by 14 cameras and three culverts of I-5 are being monitored by four cameras. As for January 2024, data from these cameras are being analyzed.

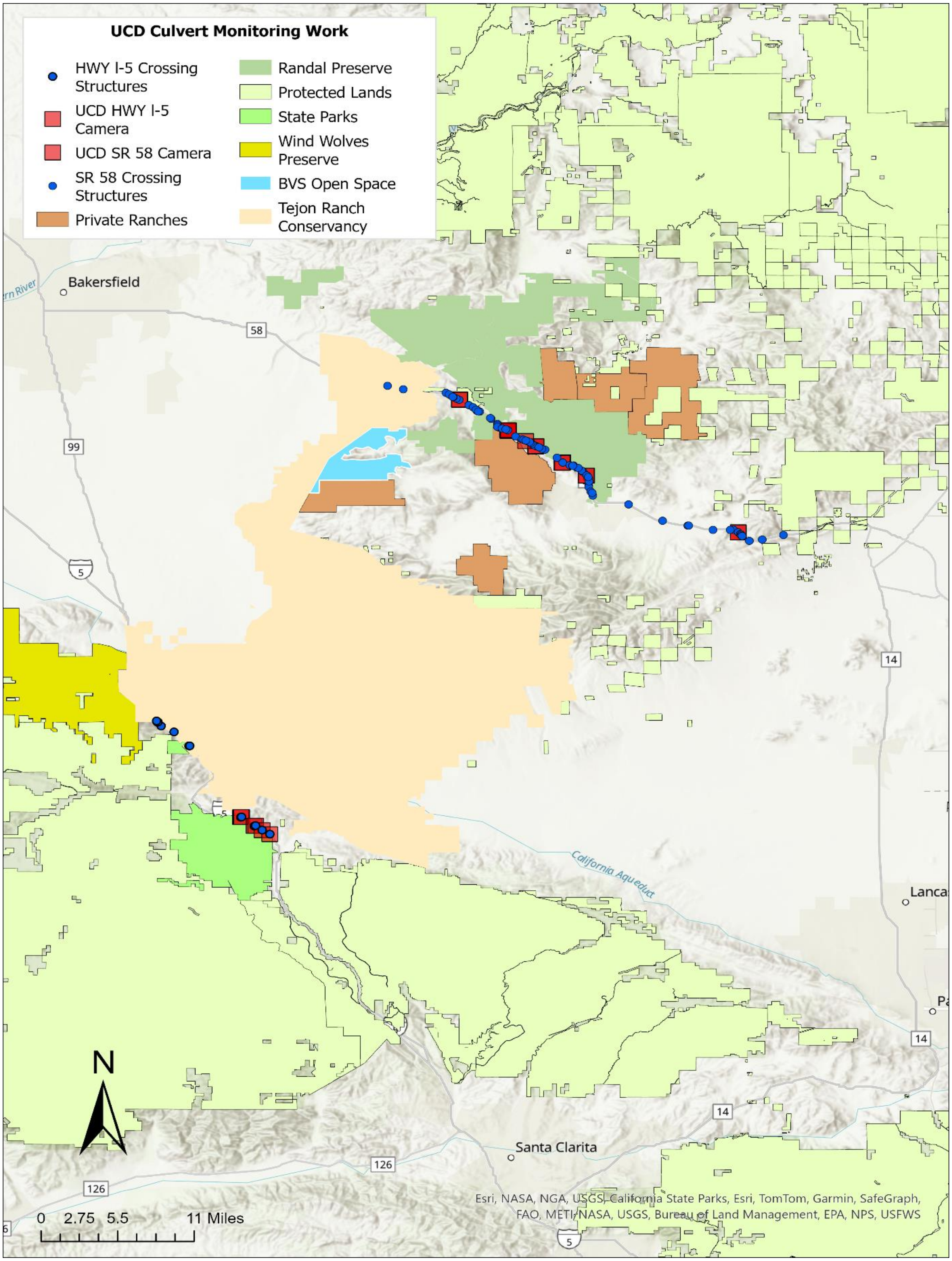


Figure 34. WHC crossing camera locations along SR58.

Site	Species Detected (August 2022- August 2023)
82.7 R1	Bobcat, gray fox, bear, deer, raccoon, striped skunk, rabbit, rodent
82.7 R2	Bobcat, coyote, gray fox, bear, deer, raccoon, striped skunk, rabbit, rodent
82.7 R3	Bobcat, coyote, gray fox, bear, deer, raccoon, rodent
84.75 R	Bobcat, coyote, gray fox, bear, deer, raccoon, rodent
84.75 L	Bobcat, coyote, gray fox, bear, deer, raccoon, striped skunk, badger, rabbit rodent
86 L	Bobcat, rodent
88.44 R	Bobcat, coyote, gray fox, bear, raccoon, striped skunk, rabbit
88.44 L	Bobcat, coyote, bear, deer
89.02 L	Bobcat, coyote, gray fox, deer, raccoon, rabbit, rodent
99.81 R	Bobcat, coyote, gray fox, bear, deer, raccoon, badger, rabbit, rodent
99.81 L	Bobcat, coyote, gray fox, bear, deer, raccoon, badger, rabbit, rodent
100.5 R	Bobcat, coyote, rabbit, rodent
100.5 L	Bobcat, coyote, gray fox, deer, rabbit, rodent
103.5 L	Bobcat, coyote, deer, rabbit
105.75 R	Bobcat, coyote, gray fox, kit fox, raccoon, rabbit, rodent

Table 5. SR58 Crossing camera data.

In addition to the wildlife monitoring effort along SR58 and I-5 at crossing structures, we have deployed up to thirty trail cameras throughout the habitat in our study area (The Nature Conservancy Randall Preserve, Wind Wolves Preserve, National Forest Lands, and Hungry Valley State Park) to help us identify mountain lion activity in proximity to the highways (Figure 35, 36, 37). From August 2022 to December 2023 there have been 385 detections of 411 individual mountain lions including 155 detections of the fifteen mountain lions that the study team had captured and GPS-collared in the Tehachapi Range from November 2022 through January 2024 (Table 6a). The total age/sex classification includes: 63 adult/subadult males, 110 adult/subadult females, 104 confirmed adult males (>30 mo), 48 confirmed adult females (>30 mo), 64 adult/subadult undetermined sex, 5 confirmed subadult male (18-30 mo), 1 confirmed subadult female (18-30 mo), 3 dependent young males, 3 dependent young females, 10 kitten or juvenile unknown sex.

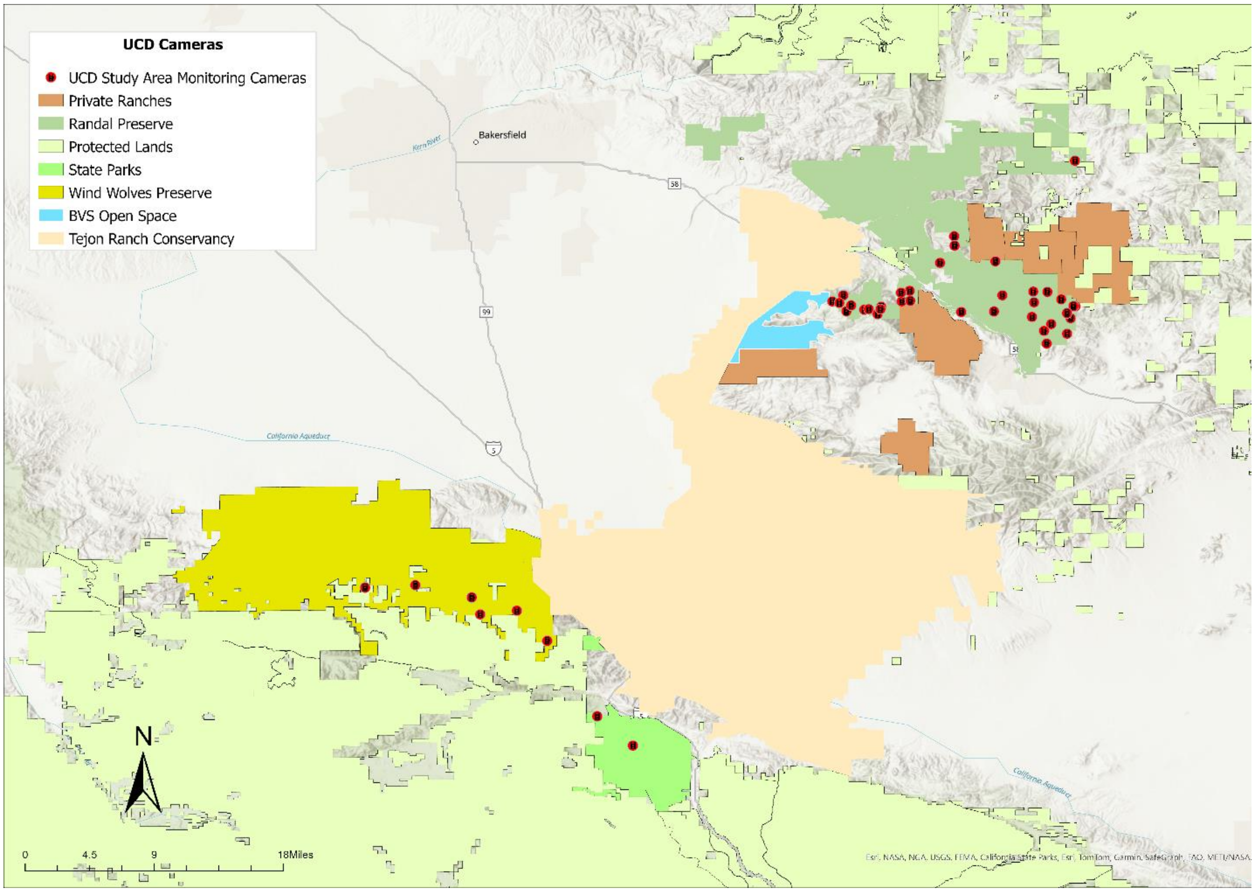


Figure 35. Monitoring camera locations in the Tehachapi Range habitat.

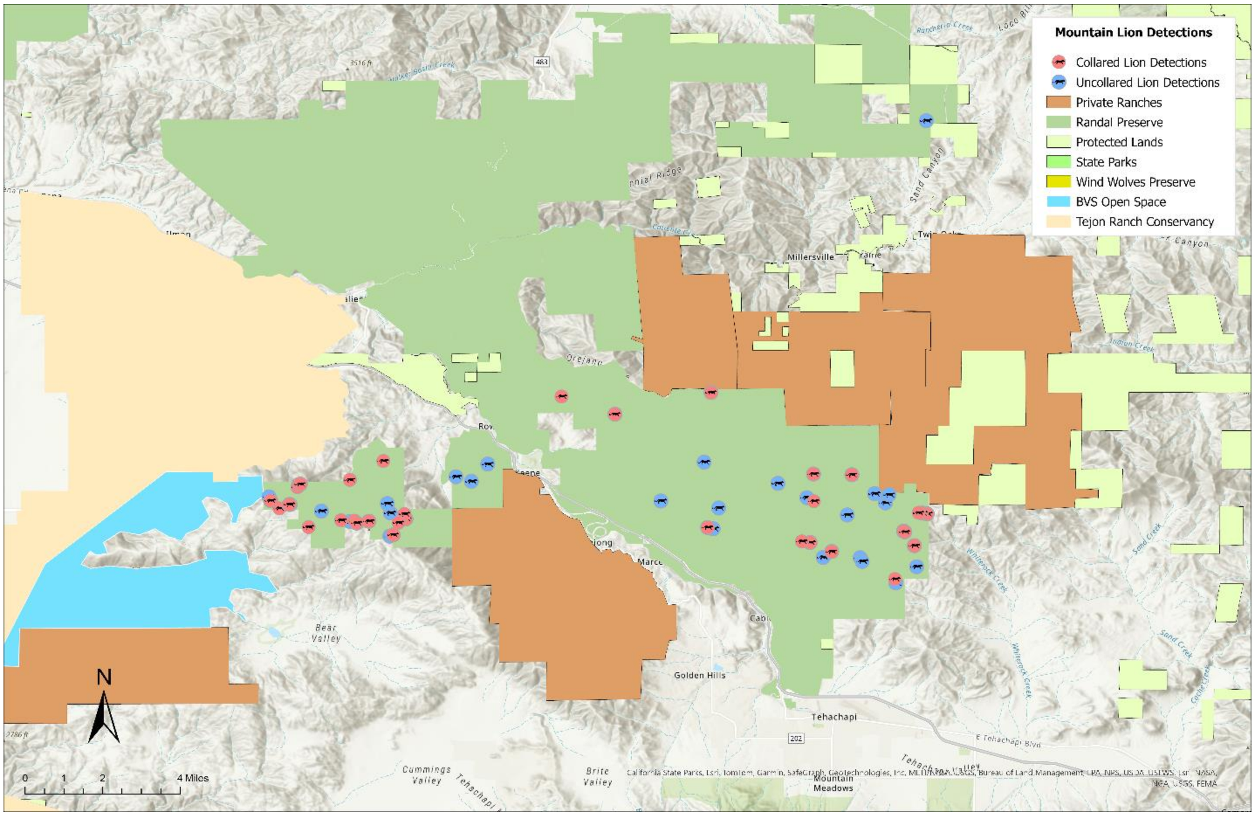


Figure 36. Mountain lion detections (collared and uncollared) near Highway 58.

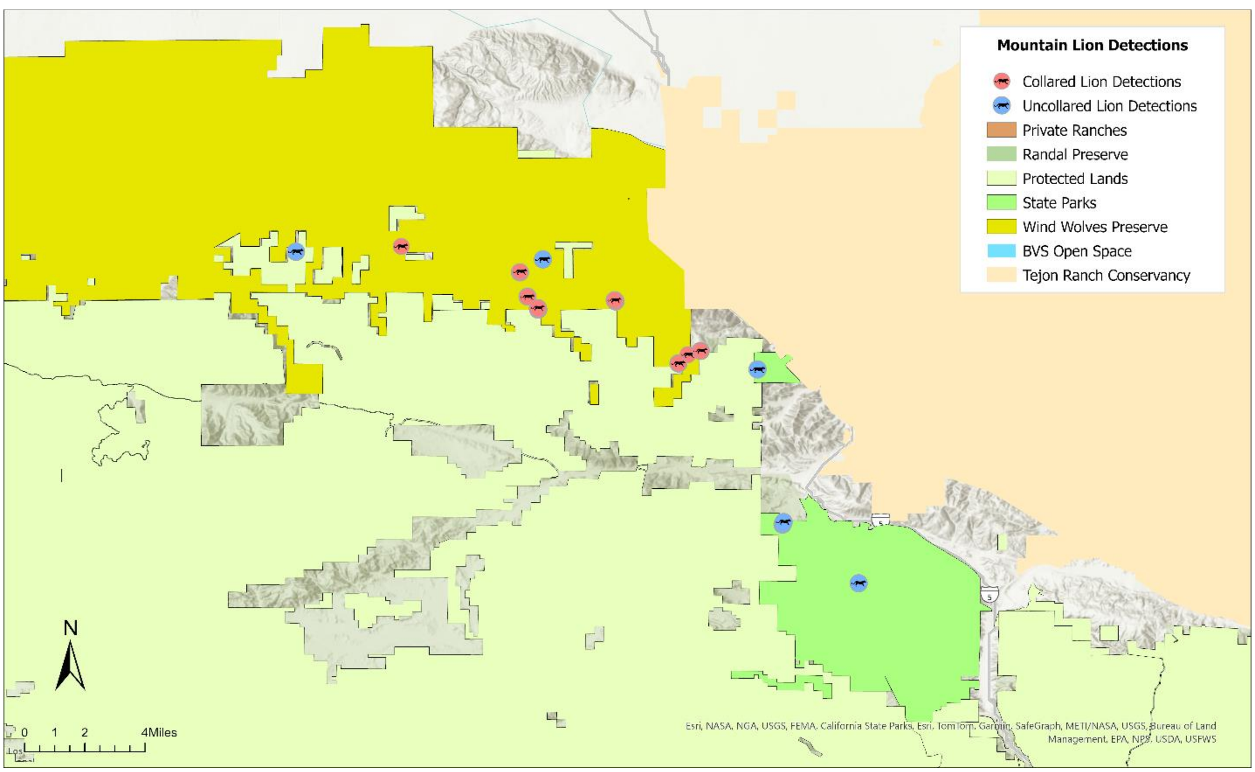


Figure 37. Mountain lion detections (collared and uncollared) west of Highway l-5.

Task 2.

Baiting for capture operations was initiated in the fall of 2022, although it had to be limited until late November in much of the study area due to bear disturbance of bait sites. However, some baiting was initiated in November west of I-5 on the Wind Wolves Preserve where bears were not as numerous as in the areas around SR58. Because of bear disturbance of bait in November of 2022, hounds were used for capture of two mountain lions over the last ten days of the month, and baiting resulted in one capture during the same period. Baiting was also limited due to weather conditions during the first trimester of 2023, with intermittent baiting operations in February. Baiting began to be steadier from April and due to bear activity and bait disturbance, trapping operations ended the first week of May. We reinitiated baiting the second week of November 2023 and it is ongoing. Tables 6a,b summarize mountain lion capture statistics, locations, along with transmitter frequencies for the fifteen mountain lions that have been captured and GPS-collared to date. Figure 35 shows mountain lion captures sites. Three GPS-collared males have died during the study period. Results are included in Table 6b.

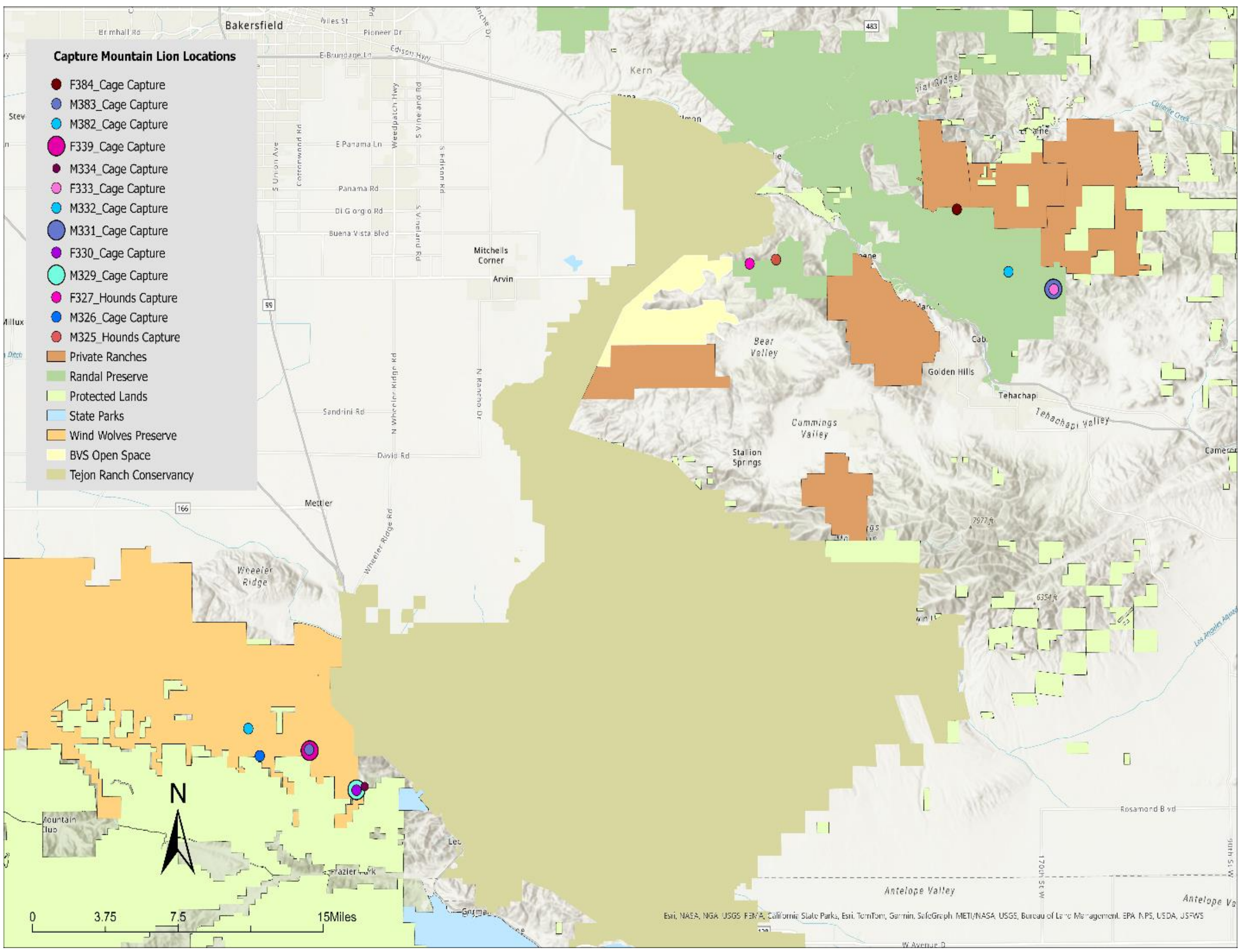


Figure 38. Mountain lion captures sites.

UCD ID	Capture Date	Method	Sex	Age (mo.)	Ear tag	Tattoo	Status
M325	11/22/22	Hounds	Male	72.0	325 orange (left)	325 (right)	Active
M326	11/25/22	Cage	Male	66.0	326 orange (left)	326 (right)	Active
F327	11/26/22	Hounds	Female	45.0	327 orange (right)	327 (left)	Active
M329	02/17/23	Cage	Male	16.0	329 orange (left)	329 (right)	Dead
F330	02/17/23	Cage	Female	80.0	330 orange (right)	330 (left)	Active
M331	02/19/23	Cage	Male	67.0	331 orange (left)	331 (right)	Dead
M332	04/08/23	Cage	Male	77.0	332 orange (left)	332 (right)	Active
F333	04/16/23	Cage	Female	55.0	333 orange (right)	033 (left)	Active
M334	04/30/23	Cage	Male	64.6	334 orange (left)	334 (right)	Dead
F339	11/16/23	Cage	Female	44.2	339 orange (right)	339 (left)	Active
M382	12/13/23	Cage	Male	18	340 orange (left)	382 (right)	Active
M383	12/19/23	Cage	Male	58.4	341 orange (left)	383 (right)	Active
F384	12/30/23	Cage	Female	18-24	342 orange (right)	384 (left)	Active
F385	01/06/24	Cage	Female	33	323 orange (right)	385 (left)	Active
F386	01/08/24	Cage	Female	18-24	324 orange (right)	386 (left)	Active

Table 6a. Statistics on mountain lions captured during reporting period in.

UCD ID	General locations	County	UTM Zone	UTM Easting	UTM Northing	Injuries	Status	Mort. Date	Mort. cause	Transmit. Freq.
M325	Bear Mountain	Kern	11	351665.35	3898735.28	None	Active	N/A	N/A	160.120
M326	Black Bobcat Canyon	Kern	11	315930.64	3863490.33	None	Active	N/A	N/A	160.160
F327	Bear Mountain	Kern	11	349875.54	3898467.49	None	Active	N/A	N/A	160.080
M329	Tecuya Creek	Kern	11	322461.94	3860960.10	Crown fracture right maxillary canine	Deceased	10/16/23	Inconclusive	160.220
F330	Tecuya Creek	Kern	11	322461.94	3860960.10	Crown fracture left maxillary canine	Active	N/A	N/A	160.320
M331	Loop Ranch	Kern	11	370447.24	3896324.41	None	Deceased	06/22/23	*Leptospira* spp. infection	160.710
M332	Loop Ranch	Kern	11	367417.50	3897608.57	None	Active	N/A	N/A	160.720
F333	Loop Ranch	Kern	11	370447.24	3896324.41	None	Active	N/A	N/A	160.715
M334	Clendenen property	Kern	11	323027.22	3861157.75	Fight wounds	Deceased	07/23/23	Road killed I-5	160.725
F339	Tecuya Creek	Kern	11	319337	3863857	None	Active	N/A	N/A	159.200
M382	Salt Creek	Kern	11	315181	3865519	None	Active	N/A	N/A	159.270
M383	Tecuya Creek	Kern	11	319337	3863857	Fight wounds Oral papillomas	Active	N/A	N/A	159.280
F384	Loop Ranch	Kern	11	363991	3902177	Oral papillomas	Active	N/A	N/A	159.230
F385	Clendenen	Kern	11	323027.22	3861157.75	None	Active	N/A	N/A	159.210
F386	Clendenen	Kern	11	323027.22	3861157.75	None	Active	N/A	N/A	159.240

Table 6b. Locations of captures and animal disposition/Transmitter frequencies.

All mountain lions but four (M329, M382, F385, F386) were considered mature adults and appeared to be maintaining territories that are similar in size to those that we have documented elsewhere in our studies (males and females home range = 235 ± 155.9 km^2; females home range = 104 ± 46.7 km^2 ; males home range = 314.3 ± 144 km^2; Figures 39 and 40). Social structure is also comparable to that described elsewhere in our studies and the literature (Figures 4).

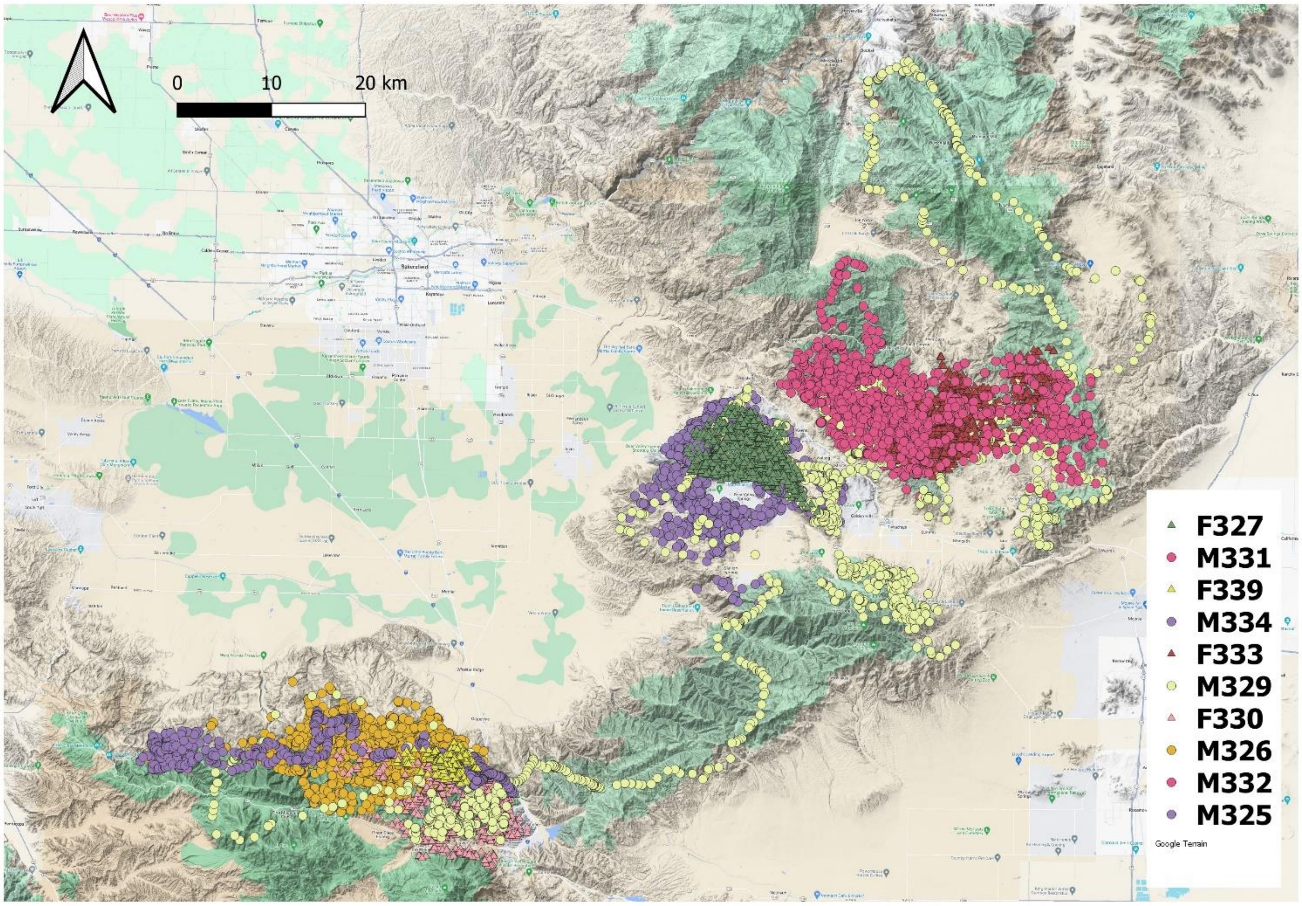


Figure 39. GPS locations from collared mountain lions in the Tehachapi Range. The mountain lions included in this figure had at least 3 months of data.

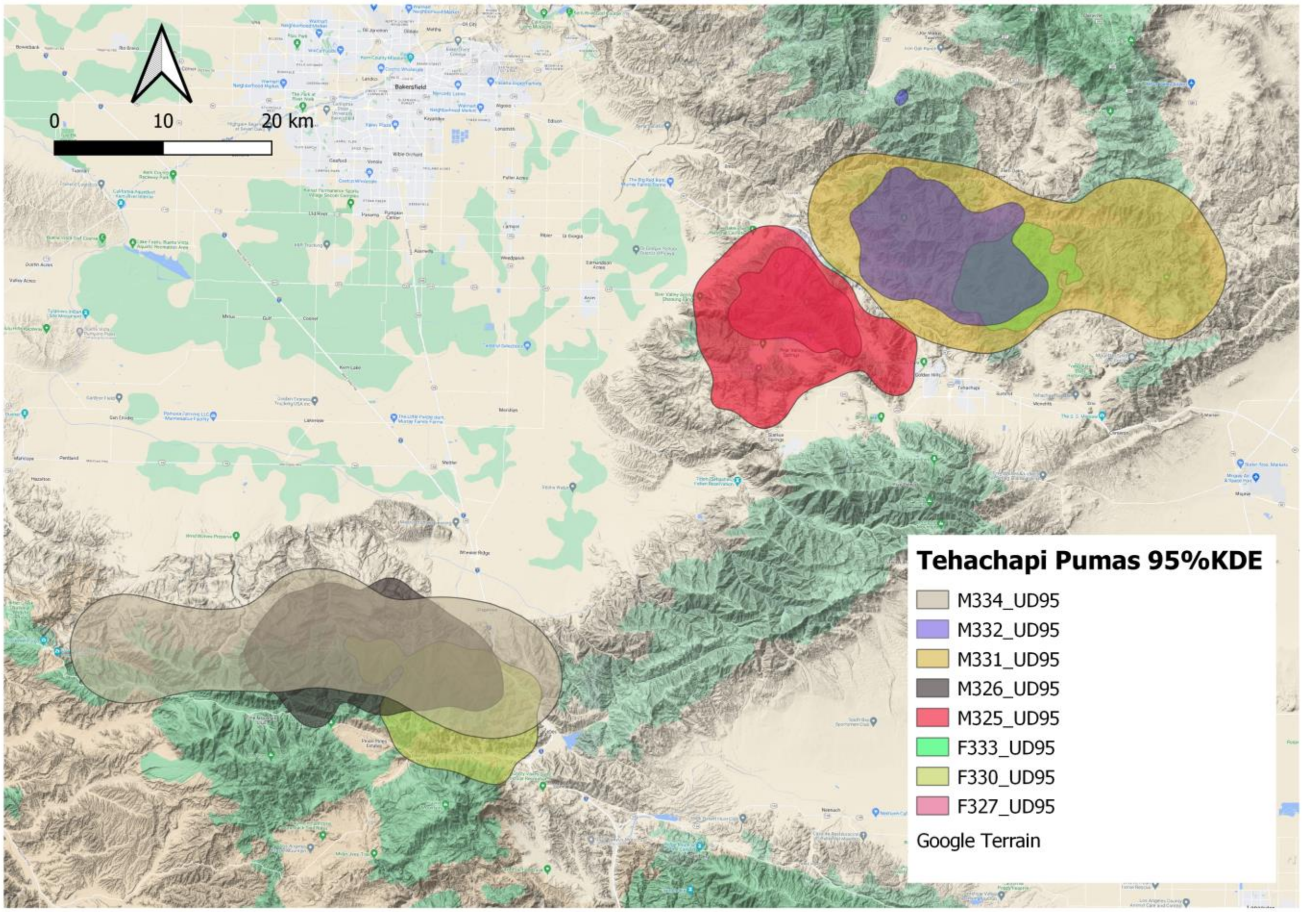


Figure 40. Males and females 95% KDE Home Range. Figure is showing individuals with three or more months of data.

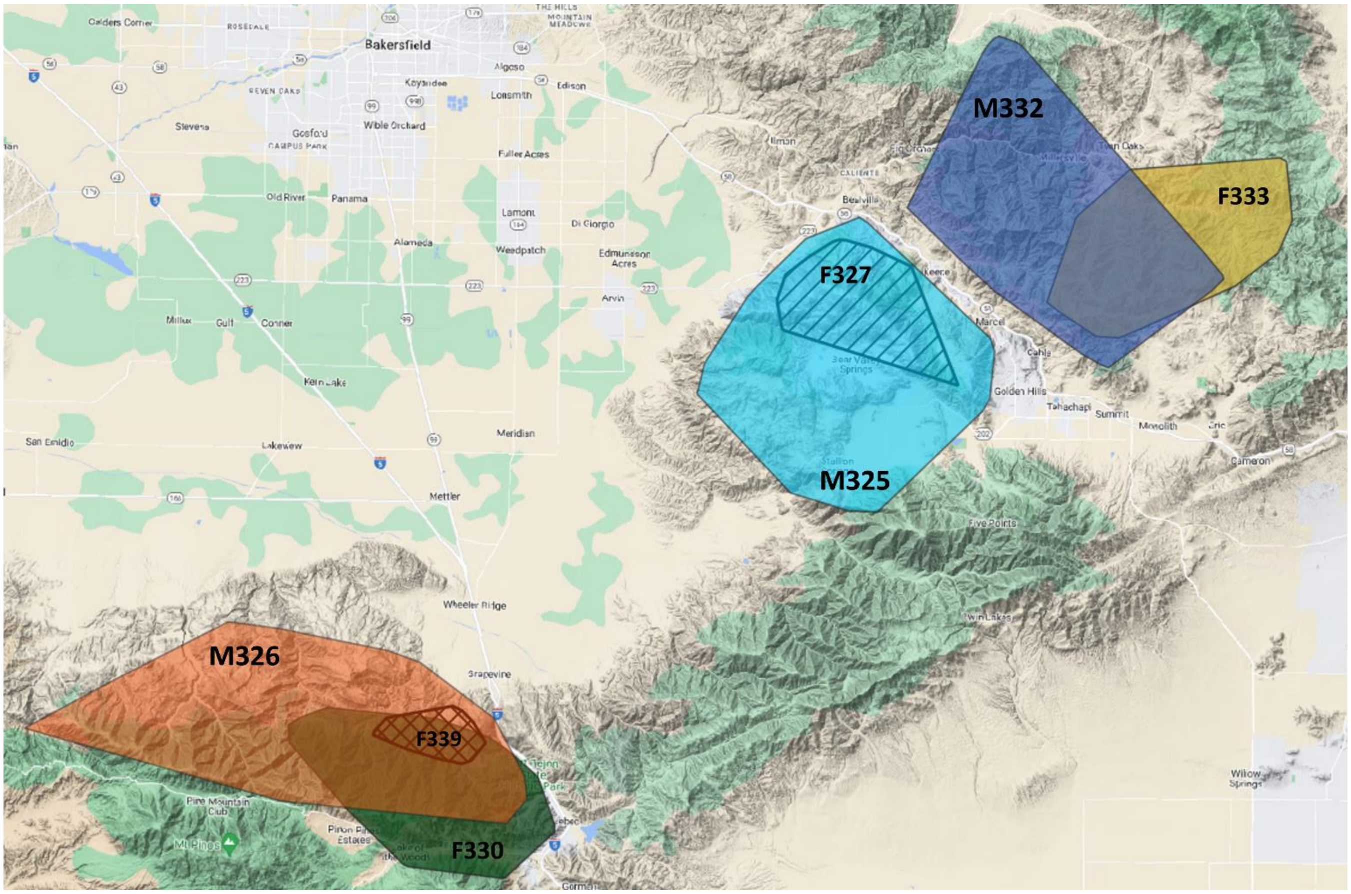


Figure 41. Mountain lion social structure along SR58 and I-5. Figure is showing individuals with three or more months of data.

None of the mature adults have crossed either of the highways that we are most focused on (I-5 and SR58) to date. Some of them (e.g., M325, M326, F327) have however crossed secondary roads periodically. Two adult males (M334 and M325) have been located close to I-5 and SR58, but they didn't attempt to make a crossing (figures 42 and 43). Similarly, F330, the mother of M329, has approached I-5 but not crossed.

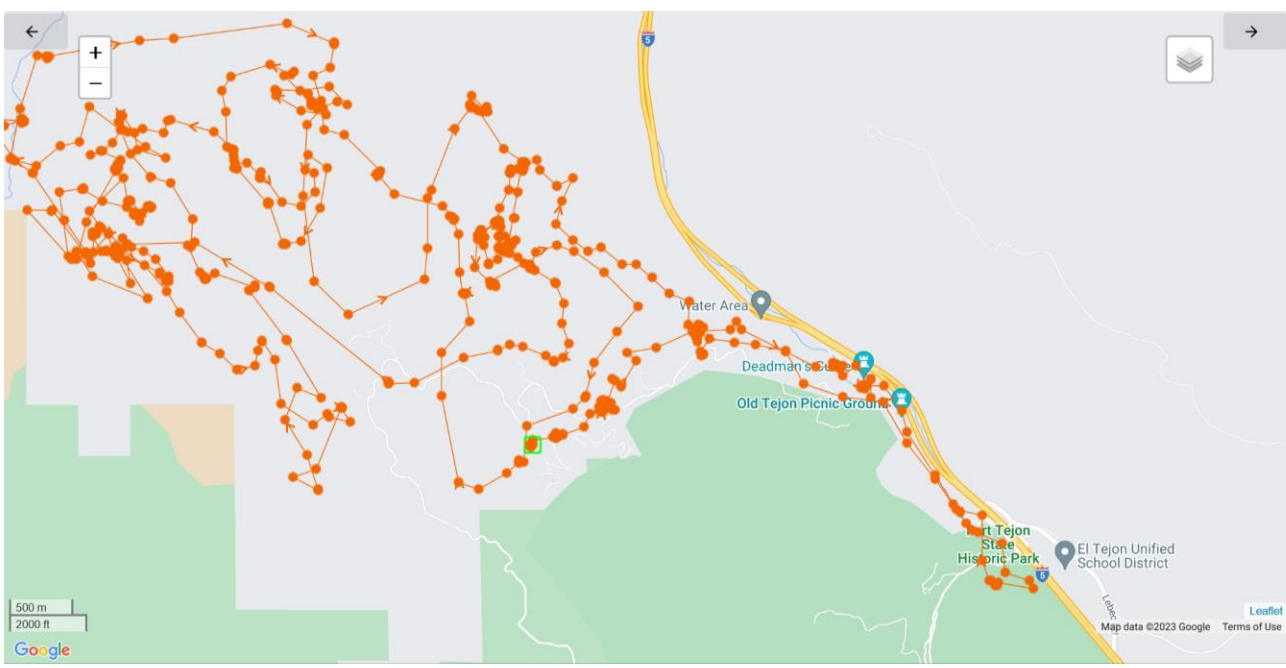


Figure 42. M334 locations near I-5.

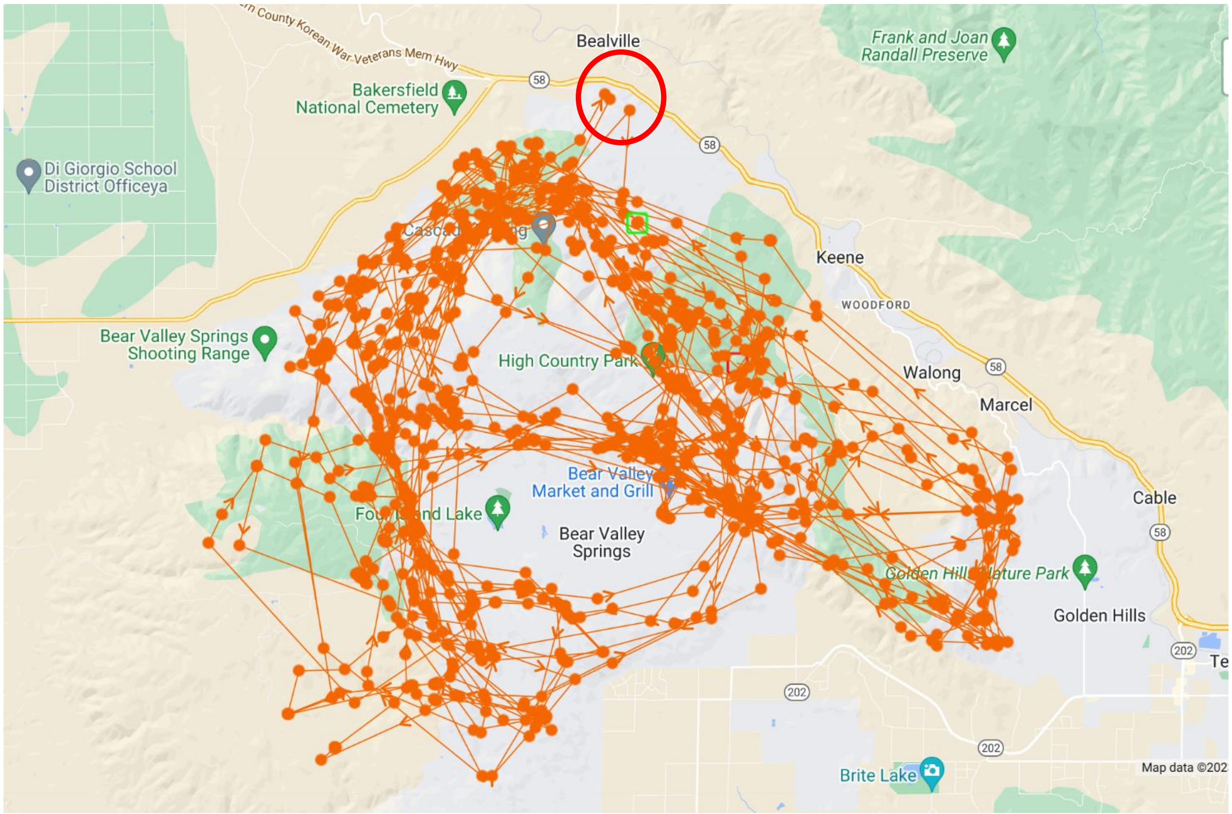


Figure 43. M325 locations near SR58.

To date, two individuals have crossed I-5 (M329 and F386) and only M329 has made successful I-5 and SR58 crossings.

M329 was captured along with his mother on February 17th, and almost two months after his capture he crossed I-5. This crossing took place on April 28th, between 12:45 and 1:15 am (figure 44). Previous to his crossing, the collar was commanded to get GPS fixes every 15 minutes, allowing a finer scale to his movement data and giving us a robust idea of where the crossing took place. Post-crossing inspection of the area by our biologist revealed that the individual crossed at grade, since no culverts or other crossing structures were located near the crossing.

Figure 44. M329 crossing of I-5.

Following his I-5 crossing, M329 kept going East, then encountered SR58 for the first time and did not attempt to cross it. Several days later, on May 15th, he successfully made the crossing of SR58 (Figure 45).

Figure 45**.** Satellite view of M329 crossing site along SR58.

After his exploratory movement east of 58, M329 came back and crossed SR58 again on August 10th. Though a culvert of adequate size for mountain lion use was located near the crossing site, a camera that had been placed there after M329's first crossing did not capture evidence of his use of the culvert for the second crossing. Therefore, it is possible but not certain that he crossed both times at grade (Figure 46).

Figure 46. Satellite view of M329 crossing site along SR58 on August 10th.

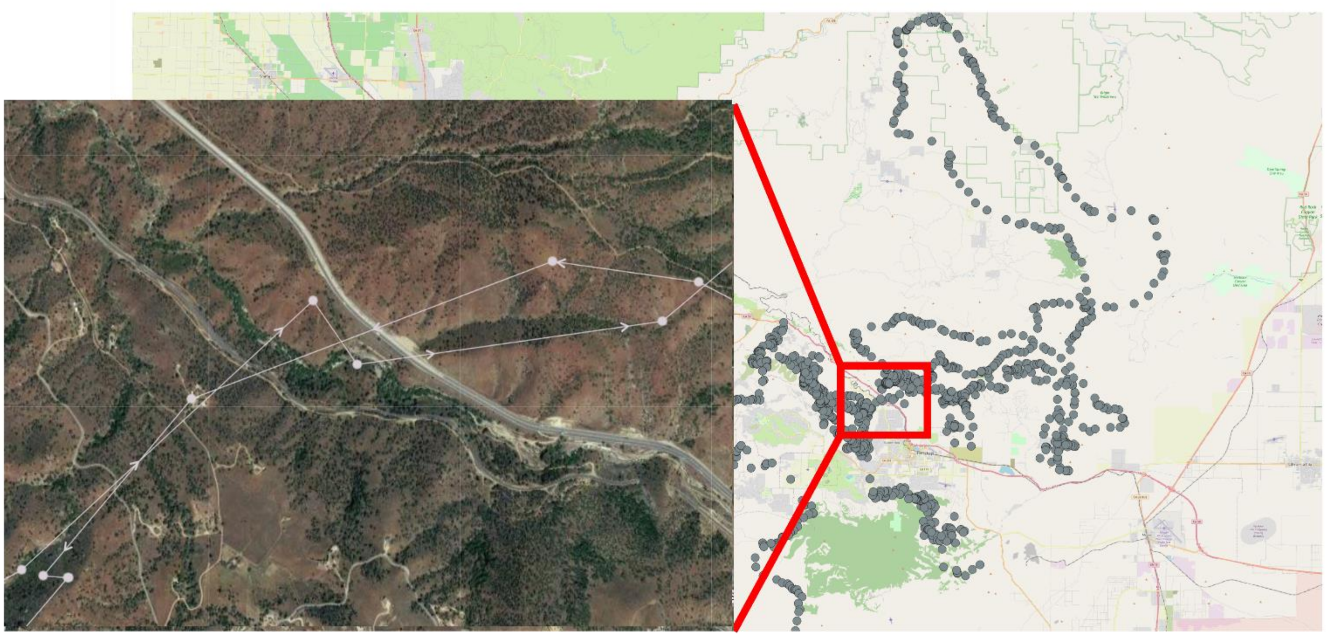


Figure 47. Satellite view of M329 crossing sites on SR58.

Unexpectedly, M329 died in October from unknown causes. The mortality alert system in his GPS collar did not send notifications to our UCD team due to mis-programming, and the carcass was not retrieved from the field until one week after he died. The carcass was in an advanced stage of decay that prevented a complete necropsy examination and determination of cause of death. No evidence of foul play or vehicle trauma was found at the site though it was within sight of a road and near a rural farm.

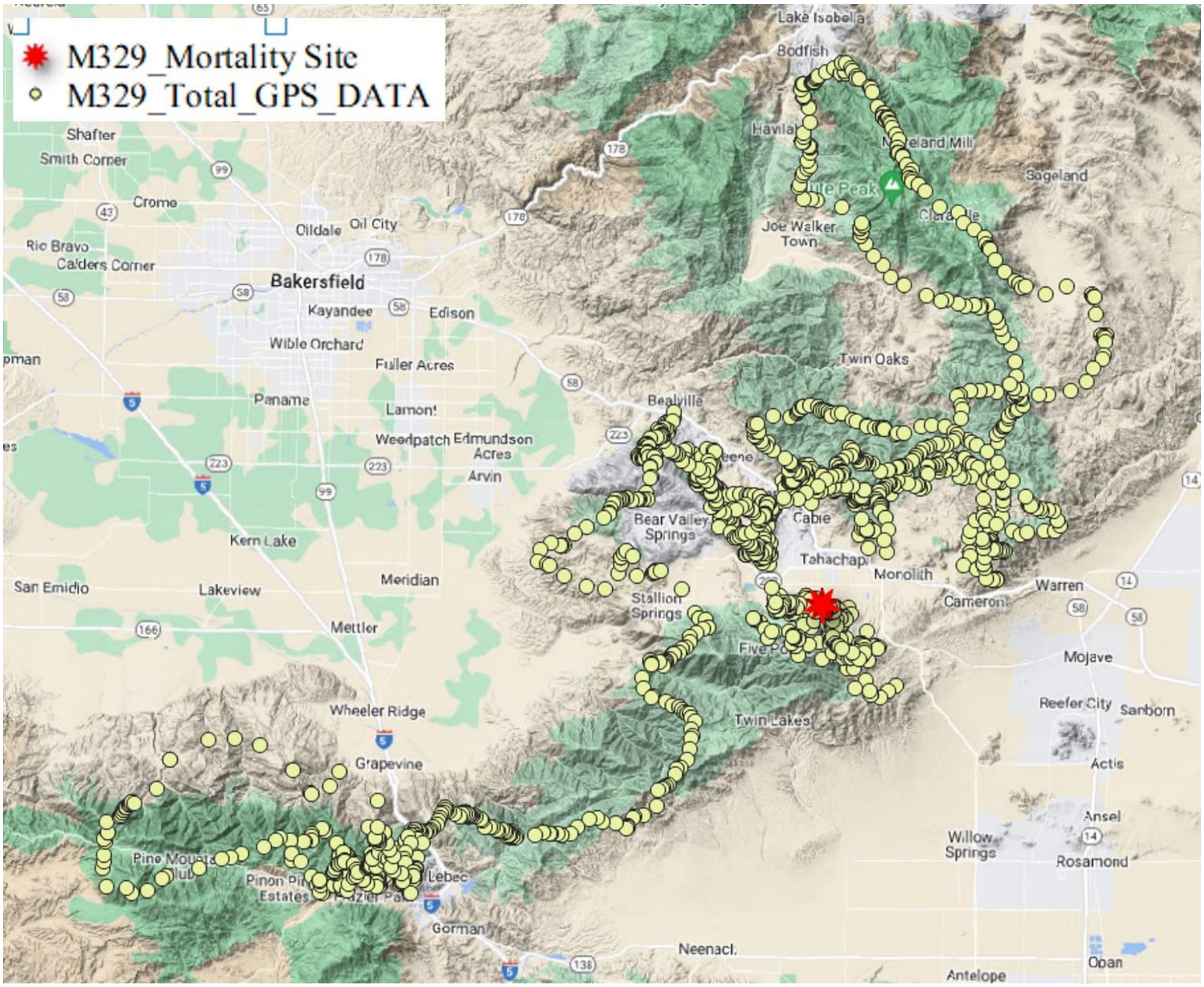


Figure 48. GPS data from M329.

Interestingly, F386 was captured on January 8th, 2024 at the same location of M329's capture. Six days later on January 14th (Saturday), she crossed I-5 at 8:00 pm. Upon investigation of the crossing location based on her GPS fixes taken every 15 minutes, we determined she crossed at grade (Figure 49a). As this report was in final preparation, F386 crossed back over I-5 at the same location at 2:45 am on January 30, 2024. Her crossing site was within an approximately three mile stretch of I-5 where M329 also crossed at grade and M334 was killed while trying to cross at grade (Figure 49b). On both occasions F386 crossed the freeway within 100 meters of a culvert large enough to accommodate mountain lion passage that our team is monitoring with cameras. Likewise, M329 crossed at grade within approximately 500 meters of monitored large culverts (Figure 49b; camera symbols on figure with mile markers). In addition, other mountain lions collared west of I-5 have approached the freeway in the same roughly three mile stretch (Figure 49c). These crossings at grade that are dangerous not only for the animals (as shown by M334) but also drivers illustrate that fencing is indicated in this section of the Interstate to funnel animals to the existing crossing structures. They also illustrate that at least one additional structure could be beneficial in that section of the freeway due to the distance between viable structures being approximately 1.4 miles and 0.9 miles in two cases.

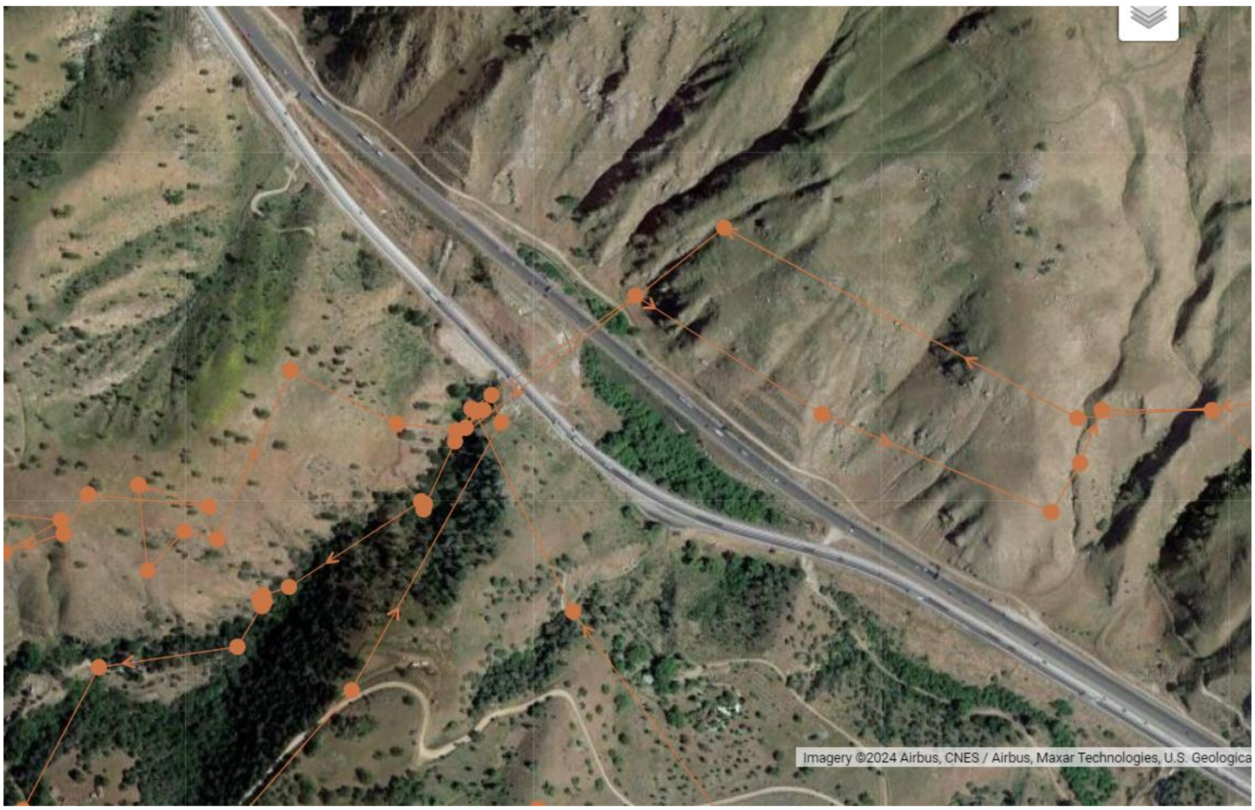


Figure 49a. Satellite view of F386 two crossings of I-5 at the same location late January 2024..

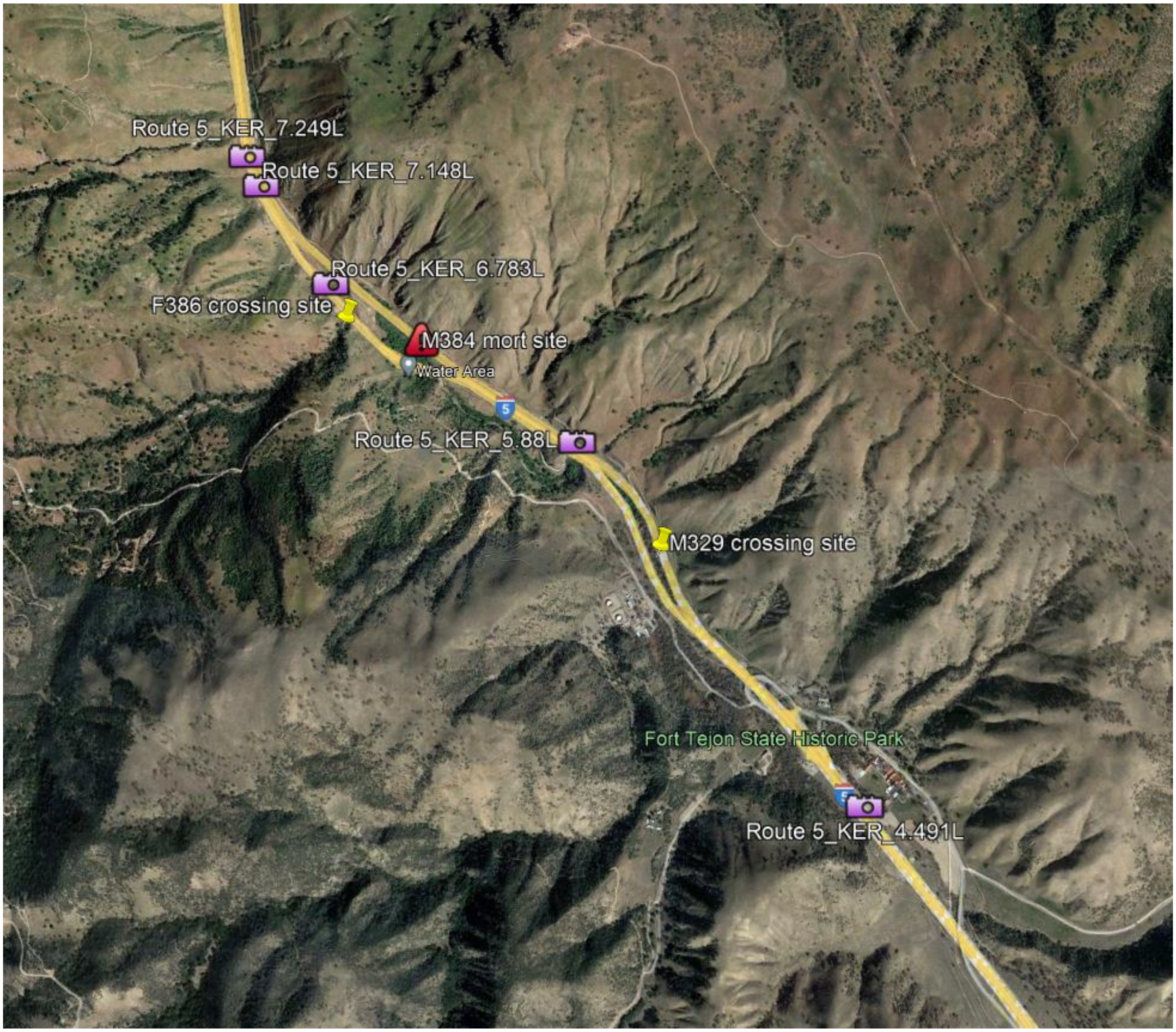


Figure 49b. Google Earth view of the approximately three-mile stretch of Interstate 5 north of the Tejon Ranch State Park where M329 and F386 crossed successfully at grade and M331 was killed by a vehicle while attempting to cross Large culverts that are suitably sized for mountain use and are being monitored by the UCD team are depicted by camera symbols and mile marker information.

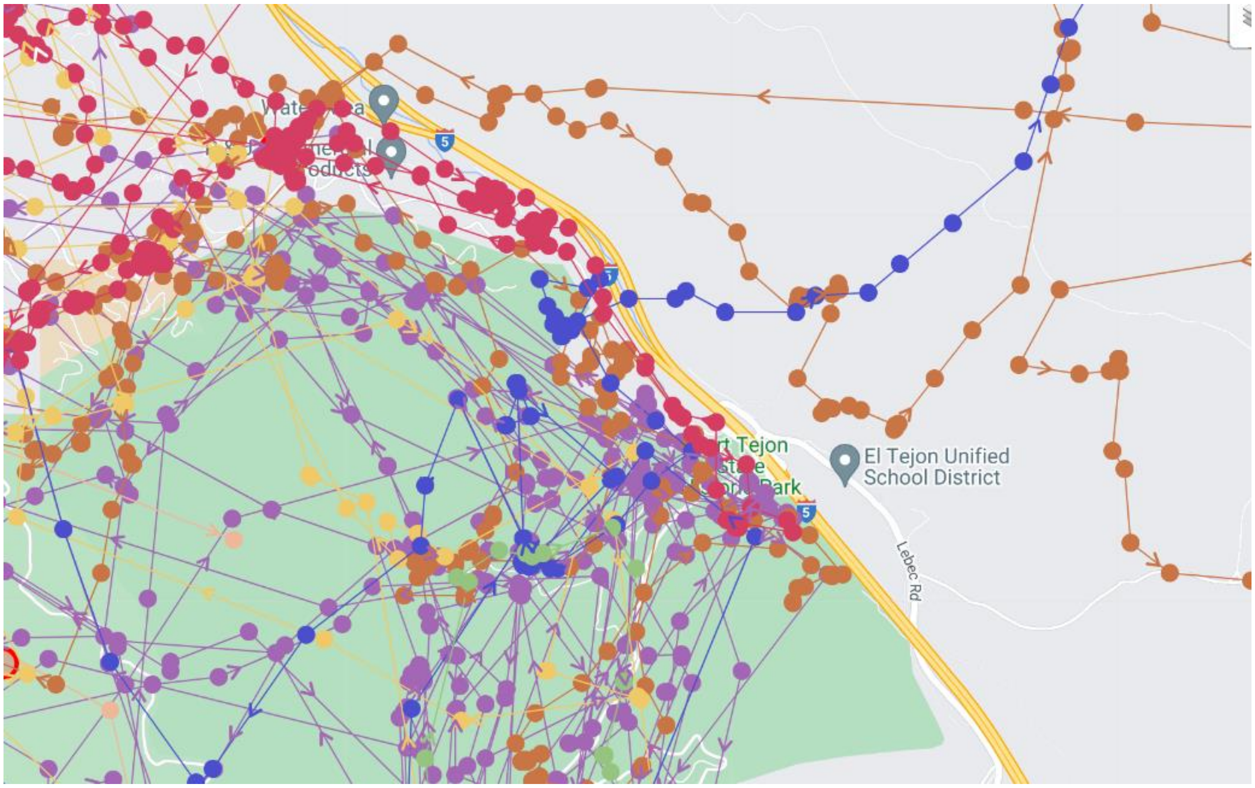


Figure 49c. GPS collar datapoints of all mountain lions that have approached I-5 from November 2023-January 2024 in the approximately three-mile section where M329 and F386 successfully crossed at grade, and M334 was killed while attempting to cross.

Task 3. Since the implementation of the project, UCD biologists opportunistically have been collecting suspected mountain lion scats at the study site. A total of 25 scats have been collected and sent to the CDFW Wildlife Genetics Lab (June 26th, 2023). As of this writing, we have not received results from these analyses. Figure 50 shows scat collection sites in the study area.

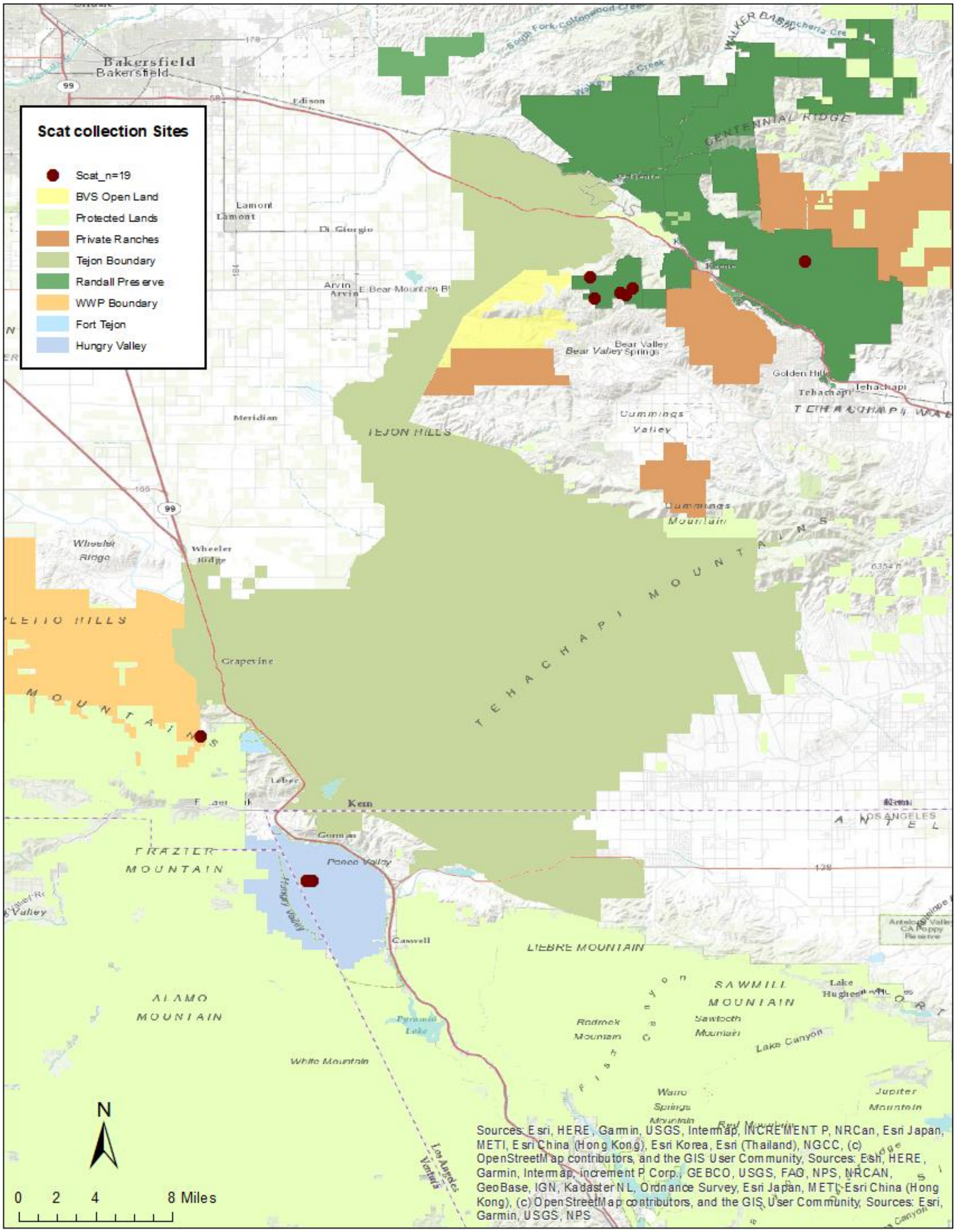


Figure 50. Scat collection sites in the study area.

A.4.2.4. Northern Gabilans and Pacheco Pass Study

In August 2022, we initiated our studies in the Gabilan Range and Pacheco Pass areas with funding from WCN and the Santa Clara Valley Habitat Agency. As in the Tehachapi study area, the goals are highly focused on connectivity across major highways in the region, especially US101 and SR152 through the

Pacheco Pass area (Figures 51,52,53). As noted previously in the report, this nexus of the Santa Cruz, northern Gabilans, the Diablo Range, and the coastal ranges to the south is a key area to understand in relation to connectivity for mountain lions and other wildlife, especially in advance of any high-speed rail construction that will likely further limit connectivity and gene flow for mountain lions and other wildlife in this area.

Mountain lion GPS collar and genetic samples can help document the current level of gene flow in relation to major roadways with existing crossing structures. Though mountain lions would be targeted, this can be done opportunistically with multiple species. CDFW's Wildlife Genetics Lab has committed to storing and analyzing all mountain lion genetic samples and would take high-quality genetic samples (e.g., tissue) from other species (Mike Buchalski – CDFW Wildlife Geneticist; michael.buchalski@wildlife.ca.gov). If connectivity was then improved, additional genetic samples could be used to monitor changes in gene flow in response to mitigation measures. Thus, understanding current connectivity and gene flow can help mitigate the impacts of future transportation infrastructure on the viability of local wildlife populations.

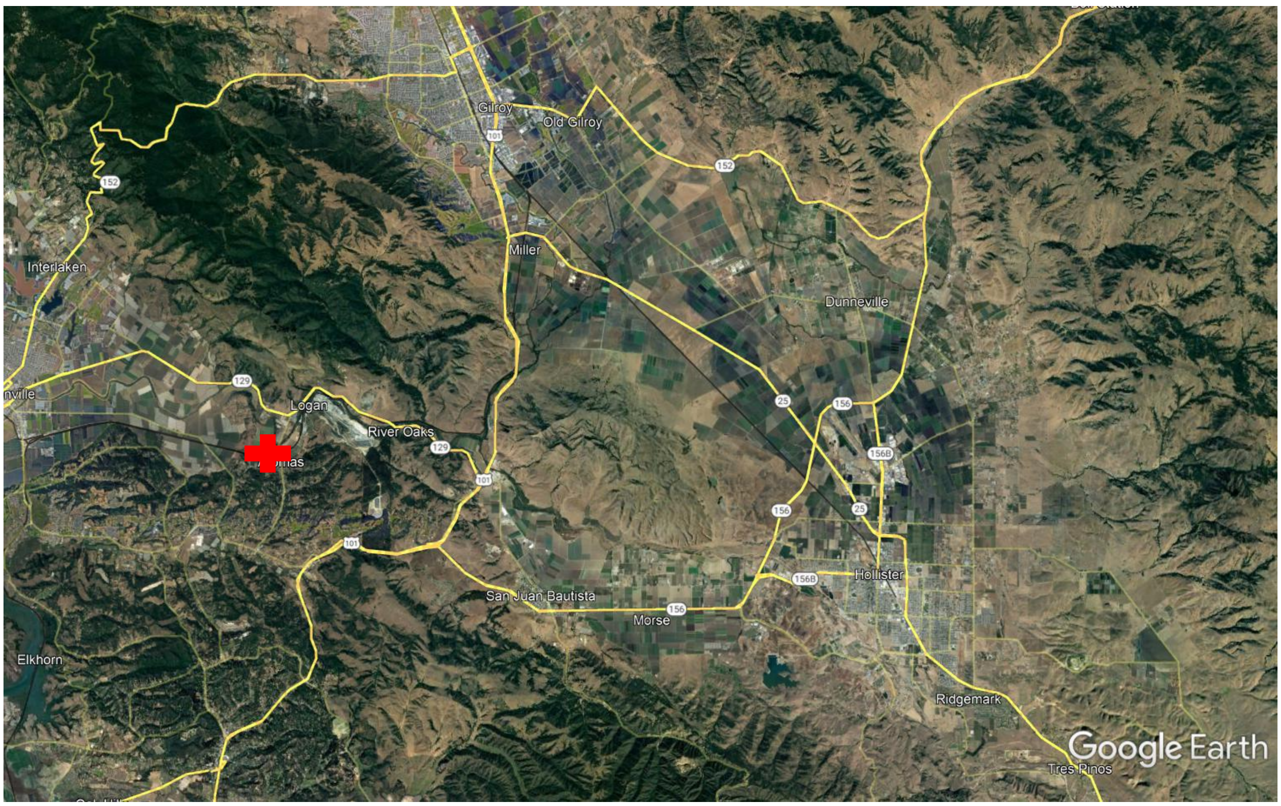


Figure 51. Highway 101 near Rocks Ranch (red cross), and SR152 through Pacheco Pass are potential barriers to connectivity and gene flow of mountain lions in this portion of California.

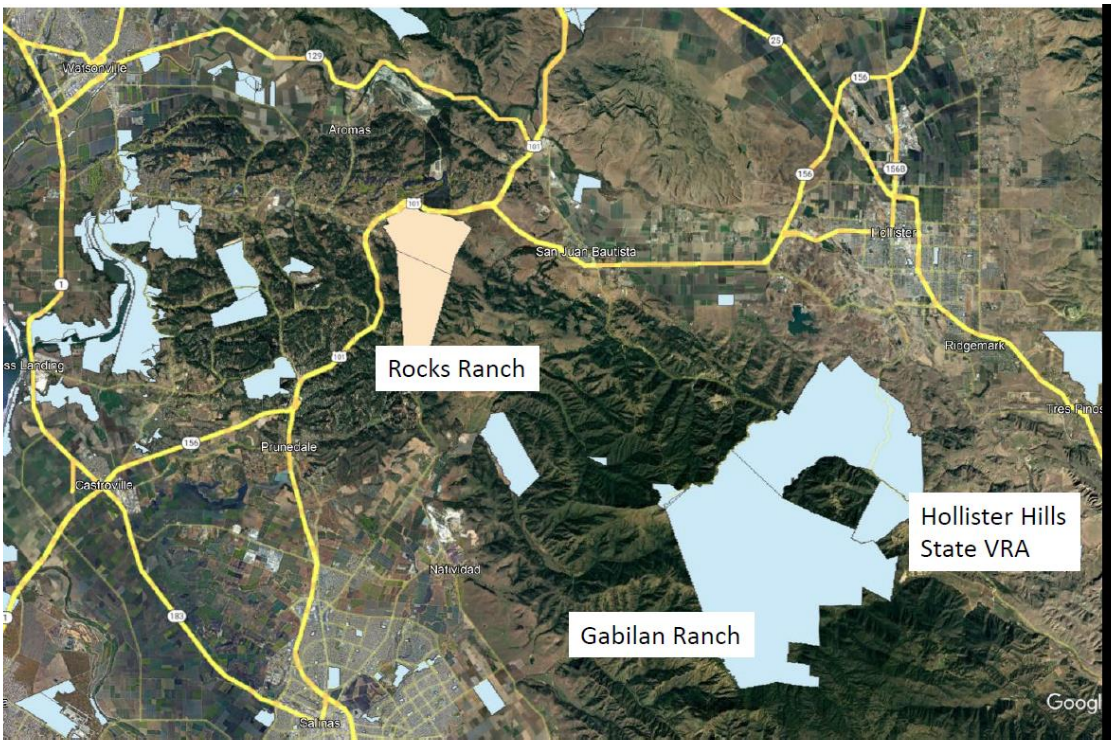


Figure 52. The northern Gabilan Range and conserved lands in blue, with Rocks Ranch (also conserved) in tan.

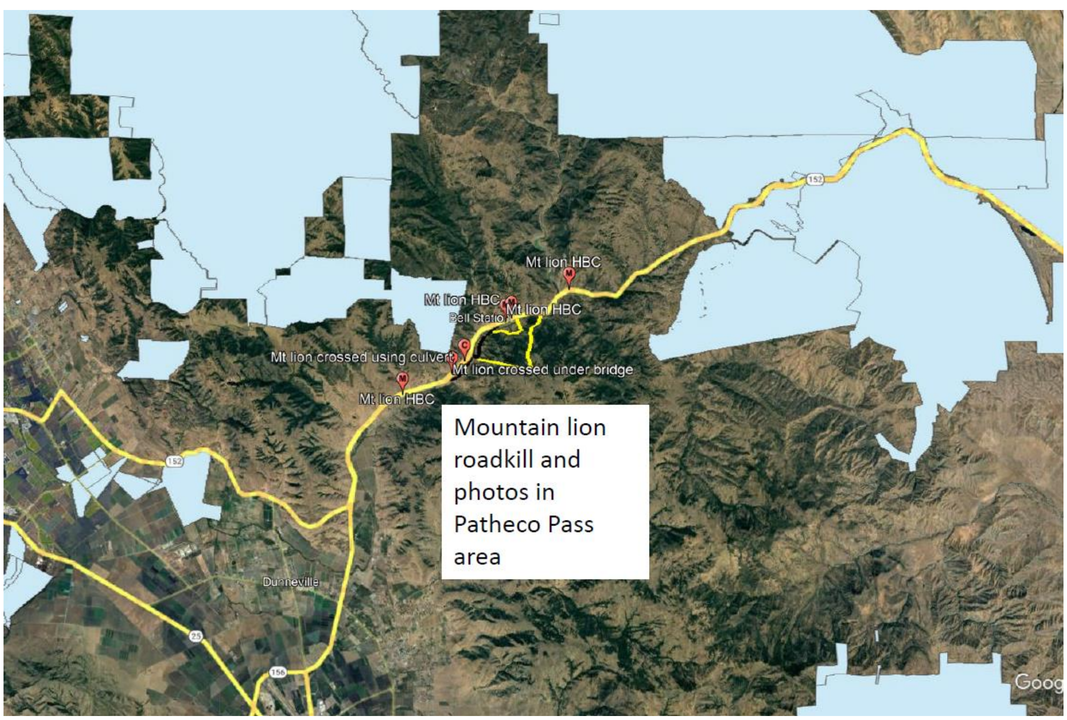


Figure 53. Pacheco Pass area and locations where mountain lion road mortalities and photos have been recorded.

Tasks and Approach:

Task 1. Determine if and where crossing structures might be needed by capturing and deploying GPS radio-collars on mountain lions (Zeller et al. 2017):

Progress made during the first six months on the Gabilan Mountains Lion Connectivity project wasprimarily in planning and preparation, including coordinating access to additional properties, filling staff positions, procuring equipment and supplies, securing permit approvals from regulatory agencies, and initiating camera surveillance of the project area.

In addition to the 2,600 acres of the Rocks Ranch and adjacent property owned/overseen by our partners at the Land Trust of Santa Cruz County (LTSCC), the WHC has successfully secured access to the southwest Nature Area of the Hollister Hills State Vehicular Recreation Area (SVCRA), and an 11,000-acre adjoining ranch owned by the Gabilan Cattle Company. This property is located in Monterrey and San Benito Counties and is approximately 18-km southwest of Rocks Ranch and HWY 101. The property was initially purchased in 1929 by Rollin Reeves and was entered into a non-developmental conservation easement with The Nature Conservancy in 2006.

Bait Collection (Roadkill Deer).

Thirty-three roadkilled deer were collected in the Santa Clara Valley area between January 1, 2023, and December 31, 2023, and deployed as mountain lion bait in the Pacheco Pass (Cañada de los Osos Reserve (CDLO), and O'Connell Ranch – SCVHA), and Gabilan Mountain Range (Rocks Ranch and the Gabilan Ranch. Seventeen lymph node samples were collected and submitted to CDFW.

Zach Mills (CDFW) also provided five roadkill deer in 2023, which were utilized on Rocks Ranch. In January 2024, Zach Mills provided four roadkill deer: three have been used on O'Connell Ranch and one is stored at our O'Connell Ranch freezer.

Mountain Lion Detections – Field Camera Maintenance (Gabilan Range).

The number and location of field cameras varied throughout the year. Fifty-eight mountain lion detections were recorded among the nine most static field cameras maintained on Rocks Ranch (Figure 54), and sixteen mountain lion detections were recorded on the nine most static field cameras maintained on the Gabilan Ranch (Figure 55). No mountain lions were detected on the two cameras maintained on the Hollister Hills SVRA side of Bird Creek (in place from 2/7/2023-7/1/2023), though scat and tracks were found in the area.

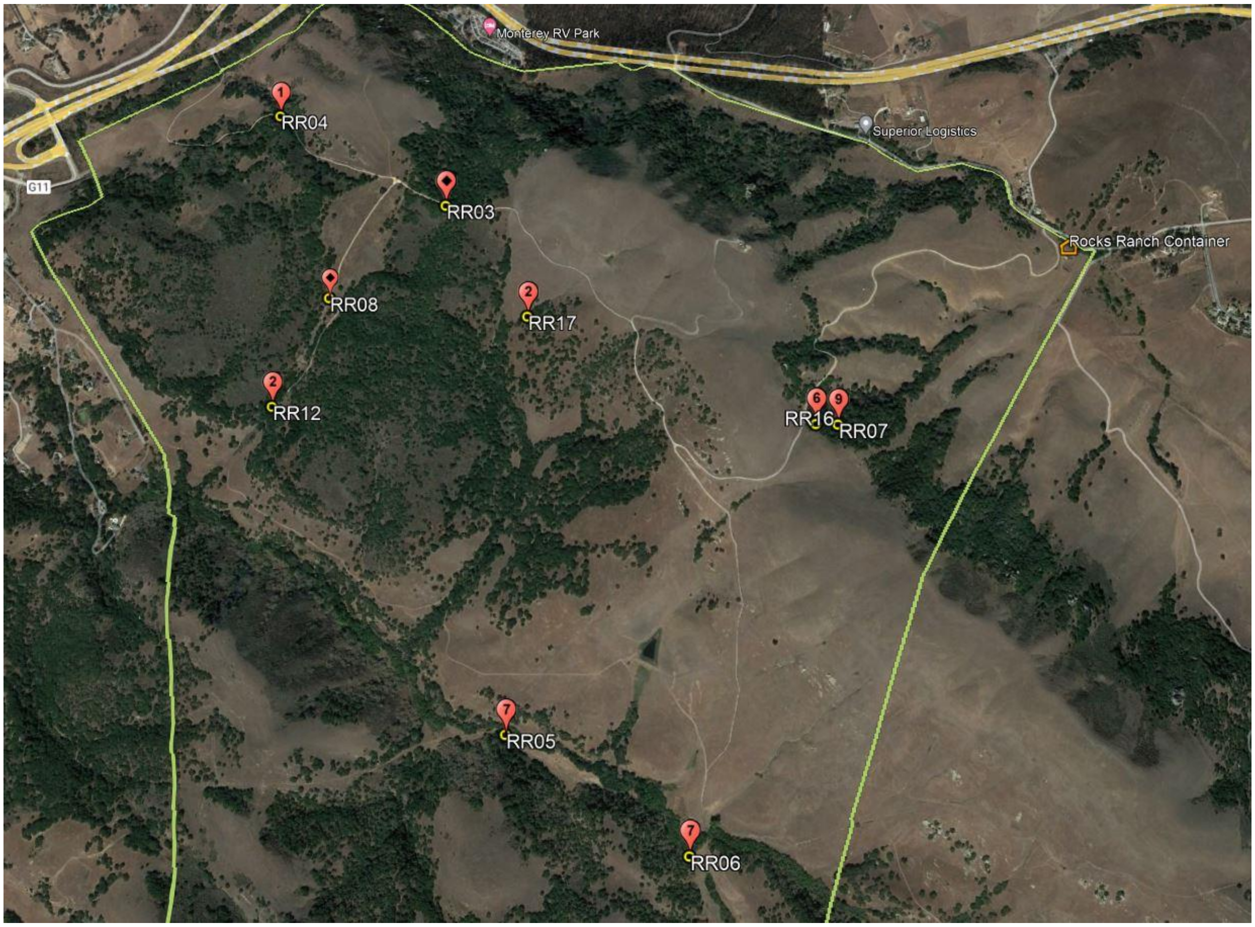


Figure 54. Fifty-eight lion detections were recorded on nine field cameras maintained on Rocks Ranch in 2023. Camera stations RR03 and RR08 each recorded 12 detections.

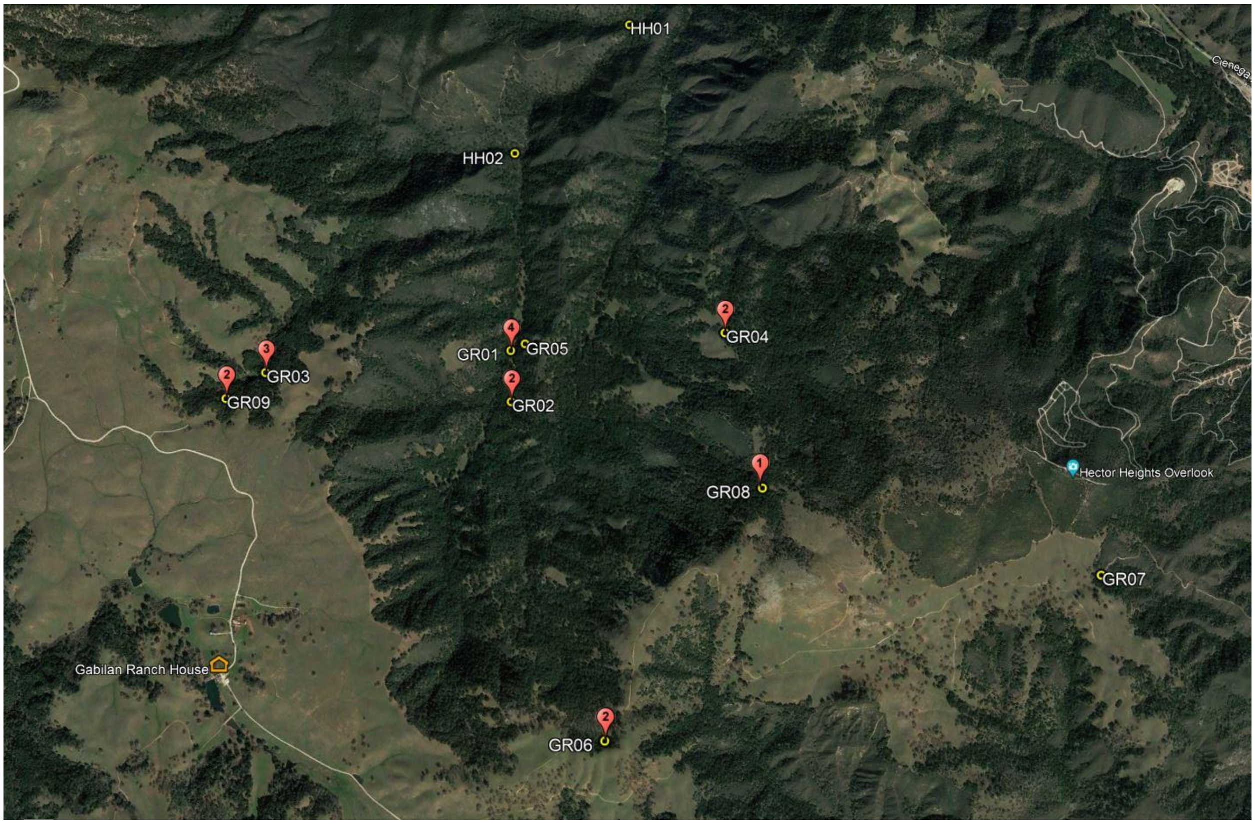


Figure 55. Sixteen lion detections were recorded on nine field cameras maintained on the Gabilan Ranch in 2023. No detections were recorded on the two cameras maintained on the Hollister Hills SVRA (RR01 and RR02).

Bait Sites and Lion Activity (Pacheco).

Six baits were deployed at five bait sites on the Cañada de los Osos Reserve (CDLO - CDFW) (Figure 56), and six baits were deployed at five bait sites on the O’Connell Ranch (SCVHA) (figure 57) in 2023. Mountain lions were detected on three bait sites on CDLO, however, on only one occasion did a mountain lion persistently feed on a bait, which led to a capture attempt (details below). A female mountain lion fed on a bait located on O’Connell Ranch in mid-December but no capture attempt was made at that time. During the preparation of this report in January 2024, on two occasions that same female mountain lion found and fed on a bait located at a different site on O’Connell Ranch leading to a capture attempt (details below).

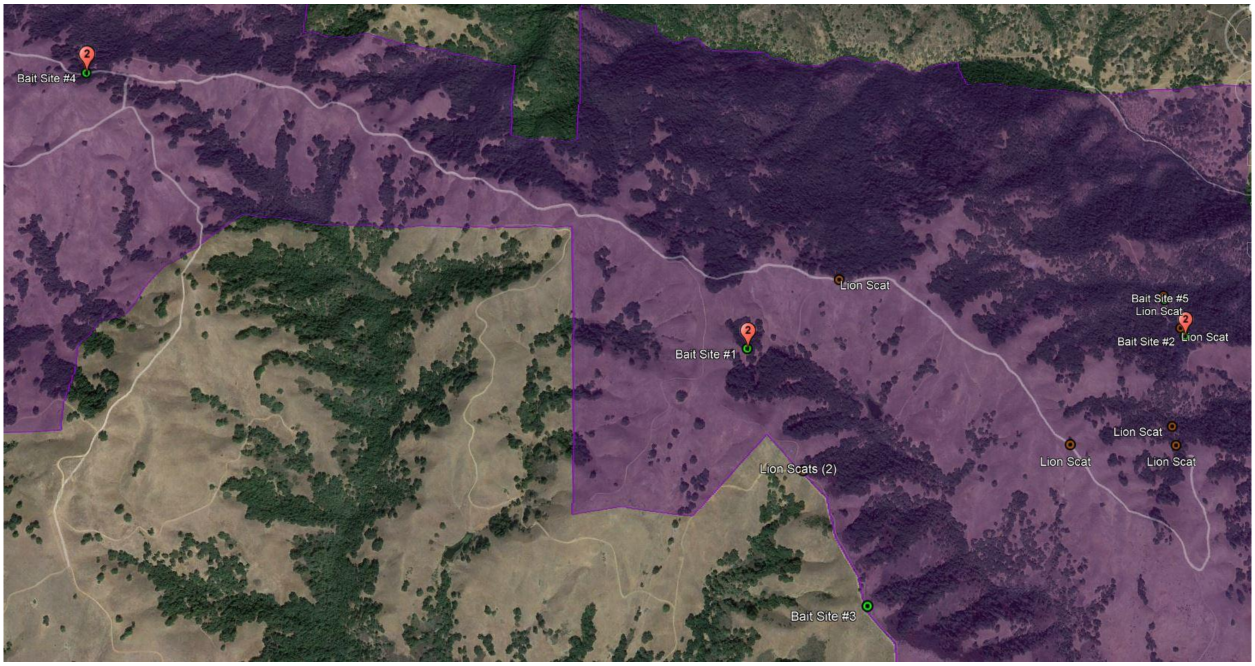


Figure 16. Six baits were deployed at five bait sites on Cañada de los Osos Reserve (CDLO - purple shading) in 2023. Mountain lions were detected at bait sites on four occasions leading to the successful capture of adult female lion F337 (11/3/2023 – bait site #1). Eight mountain lion scat samples were collected on CDLO in 2023.

Figure 57. Six baits were deployed at five bait sites on O’Connell Ranch in 2023. Mountain lions were detected at one bait site on two occasions leading to one capture attempt (11/5/2023). Four mountain lion scat samples were collected on O’Connell Ranch in 2023.

Bait Sites and Mountain Lion Activity (Gabilan Range).

Twenty baits were deployed at 11 bait sites on Rocks Ranch in 2023 (Figure 58), and two baits were deployed at two bait sites on the Gabilan Ranch (Figure 59). Mountain lions were detected at four of the bait sites on Rocks Ranch, however, on only one occasion did a mountain lion persistently feed on a bait, which led to a capture attempt (details below). Mountain lions found both Gabilan Ranch baits, however, on only one occasion did one or more lions persistently feed on a bait, which led to a capture attempt (details below). A consistent challenge in both the northern Gabilan and northern Diablo Range study areas has been the presence of large numbers of wild pigs. The pigs have often fed on baits before mountain lions had a chance to find them. In addition, cattle present on the Rocks and Gabilan Ranches have at times disturbed bait sites or in one case actively appeared to drive a feeding mountain lion away.

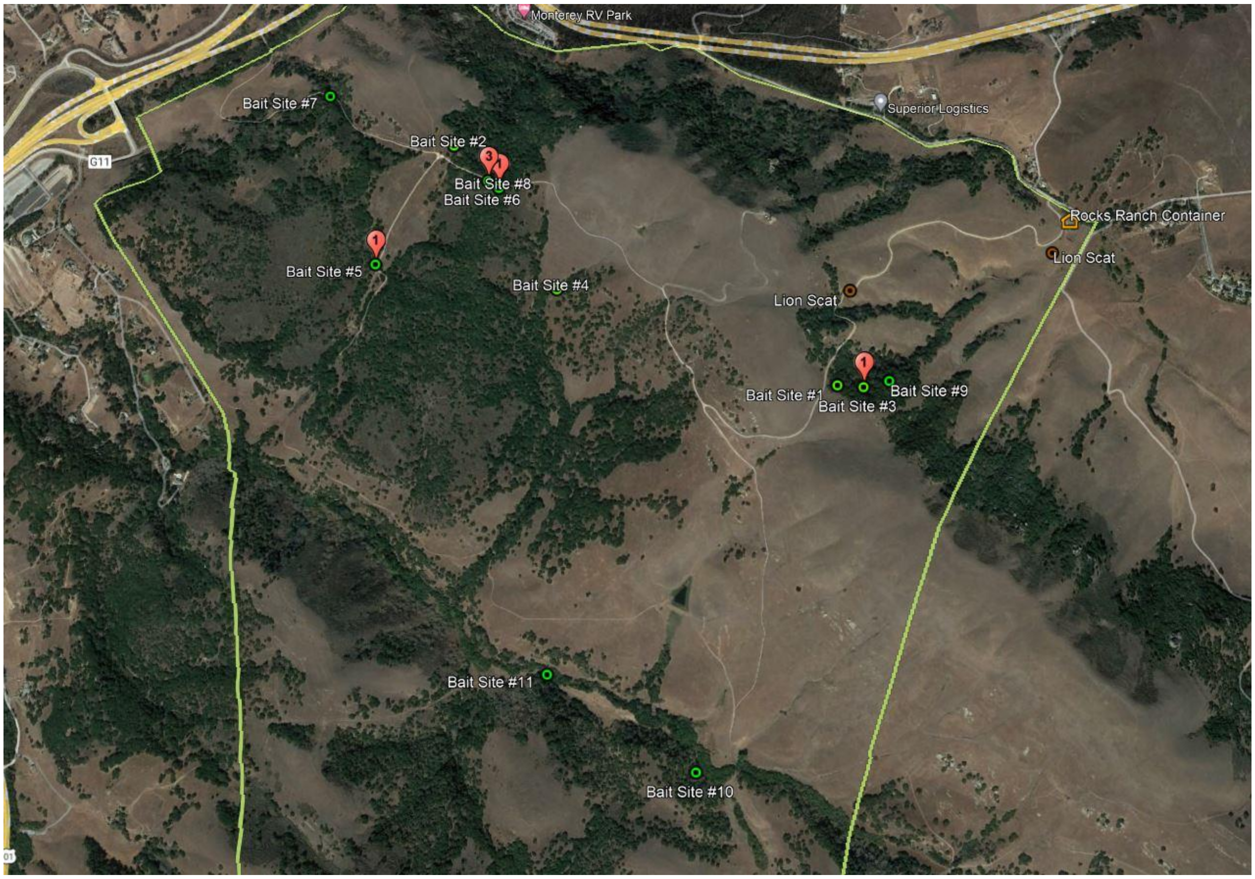


Figure 58. Twenty baits were deployed at 11 bait sites on Rocks Ranch in 2023. Mountain lions were detected at bait sites on six occasions leading to one capture attempt (12/5/2023 – unsuccessful). Three mountain lion scat samples were collected on Rocks Ranch in 2023.

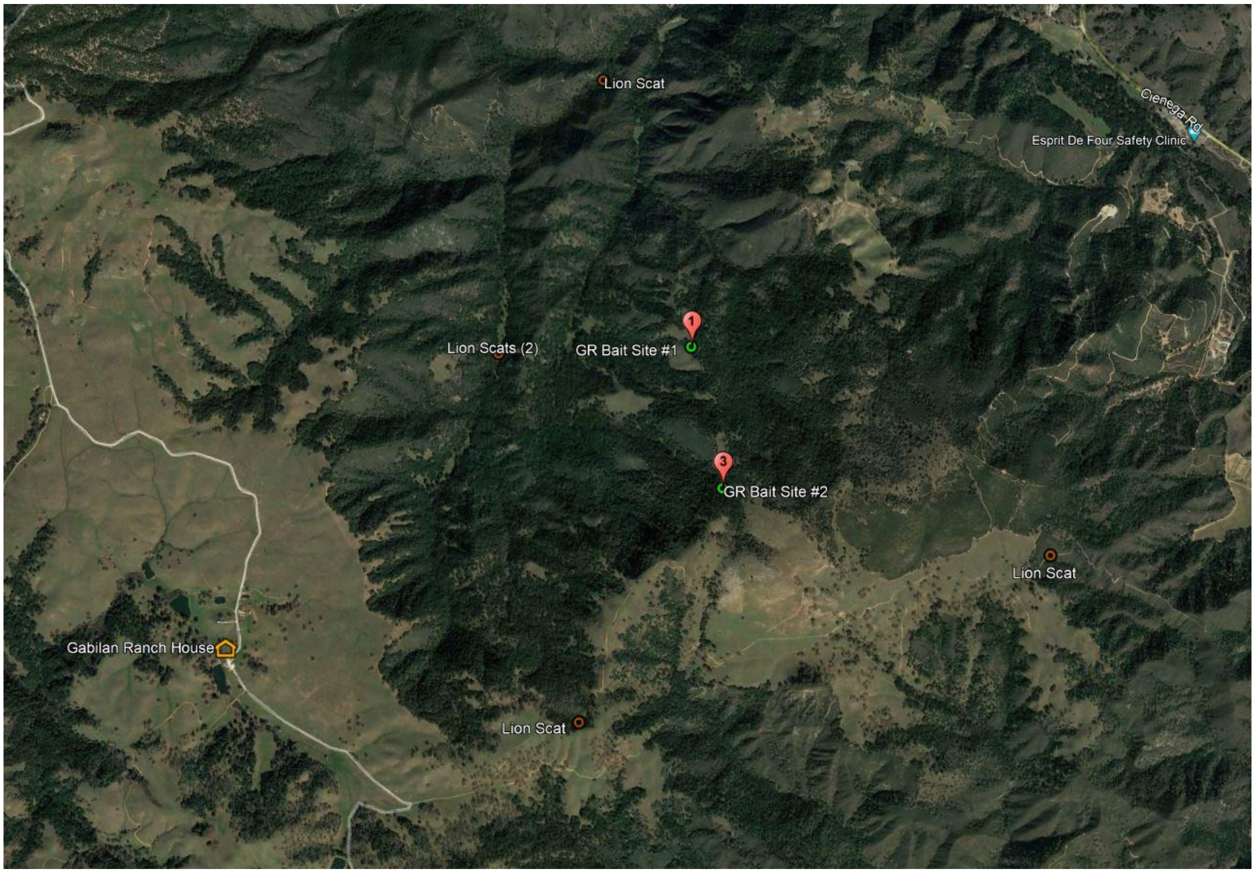


Figure 59. Two baits were deployed at two bait sites on the Gabilan Ranch in 2023. Mountain lions were detected at bait sites on four occasions leading to one two-night capture attempt (6/9/2023 & 6/10/2023 – unsuccessful). Four mountain lion scats were collected on the Gabilan Ranch in 2023, and one scat was collected on the Hollister Hills SVRA.

Lion Captures and Capture Attempts (Gabilan Range and Pacheco).

Gabilan Ranch – Capture Attempt (6/9/2023 & 6/10/2023) - Unsuccessful. In response to a mountain lion or multiple lions feeding on a bait (GR Bait Site #2) on 6/8/2023 and 6/9/2023, the UCD capture team (Fernando Nájera, Juan Gonzalez, and Calvin Duncan) accompanied by Jason Lombardi (CDFW) (6/9 only) set and monitored traps on 6/9/2023 and 6/10/2023. Three mountain lions (1 adult female, 1 juvenile, and 1 adult male) investigated the traps on 6/9/2023, but none of the animals entered the traps. No mountain lions returned to the trap site on the evening of 6/10/2023.

Rocks Ranch – Capture Attempt (12/5/2023) – Unsuccessful. In response to an adult female mountain lion feeding multiple times on a bait (RR Bait Site #6) on 12/4/2023 and 12/5/2023, the UCD capture team (Fernando Najera & Calvin Duncan), accompanied by Zach Mills, Jason Lombardi, Aidan Branney (CDFW) and Matthew Timmer (Land Trust of Santa Cruz – Observer), set and monitored traps on 12/5/2023. The mountain lion returned and investigated the traps, but did not enter.

100 Hillside Road, San Juan Bautista, CA – Mountain lion Capture (11/15/2023). In response to a mountain lion depredation incident, Zach Mills (CDFW) enlisted the assistance of the UCD capture team (Fernando Najera and Calvin Duncan) to capture a juvenile male mountain lion (M338) on the evening of 11/15/2023.

CDLO – Mountain lion Capture (11/3/2023). In response to two mountain lions (1 adult female, 1 unk) feeding consistently at CDLO bait site #1, the UCD capture team (Fernando Najera, Juan Gonzalez, and

Calvin Duncan), accompanied by Zach Mills (CDFW), Julie King, and Matthew Fogarty (SCVHA), set traps and captured an adult female mountain lion (F337).

O'Connell Ranch – (11/5/2023) - Unsuccessful. In response to an adult female mountain lion feeding consistently at OC bait site #1, the UCD capture team (Fernando Najera, Juan Gonzalez, and Calvin Duncan), accompanied by Zach Mills (CDFW), Julie King, and Matthew Fogarty (SCVHA), set and monitored traps on 11/5/2023. The mountain lion did not return. After reviewing additional camera footage, the target mountain lion was determined to be nursing and would have been released without a workup, had she been captured. Additional mountain lions had been detected in the area, so the bait was reset in mid-December. The nursing mountain lion returned to the bait site on 12/21/2023 and fed consistently for six days, but no capture was attempted due to concern with disrupting her nursing of kittens at a den site. As this report was being completed in late January, a female presumed to be the same animal was seen on camera at the same bait site, accompanied by kittens that appeared to be approximately 3-4 months old based on their size. A capture attempt on 1/22/24 was unsuccessful when the animal did not return to the bait site, but a second attempt after she returned to feed was successful on 1/27/24.
Tables 7a,b summarize mountain lion capture statistics, locations, along with transmitter frequencies.

UCD ID	Capture Date	Method	Sex	Age (mo.)	Ear tag	Tattoo	Status
F337	11/22/22	Cage	Female	62.3	337 orange (right)	337 left)	Active
M338	11/25/22	Cage	Male	18	338 orange (left)	338 (right)	Active
F387	01/27/24	Cage	Female	31	128 yellow (right)	387 (left)	Active

Table 7a. Statistics on mountain lions captured during reporting period in

UCD ID	General locations	County	LAT (WGS 84)	LONG (WGS 84)	Injuries	Status	Mort. Date	Mort. cause	Transmit. Freq.
F337	Canada de los Osos	Santa Clara	37.03918	-121.35621	None	Active	N/A	N/A	150.020
M338	San Juan Bautista	San Benito	36.7972	-121.47225	None	Active	N/A	N/A	159.160
F387	O'Connell Ranch	Santa Clara	36.9882	-121.37107	None	Active	N/A	N/A	159.265

Table 7b. Locations of captures and animal disposition/Transmitter frequencies

After the capture and collaring of M338, several depredations on livestock by that animal (mainly goats) were reported to CDFW. This prompted a community meeting with the neighbors of San Juan Canyon organized by CDFW (Zach Mills and Dave Hacker), with the participation of the UCD team (Calvin Duncan). Most of the attendees seemed receptive to implementing deterrence techniques, as well as to improving husbandry practices.

Spatial data

All the GPS fixes recorded to date from F337 showed movement range similar to other females. Her movement pattern seems constricted to the NW side of SR152 (Figure 60). She approached SR152 at several locations but she has not crossed the highway to date.

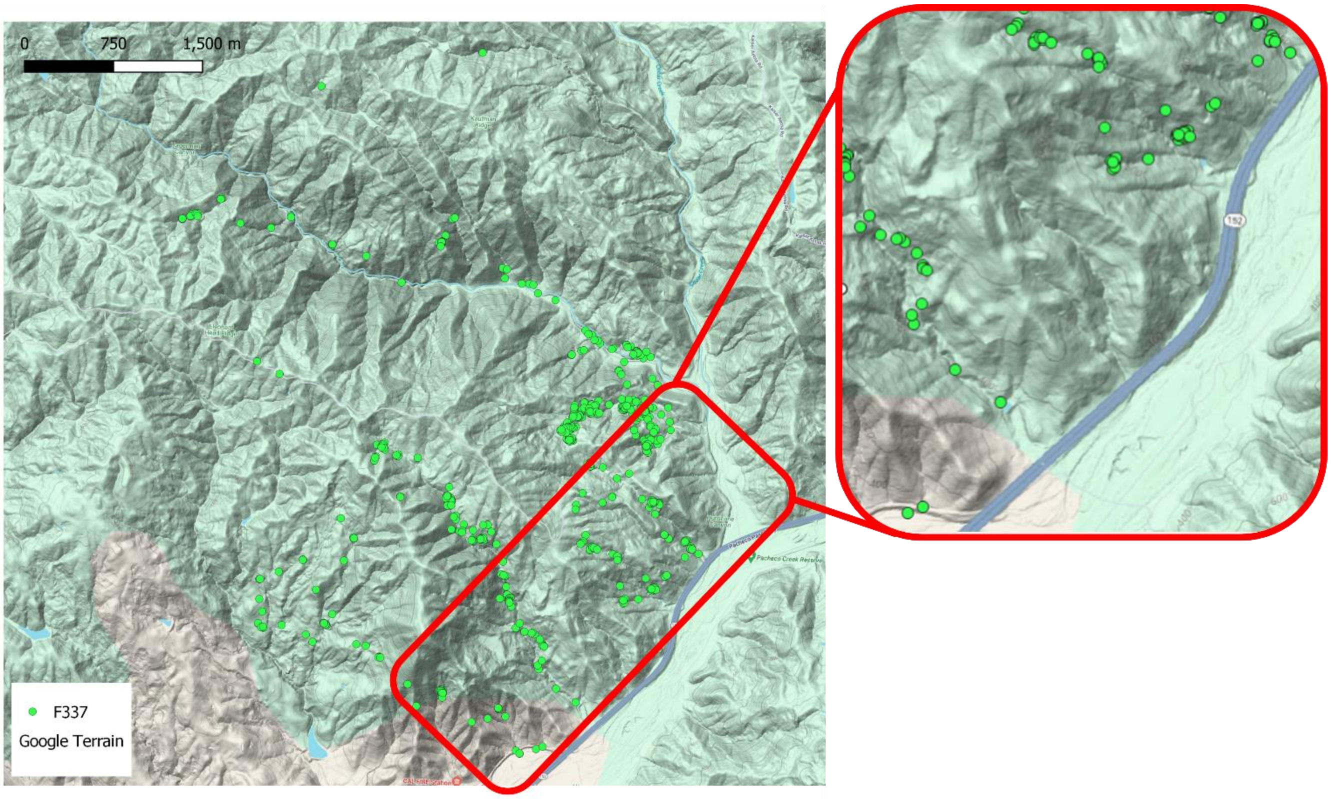


Figure 60. F337 GPS locations to date. In red: detail of F337 fixes taken every 15 minutes within the SR152 geofence.

During the first weeks after his collaring, M338 focused his movement on San Juan Canyon which could be motivated by the easy access to unprotected livestock and his depredation events. Since the beginning of January, he has made exploratory movements NW and SW from his capture location. No attempts to approach SR156 (closer roadway) have been registered to date (Figure 61).

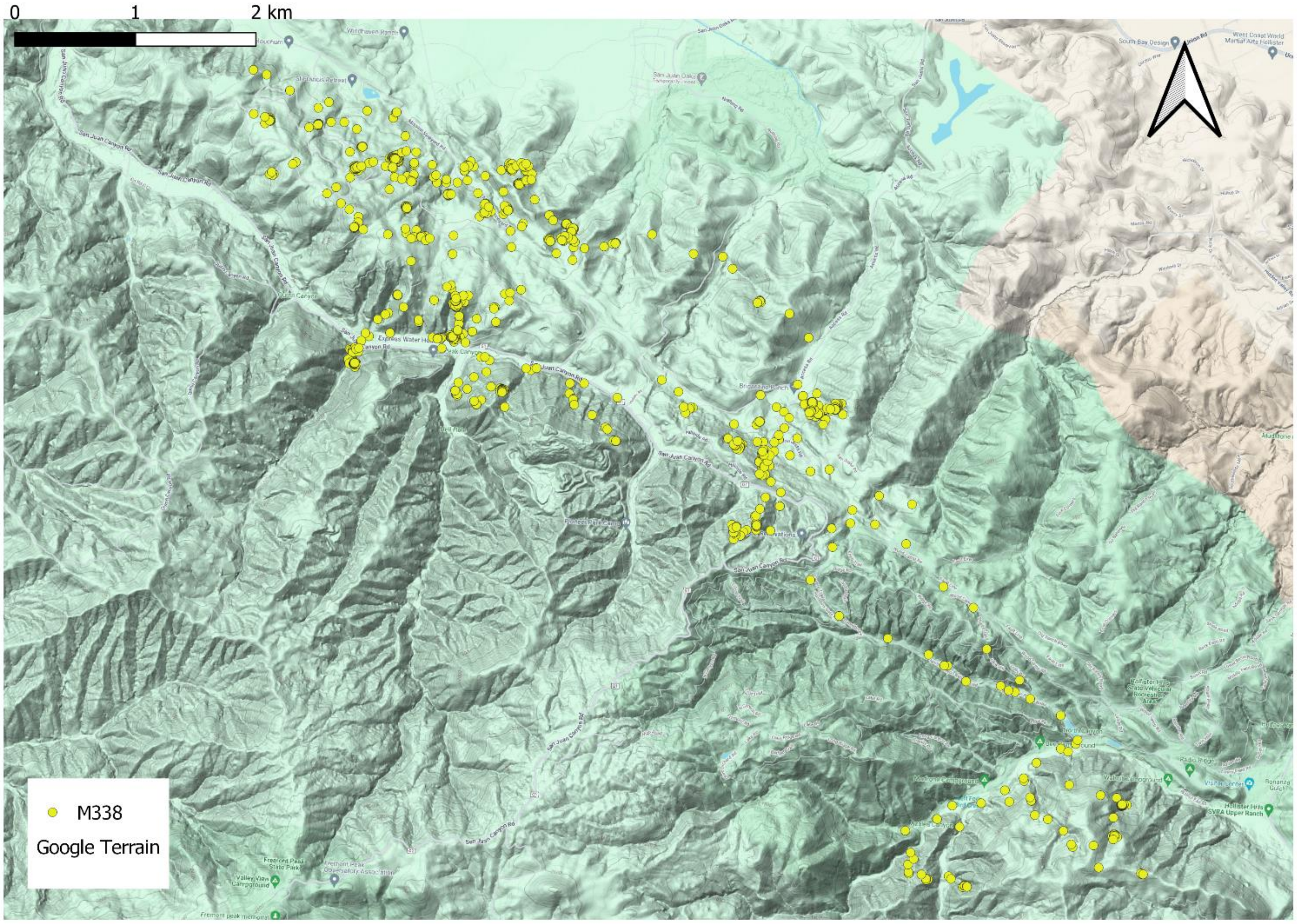


Figure 61. M338 GPS data to date.

Task 2. Understand gene flow of mountain lions and other mammals by opportunistically gathering genetic samples e.g., from captures, roadkill, scat, and hair; Gustafson et al. 2017):

Twenty-seven mountain lion scat samples were collected in the Santa Clara Valley and Gabilan Mountains areas in 2023. Twenty-two have been sent to Michael Buchalski (CDFW lab) and five remain in storage awaiting shipment.

Task 3. Compile results for and consult on efforts to improve/maintain connectivity given existing and future transportation infrastructure.

As GPS collar data becomes available, WHC biologists will process it and develop resource and step selection function models to understand spatial connectivity (Dellinger et al. 2020b). Genetic samples will be analyzed using a single nucleotide polymorphism (SNP) assay to determine individual ID's and contribute to understanding of genetic connectivity (Buchalski et al. 2022). In addition, this Task includes attending/participating in meetings and consulting with pertinent groups to improve/maintain connectivity in the study area. Lastly, this Task also includes overall project funds management with assistance from administrative support personnel at the UC Davis WHC, and overall reporting.

In both the Gabilan Range and Pacheco Pass areas extensive pre-existing data exists from crossing monitoring cameras (Diamond et al. 2022, J. King/C. Duncan pers comm) but little data exists about mountain lion activities in the habitat on either side of the highways. We hope that our efforts will provide substantially more information to help secure or improve connectivity in those areas.

A.5. Discussion

Low annual survival rates of mountain lions in southern California, combined with restricted genetics and connectivity are a major concern, especially for the population in the Santa Ana Mountains which is estimated to number between 16 and 21 adults (Beier et al. 1993, Benson et al. 2019) or 41 individuals based on our DNA scat analysis, but evidence is mounting of concerns for the populations elsewhere in southern California as well.

These findings suggest that with projected increases in human population size in the region and the attendant loss of habitat and increased traffic and roads, that mountain lions in this mountain range are under increasing threat. The population east of I-15, though larger and potentially more robust, also faces the same array of increasing threats.

Even under current conditions of immigration into the Santa Ana's, the likelihood of extirpation of the population over the next 50 years is uncomfortably high (1 in 5 or 6 chance), and if inbreeding depression develops then extirpation in a much shorter time frame (<15 years) becomes much more likely without intervention (Benson et al. 2019). Thus the key to keeping robust populations of mountain lions in southern CA is to assure and improve connectivity, and reduce mortality rates. It is critical that monitoring of the SAM and ePR populations be regular and at a frequency and with protocols that can potentially detect significant population declines in a timely fashion should they occur. Interventions that could include translocation of mountain lions into the SAM should be prepared and planned for in case they become necessary.

In 2023, our team has been involved with extensive communication and collaboration with the three counties in the region, transportation agencies, non-profits such as The Nature Conservancy, and wildlife agencies, and others in an attempt to accomplish these goals. It is also critical that mortality levels be reduced across the region as much as possible. Our work on both depredation prevention and road crossings and fencing are aimed at this goal.

We feel that education, investment in proper road crossings and fencing, habitat conservation, prevention of habitat fragmentation, and proper domestic animal husbandry all must be employed to improve the potential for mountain lion population persistence in the region, especially the Santa Ana Mountains population. These mitigation measures are under human control but require increased financial and political support in order to accomplish real reductions in current rates of mortality. In light of the need for greater movement of males across I-15 into the Santa Anas, reducing mortalities of males (especially young males) east of the freeway should be a major priority. We are exploring other potential programs and pathways that might also be utilized to reduce threats to these populations.

In northeastern California collared mountain lion survival is substantially higher than survival of collared lions in southern California, despite some losses of collared animals over time to depredation permits and disease. It is hoped that our work on depredation reduction that is focused in southern California may also benefit this population. Information from this study area on dispersal, female behavior, and prey choice may inform management decisions by CDFW in this area. The emergence of a case of Highly Pathogenic Avian Influenza in this study site highlights the need to keep monitoring infectious diseases in order to determine their impact on mountain lion populations. Our collaborative work with SDZWA will shed light into this topic.

Our work on connectivity assessment in the Tehachapis and northern Gabilan Range and Pacheco Pass will be informative to many levels of conservation action in those areas. Connectivity in both areas is critical to mountain lion persistence not only in those specific areas but in areas distant to those ranges. It is more and more evident from genetic studies and roadway studies that solutions need to be found via the movement of dispersing animals through the likely bottlenecks that exist. It is our hope that our data generated will also spur increased interest in conservation among the general public in that area.

A.5.1. California Mountain Lion Project Plans for 2024

We will continue collaborations with most of the mountain lion researchers in CA and CDFW to conduct further connectivity and roads research, genetic and disease research, and research into deterrence devices to help protect livestock from predation. We are also actively collaborating with TNC, SANDAG, Caltrans, UC Davis Road Ecology Center, and others on roads improvements.

Mountain lion populations in both the central coast and southern California depend on connectivity through the Tehachapi Mountains to remain genetically viable. For southern California, additional work will still be required in the Transverse Range to ensure viability of mountain lions in the Santa Ana's and Peninsular Range (Figure 62).

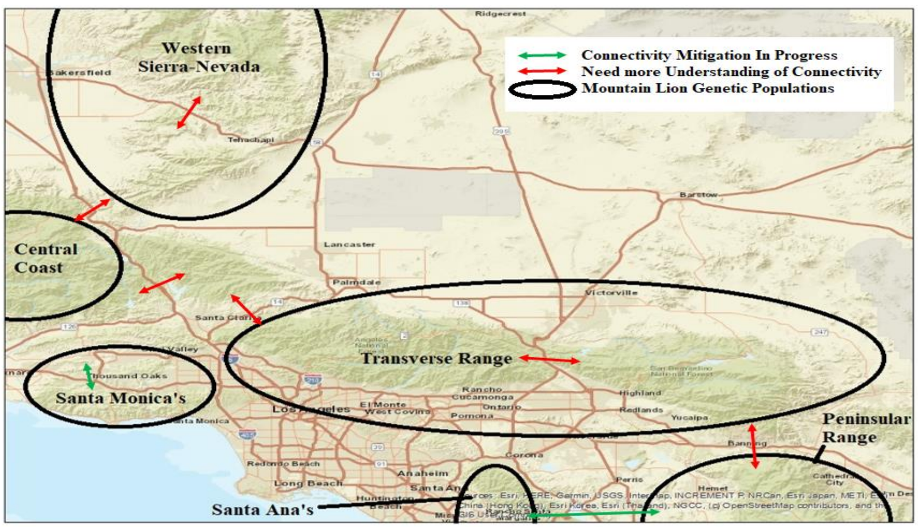


Figure 62. Areas of connectivity focus in southern California that are necessary for long term persistence of mountain lion subpopulations there.

We will be continuing all the efforts detailed in this report through 2024.

The UCD team will continue to monitor the mountain lions with active collars in the eastern Peninsular Range and the Santa Anas. We will be continuing collaring efforts in the Santa Anas to test lighter weight collars that could be worn longer term to assist in ongoing monitoring of the population and in San Diego County near I-15. We will test deterrent devices and strategies on those animals as opportunities arise.

We will be continuing collaring efforts in the Tehachapi and Gabilan/Diablo Ranges that will inform connectivity in these areas. We will also work on deterrence device testing in these ranges opportunistically and we plan to be involved in human-livestock-mountain lion conflicts along with CDFW to mitigate depredation on domestic animals as well as lethal removal of mountain lions.

We will continue our collaboration between IWS and the NE California study and the Audubon Canyon Ranch Living with Lions Project in Sonoma-Napa-Mendocino Counties, as well as the San Diego Zoo to conduct comparisons between the three areas relating to disease and toxin exposure, habitat utilization at fine scales, prey studies, and others.

In the NE CA study area specifically, IWS will also focus on:
-Determining mountain lion kitten survival through non-invasive photo monitoring.
-Continuing to investigate the diet of lions in northeastern California through visiting GPS clusters to identify kill sites.
-Determining mountain lion habitat use, density and range overlap to better understand changes in landscape use patters if/when wolves establish in the study area.
-Developing further peer reviewed publications via another MS graduate student that IWS has taken on to work on mountain lion biology, specifically dispersal patterns and distances in the study area.

Samples and data from all study areas have, and will continue to be, contributed to statewide analyses of survival, genetics, habitat selection, and connectivity, and be available to CDFW.

B. GREY WOLF ECOLOGY

B.1. Introduction

Gray wolves are in the early stages of re-colonizing California. Such an event brings about opportunities and challenges to: 1) monitor population expansion and growth, 2) understand changes in community ecology, and 3) mitigate human-wildlife conflict with this re-colonizing large carnivore. The Conservation Plan for Gray Wolves (Wolf Plan), developed by the California Department of Fish and Wildlife (CDFW), was developed in 2016 to conserve and manage wolves as their population and geographic range increases. A key conservation strategy identified in the Wolf Plan is to: outreach with affected and interested publics to educate and reduce likelihood of perceived or real conflicts with wolves. Thus, there is an identified need to communicate to the public about wolf biology and ways to reduce conflict. Such education and outreach will only serve to bolster the worth of this project.

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Our UC Davis wolf project has been funded by the Wildlife Conservation Network to further elucidate wolf ecology and improve coexistence in California by: 1) understanding the ability of non-invasive techniques to monitor wolf pack size and reproductive status, 2) elucidating community ecology interactions with native ungulates and co-occurring carnivores, 3) utilize genetic techniques for elucidating aspects of wolf demographics and foraging ecology, and 4) outreaching agricultural producers and attempting to educate them on ways to mitigate wolf-livestock conflicts using information from the previous tasks. Such increased understanding will help clarify ecological impacts of a recolonizing large carnivore in California and inform programs seeking to promote coexistence. Providing this data is a role that our organization has played for 20+ years in many areas and with numerous other species.

B.2. Task 1. Monitor wolf pack size and reproductive status using game cameras and acoustic monitoring devices.

Previous research shows that pack cohesion is lower in summer (~35%) than winter (~55%) and thus movements of radio-collared animals may not readily represent movement patterns of other pack members (Benson and Patterson 2015). A first step in assessing ability of game cameras and acoustic monitoring to aid in understanding wolf presence, reproduction, activity centers, and variation in intensity of use across a home range is examining correlation between the utilization distribution of wolf packs (e.g., sign surveys, etc.) and detection rates of wolves in the same pack via game cameras and acoustic tools. Camera and acoustic device deployment and placement would be guided by a-priori knowledge of distribution of wolf sign within known home ranges (Ausband et al. 2022; Lannarilli et al. 2018; Garland et al. 2020). If there is strong correlation between utilization distribution values from presence data (e.g., sign surveys, etc.) and detection rate values from camera and acoustic data, then future monitoring would be able to rely on a greater diversity of tools to monitor wolf packs in California.

Information on intensity of use (or utilization distribution) within a home range is a critical element in calculating amount owed to livestock ranchers in a pay-for-presence compensation program (Dickman et al. 2011). Ranchers operating in areas of higher intensity of use are generally paid more than ranchers operating in a wolf pack home range but in an area of lower utilization. Thus, the more we can understand about the ability of various tools to monitor and detect variation in wolf utilization within a home range, the more informed wildlife managers can be regarding decision making concerning wolf-livestock conflict management actions including a compensation program.

Methods:

Data collection has begun and is being accomplished by deploying game cameras, and deploying acoustic devices in collaboration with the Cornell Ornithology Lab (contributing in-kind support of this work). Camera and acoustic grid development was initially being guided using the known distribution of current packs as for 2022 (Figure 1) in a single-blind experiment approach. It is expected that analysis of data collected over the whole 3-year period will be done in a multi-season occupancy and detection framework (Dellinger et al. 2019).

Figure 1. Current distribution of wolf packs in California as for 2024

A 20-cell grid system was created in each known pack area. Prior to formal initiation of the project on September 1, 2022, scouting and camera placement occurred in approximately one fourth (14 of 60) of the 4x4 km grid cells in summer 2022 (figure 2), with a focus in areas where tracking suggested wolves were occurring most frequently. Cameras were moved to new locations in most cases after 30 days, though some were left in place for 60 or 90 days. Cameras were placed at a total of 31 sites in all. This was done to test the concept and better gauge the challenges that could occur to the field crews going forward. Acoustic devices were also deployed in 5 grid cells to test their function. Our initial design would lead to choosing one of these grid cells to be kept at the same location for the reminder of the study, after the analysis of the initial 2022 pilot period results.

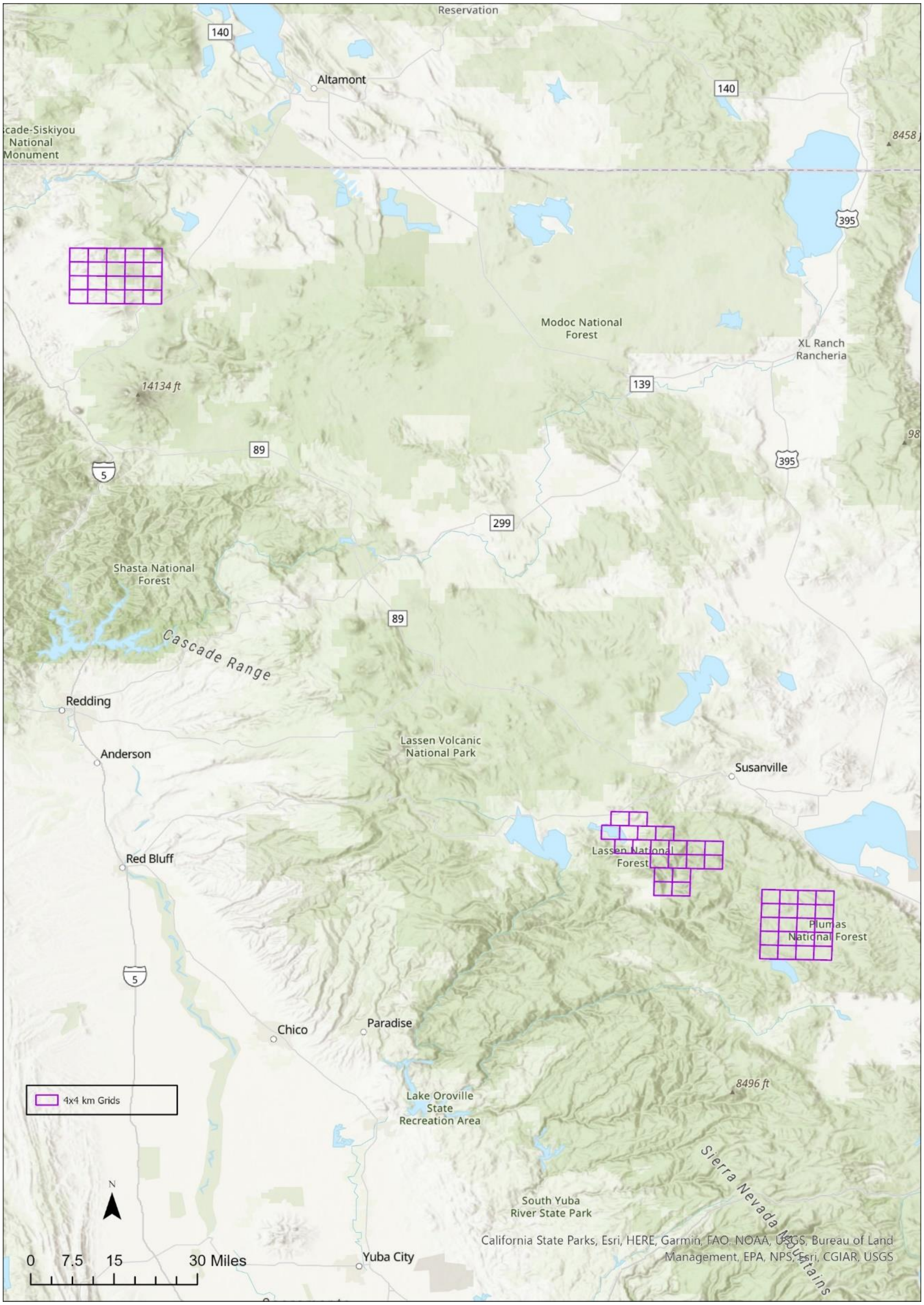


Figure 2. Grid structure employed during our 2022 fieldwork.

However, as part of our collaboration with the California Department of Fish and Wildlife, our research team met with Dr. Jason Lombardi (CDFW Statewide Large Carnivore Research Coordinator) about new CDFW plans to also place cameras on the landscape for wolf monitoring. After examining the resources that each team could bring to the project, a new research approach was developed that would benefit existing efforts by CDFW to monitor wolf packs and coordinate and combine data from both efforts. Results from our data collection will be now included in the wolf demographics statewide analysis carried out by CDFW.

Our current approach utilizes just two grids that cover all the current packs confirmed by CDFW in the Northern area of the State, with 5x5 km cells (figure 3). We have combined the previous two grid structures from the Lassen and Beckwourth packs into a new 205-cell grid that covers home ranges from the Lassen and Beyem Seyo packs. At this time, the fate of the Beckwourth pack is unknown (CDFW communication). The Whaleback grid is composed of 120 cells.

A newly discovered pack in Tulare County is not yet well enough documented as to its range to allow a grid structure to be developed for this type of monitoring, but that may occur in the future.

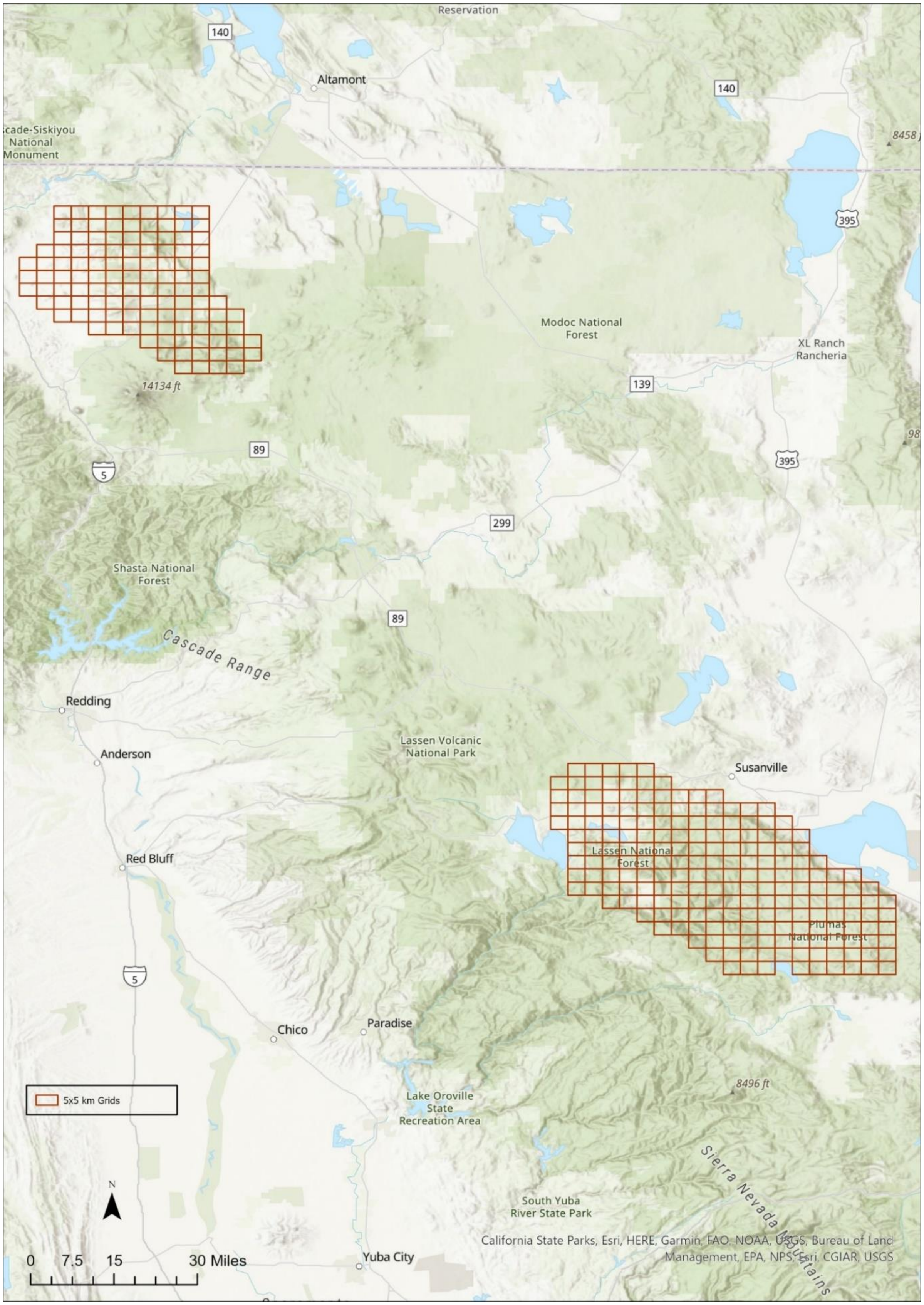


Figure 3. Current grid structure employed during our 2023 fieldwork.

In July and early August of 2023, single cameras were placed in grid cells randomly chosen along a game trail, dirt road or other feature that promotes wildlife travel (Shores et al. 2019). Acoustic devices were paired with cameras, where possible, but in locations where the field team estimated that sound would carry furthest (based on landscape character) to increase detection rates. These cameras and audio devices will remain for a minimum of three years and be checked regularly (e.g., once per three to six months) to replace SD cards and batteries, if needed. Longer deployments may be necessary to derive robust detection rates (i.e., reduced standard errors).

This random camera/audio device deployment intends to:

1. Detect individuals from the known packs.
2. Detect reproduction/wolf pups from the current packs.
3. Detect dispersing individuals outside the boundaries of the current home ranges.

While checking cameras and acoustic devices, surveys will be conducted looking for wolf signs (e.g., tracks, scats; see photographic evidence at the end of this report). Wolf sign will be documented, and presence/distribution of sign will be compared with detection rates of camera and acoustic data. It should also be pointed out that data from this effort could also be used to inform habitat conservation efforts including, but not limited to, developing conservation easements on private lands occupied by wolves. Further, data may help detect wolf presence near high traffic roads.

Results:

Results from the initial pilot study using the 2022 grid structures are outlined in our last progress report. Our camera and acoustic device placement respected the 2023 wolf breeding activity by avoiding placing cameras near dens or rendezvous sites. Before any camera/audio device placement took place, CDFW personnel ensured that our work in the randomly selected grid cells would not interfere with wolf behavior at this time.
In total, we deployed 30 cameras and 9 audio devices in the Siskiyou grid. For the Plumas area, we deployed 28 cameras and 11 audio devices (Figure 4).

From the audio devices, we could only detect wolf howling at two locations, one in Siskiyou and one in Plumas.
While in Plumas we recorded three audio detections over two months (August/September), in Siskiyou we recorded 39 audio detections in three months: July (20 detections), August (18 detections), and October (1 detection).

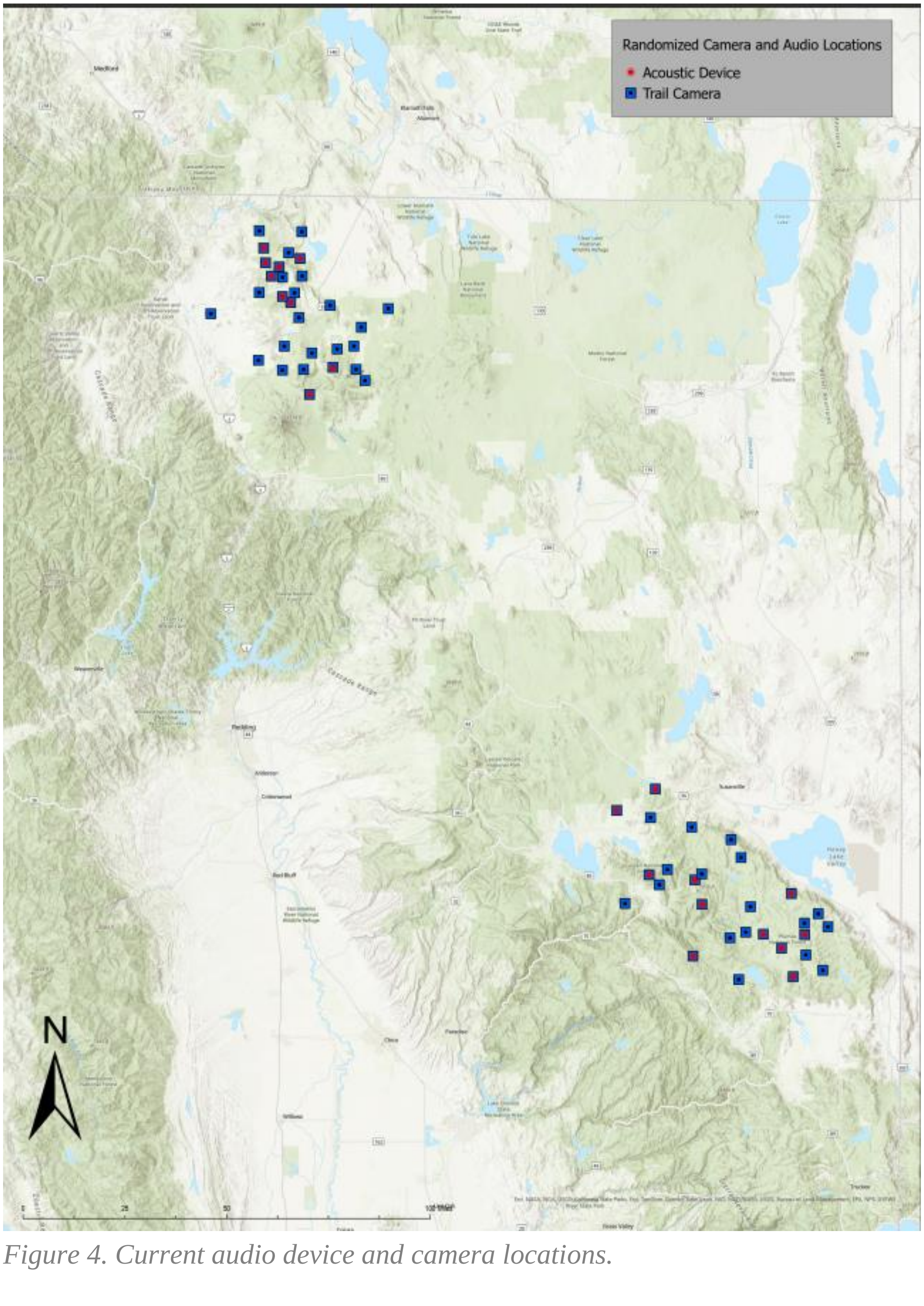


Figure 4. Current audio device and camera locations.

B.3. Task 2. Understand wolf community ecology.

Methods:

The camera grid efforts mentioned above will serve a dual purpose by also providing data on occupancy and detection rates of wolves and other wildlife in relation to one another (Keim et al. 2019; Rossa et al. 2021). Processing of photo data (e.g., culling blank photos and identifying species in remaining photos) will be aided by in-kind collaboration with CDFW. Ultimately this work could elucidate indirect impacts of wolves on livestock (Muhly et al. 2010) and interactions with native species (Shores et al. 2019). Both of these facets are identified as key research topics in the Wolf Plan (Kovacs et al. 2016).

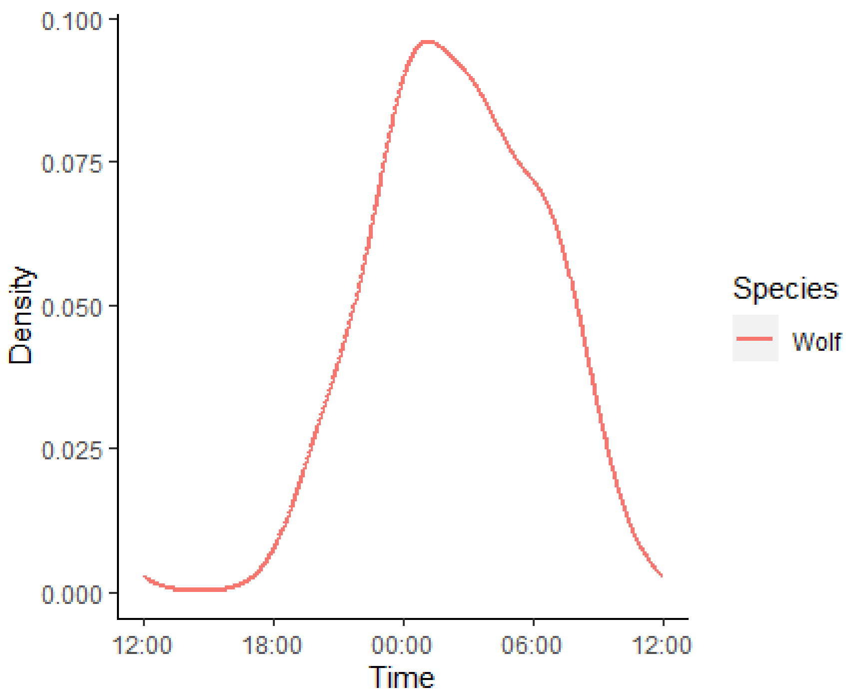
Results:

Analysis of camera cards for documentation of other species has occurred after our first deployment. Processing of photo data has been undertaken by CDFW thanks to our collaboration. Some of the camera analysis has been performed using the software Wildlife Insights (e.g., detections).
The major findings in relation to species detections are as follows:

- 390 images of 33 detection sequences of wolves.
- Whaleback pack area (5 cameras) – 19 detection sequences including one collared wolf.
- Lassen pack area (2 cameras) – 2 sequences
- Beyem Seyo pack area (4 cameras) – 12 sequences including one collared wolf.
- Mountain lions: 26 detection sequences of 394 photos. Mountain lion detections did not occur at sites with wolves. Mountain lions and wolves spatially overlapped at just one camera location.
- Black bears: 4325 images across >150 detection sequences
- Other species detected by our cameras include: feral horses, badgers, elk, mule deer, coyotes, grey fox, and bobcat.

One of our most notable findings during this time frame relates to the >15,000 detection sequences of motorized vehicles in the national forest at all times of day which highlights the potential for serious impacts on species detection/occurrence.

Aidan Branney, Large Carnivore Scientific Aide, also analyzed the diel activity pattern for the three large carnivore occurring in our study site. See below.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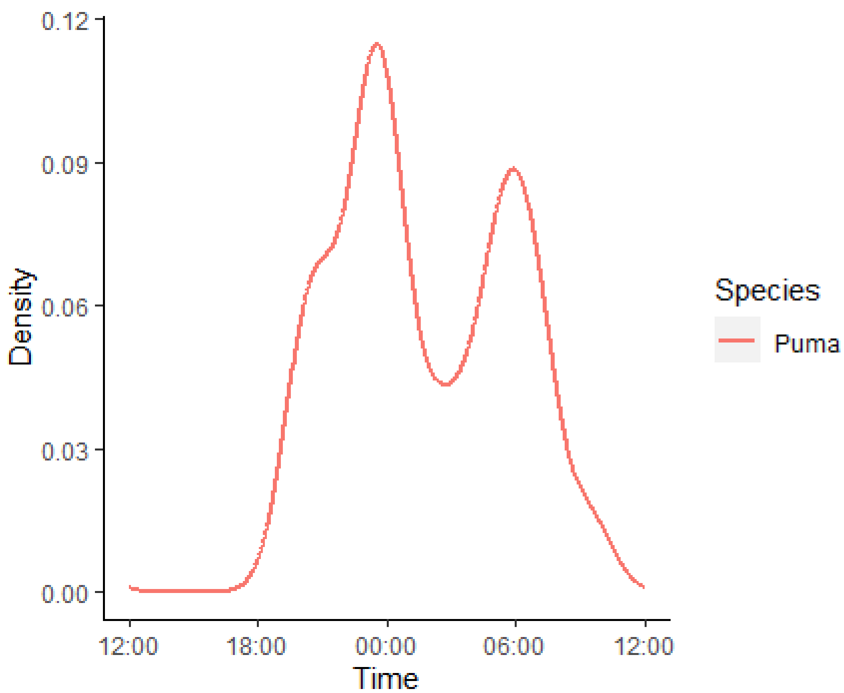
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0.06
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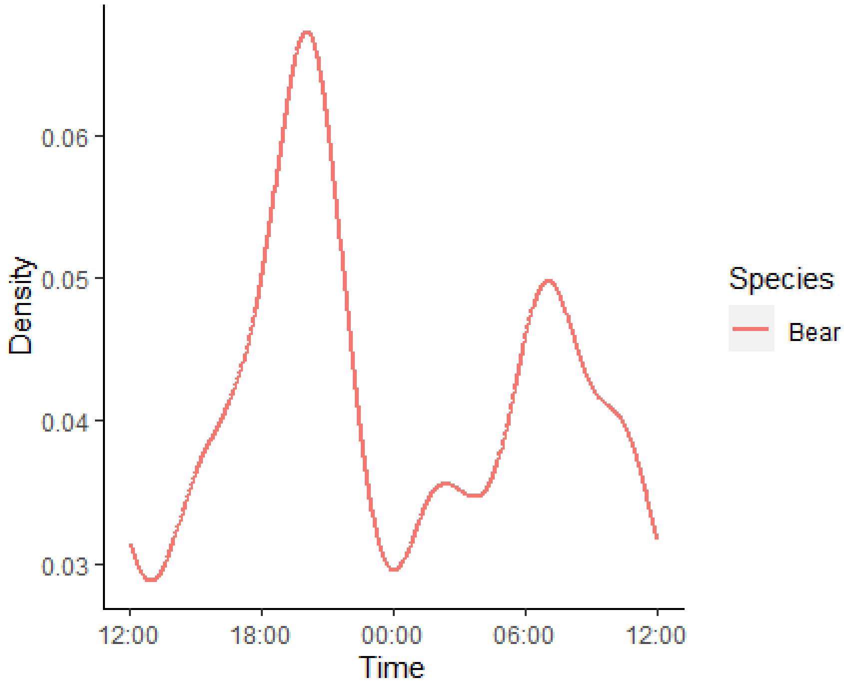
0.00
Density
12:00
18:00
00:00
06:00
12:00
Time
Species
Puma



B.4. Task 3. Use noninvasive genetics to elucidate wolf demographics and community ecology

Methods:

For the sign surveys mentioned in Task 1, during the 2022 field work, any wolf scat encountered was collected for analysis. Analysis by our collaborator at UC Davis, Dr. Ben Sacks, was done to determine: 1) unique genotypes of wolves, 2) relatedness of individuals if possible, and 3) diet of wolves.

The same process will be followed with scat collected in subsequent field seasons, though at the request of CDFW, identity assignment via scat-based DNA will be done by the CDFW Wildlife Genetics or Forensic Genetics Lab. This is due to their pre-existing database of wolf DNA from the state. These results will give insight into wolf demographics and ecology, intuitively bolstering insights from Tasks 1 and 2 above. Deriving individual genotypes of wolves from scat using microsatellite and SNP techniques can help with understanding pack composition and possibly relatedness within and between packs. For example, this effort could prove effective at helping identify and track dispersing animals and understand their pack of origin.

Concerning community ecology, a dataset exists in which the diet of wolves in California was examined previously (Dellinger et al. 2021). Growing and refining this dataset using metabarcoding techniques would prove beneficial to further understanding wolf ecology and interactions with domestic and native prey. Metabarcoding of scats for diet assessment will be done either by Dr. Sacks's lab as before or by one of the CDFW labs.

Results

During the 2023 fieldwork season, we have been able to collect 6 potential wolf scats based on size, content, and morphological features. One corresponded to the Lassen/Beyem Sayo packs grid and five corresponded to the Whaleback pack.

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- San Diego County Association of Governments
- Santa Clara Valley Habitat Agency
- The Nature Conservancy
- Orange County Natural Communities Coalition
- Orange County Parks
- San Diego Zoo Wildlife Alliance
- California Department of Fish and Wildlife Local Assistance Grant Program
- The Foothill East Transportation Corridor Agency
- Land Trust of Santa Cruz County
- South Coast UC Research and Extension Station
- Caltrans
- San Diego State University Santa Margarita River Ecological Reserve
- UC Riverside Emerson Oaks Preserve
- Western Riverside County Regional Conservation Authority
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- The Irvine Ranch Conservancy
- City of San Diego Water District, Parks, and Environmental Services Departments
- Caltrans
- Foothill East Transportation Corridor Agency
- The Nature Conservancy
- San Diego State University
- Western Riverside County Conservation Authority
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- The Wildlands Conservancy
- The Conservation Fund
- Rivers and Lands Conservancy
- Rancho Mission Viejo

- Wind Wolves Preserve
- Gabilan Ranch
- Land Trust of Santa Cruz County
- Santa Clara Valley Habitat Agency
- Private landowners

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APPENDIX A. A Review and Recommendation of Techniques for Long-Term Monitoring of Mountain Lions (*Puma concolor*) in Southern California.

A Review and Recommendation of Techniques for Long-Term Monitoring of Mountain Lion's (Puma concolor) in Southern California

University of California – Davis Agreement A37682
Amendment #2 SANDAG Contract #5005298 Amendment #2
(S890571)

Task 6

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A Review and Recommendation of Techniques for Long-Term Monitoring of Mountain Lion's (Puma concolor) in Southern California

University of California – Davis Agreement A37682 Amendment #2
SANDAG Contract #5005298 Amendment #2 (S890571)

Task 6

Update:
San Diego Association of Governments
October 20, 2023

Executive Summary

This document is a fulfillment of Task 6 in the contract/agreement referenced above wherein the University of California Davis Wildlife Health Center (UCD-WHC) seeks to provide the San Diego Association of Governments (SANDAG) with a thorough review of relevant literature on scientific techniques for monitoring mountain lions in southern California.

For each technique discussed herein, we were asked to critique them for their applicability to:

1. Mountain lions specifically, including any relevant local past efforts using the technique,
2. Special considerations related to application of technique,
3. Feasibility of employing the technique to obtain meaningful and repeatable results,
4. Quality of data and robustness of results associated with the technique, and
5. Costs and intervals associated with employing the method through time.

Key Findings:

1. More intensive approaches, such as radio-collaring, provide the most data (e.g., survival, recruitment, high quality genetic samples, health/disease exposure, and habitat use data); but this approach is financially and logistically intensive.
2. Non-invasive approaches have potential but some, such as cameras, have not been thoroughly investigated as to their utility and robustness.
3. All non-invasive approaches should, depending on sample size, provide insight on some aspects of demographics (i.e., density and abundance) and some, like scat detection dogs, can also provide limited insight on genetic metrics; understanding population status beyond these two metrics is limited with current non-invasive approaches.
4. Opportunistic data collection from roadkill animals could bolster insight gained from non-invasive approaches by providing high quality genetic and health/disease samples as well as providing insight on areas of potential connectivity concern.
5. Insight from non-invasive methods can be improved if informed with existing data from radio-collars.
6. Long-term low fix-rate collars could be used to get coarse demographic data at a lower cost than traditional collaring efforts, which could then inform more financially and logistically feasible non-invasive methods. This integrated approach could provide an in-depth understanding on population status and trend at a reduced cost.

Introduction

Mountain lions (*Puma concolor*) are the most widely distributed large carnivore in the western hemisphere and can be found inhabiting areas from 64° N to -55° S latitude (Franklin et al. 1999, Jung and Merchant 2005). Given this expansive distribution, substantial spatial requirements, and their ecological niche as an obligate large carnivore (Nielsen et al., 2015), mountain lions have considerable ecological impacts over vast areas (Ripple et al. 2014). Mountain lions can influence ecosystem level processes such as nutrient flow (Barry et al. 2019); impact prey population dynamics (Conner et al. 2018) and behavior (Laundre 2010); indirectly affect plant communities (Ripple and Beschta 2008); alter competitive interactions between herbivores (Logan and Sweanor 2001); and provision (Elbroch et al. 2015) and compete with other carnivores (Bartnick et al. 2013). However, their wide distribution, and food and spatial requirements present management challenges including range expansion (LaRue et al. 2012); habitat fragmentation that isolates populations (Benson et al. 2019); predating endangered species (Conner et al. 2018); and damaging private property (Dellinger et al. 2021a). These ecological benefits and management issues often cause special interest groups, and the public in general, to advocate for wildlife management agencies to take actions to: promote mountain lion populations for their ecological benefits; limit populations to address management challenges; or some combination of these two ends of this spectrum (Greenspan et al. 2021). Thus, wildlife management agencies need scientifically robust and up-to-date mountain lion density and abundance estimates using methods that are repeatable, and economically and logistically feasible to implement (Beausoleil et al. 2021). However, few wildlife management agencies have reliable demographic information on mountain lions in even a portion of their management jurisdiction where mountain lions occur (Murphy et al. 2022).

While wildlife managers, researchers, and the public all have a keen interest in, and place a high priority on, having current information regarding mountain lion densities in a given area (Jenks 2011), methods used to derive estimates have varied considerably (Choate et al. 2006, Cooley et al. 2009, Russell et al. 2012, Davidson et al. 2014, Proffitt et al. 2015, Stoner et al. 2018, Alldredge et al. 2019, Murphy et al. 2019, Loonam et al. 2021). Mountain lion density and abundance is estimated, generally speaking, using two approaches: a minimum count of known animals with associated monitoring data (e.g., radio-collar data and age class data) divided by the amount of habitat in a demarcated study area (Lambert et al. 2006, Cooley et al. 2009), or a spatial capture-recapture (including spatial mark-resight) framework using a variety of methods including, but not limited to, DNA from scat (Davidson et al. 2014), skin biopsies (Proffitt et al. 2015), or hair (Alldredge et al. 2019), or collars and/or cameras (Alexander and Gese 2018, Murphy et al. 2019, Loonam et al. 2021). The minimum count approach has been used for many decades and has the benefit of generating a lot of detailed site-specific data on mountain lion populations (Beausoleil et al. 2013). However, such an approach is very resource intensive and difficult to replicate. Murphy et al. (2022) conducted a review of techniques and determined approaches employing a spatial capture-recapture framework could estimate mountain lion populations but precision of estimates, primarily due to size of the study areas, was a concern that should be addressed prior to initiating any data collection efforts. In general, there is uncertainty related to how different data collection and analytical methods relate to one another (Beausoleil et al. 2021, Murphy et al. 2022). Additionally, some of the difficulty in comparing results is simply related to differences how studies define spatial and temporal scales (Cooley et al. 2009, Dellinger et al. 2018) and what segment of the population (e.g., adults and/or subadults) density is being estimated for (Rinehart et al. 2014, Beausoleil et al. 2016). To this point, most efforts to estimate mountain lion density have been conducted once over a short time period (e.g., < 5 years) equal to or less than 1-2 generations as it relates to mountain lions, and/or most efforts occur at a spatial scale that only encompasses an area the size of an average male mountain lion's home range (e.g., < 300 km^2; Beausoleil et al. 2013, Murphy et al. 2022). Given that processes influencing mountain lion population viability occur over much longer temporal (van de Kerk et al. 2019) and larger spatial scales (Dellinger et al. 2020), there is a need to standardize efforts across a given jurisdiction (e.g., regional, state, or provincial level; Beausoleil et al. 2013) and replicate efforts regularly (e.g., every 5-10 years given mountain lion generation times; Jenks 2011) to understand population trends.

Mountain lion management in California has gone through many changes, resulting from changing attitudes of public stakeholders, policy makers, and elected officials (Bruskotter and Shelby 2010; Davenport et al. 2010). Mountain lions were subject to a bounty system in California from 1906-1963 wherein, according to records, 12,580 individual mountain lions were lethally removed by members of the public and state wildlife management agency staff (Dellinger and Torres 2020). From 1964-1969, mountain lions were designated as vermin and could be lethally removed year-round without a bag limit or hunting license. For two years (1970-1971) mountain lions were designated as a game species, wherein a hunting license and tag were required to lethally take a mountain lion. During this time 118 individual mountain lions were reported taken (Mansfield and Weaver 1989). In 1972, the state legislature enacted a moratorium on the hunting of mountain lions, due to growing public concern over the status of the species in California. The moratorium lasted until 1986 but was made permanent by a public referendum in 1990 that was enacted in 1991 wherein mountain lions have since been designated as a 'specially-protected' species (Dellinger and Torres 2020). Since the cessation of hunting in 1972, citizens could get a permit from California Department of Fish and Wildlife (CDFW) to lethally remove a mountain lion if it was deemed they had lost domestic animals to a mountain lion. Since 2017, CDFW has issued fewer permits authorizing lethal removal of mountain lions and increasingly permitted citizens to utilize non-lethal measures to deter mountain lions from depredating domestic animals (Dellinger et al. 2021b). The specially-protected status has resulted in a wide array of concerns from various parties. Ungulate conservation groups have expressed concern that the mountain lion population is growing unchecked and is negatively impacting deer (*Odocoileus hemionus*) herds due to high rates of predation (Proposition 197 [1996]; Walgamuth 2017). Conversely, predator conservation groups have suggested that a combination of negative factors (e.g., habitat loss, degradation, and fragmentation) threatens the viability of mountain lions in some parts of California (Benson et al. 2019, Dellinger et al. 2020, Gustafson et al. 2022). Dellinger and Torres (2020) demonstrated that statewide it is likely that mountain lion abundance has rebounded and could be as high as 5,000 or more individuals. However, the authors acknowledge that their findings are limited and urge for more rigorous efforts be undertaken to estimate mountain lion density and abundance statewide as well as in regions where mountain lion populations are at higher risk in order to inform decision-making related mountain lion conservation and management issues. (Benson et al. 2019, Dellinger et al. 2020, Dellinger et al. 2021a, Gammons et al. 2021, Gustafson et al. 2022).

Mountain lions exist throughout San Diego County, and have relatively low overall survival rates (Vickers et al. 2015, Benson et al.2023). As a result, concerns have been raised about the possibility of long term overall population decline and the need to monitor the population trajectory over time.

Herein we detail and discuss three non-invasive techniques for monitoring mountain lion populations: 1) hair sampling; 2) scat sampling; 3) and camera monitoring. We also discuss the utility of opportunistic data collection (e.g., sampling carcasses of recently deceased animals) and modifications of more invasive approaches (i.e., radio-collaring) to inform long-term population monitoring of mountain lions in southern California. Lastly, we provide a decision matrix of techniques, or combination of techniques, for mountain lion monitoring with +/- and estimated costs associated with each option. Additionally, this decision matrix includes insight that each technique, or combination of techniques, may provide, including but not limited to: 1) demographics; 2) population genetics; 3) habitat connectivity; 4) health/disease exposure; and 5) foraging ecology.

Techniques

Hair Sampling

To our knowledge, research involving non-invasive collection of mountain lion hair samples for understanding aspects of population status are limited. Alldredge et al. (2019) reported on an effort along the Front Range in Colorado. This effort used predator calls and scent lures to attract mountain lions. Predator calls and scent lures were placed within cubbies made of brush/wood (Figure 1). Barbed wire was erected around the cubbies such that a mountain lion would have to cross the barbed wire to get to the attractant. Sites were monitored by game cameras. The study area was gridded off into 5 x 5 km^2 cells and 5 sites were established in each cell. A total of 165 sites were established though not all were operational at the same time or for the entirety of the study. Sites were monitored once per week and active for a total of 12 weeks per year for three years. Due to logistical difficulties, there were a total of 8,200 trap nights. Of those trap nights, there were 246 detections of mountain lions on game cameras (0.03 detections per trap night). Fifty-six percent (n = 138) of game camera detections were associated with hair samples being deposited and 33% (n = 46) of hair samples were of high enough quality to provide individual genotypes. Thus, the rate of individual identification of a mountain lion via DNA from hair was 0.006 per trap night. The raw number of genotypes derived over the extent of study was too few to allow for modeling of local mountain lion density.

Rossettie et al. (2022) reported on a similar effort in southwestern New Mexico that occurred over a 6-month period of time. This effort used modified foothold traps, monitored by game cameras, placed along travel routes to pull hair from the ankle/foot of a mountain lion that stepped into the trap. The trap allowed for the mountain lion to quickly pull free of the trap after the trap was triggered. Sixty-six sites were established for this effort for a total of 1,618 trap nights with traps being checked every 5-7 days. During this effort there were 20 instances where a mountain lion passed through a site and hair was collected on 7 (35%) of those instances; and 6 of the 7 contained high enough quality DNA to derive an individual genotype. Thus, the detection rate for mountain lions in this study was 0.01 detections per trap night and the rate of individual identification of a mountain lion via DNA from hair was 0.004 per trap night. Again, the raw number of genotypes obtained, and low number of recaptures per genotyped individual (< 2 detections per individual), does not allow for modeling local mountain lion density.

Vickers et al. (2022) conducted a hair snare effort in the Santa Ana Mountains of southern California. This effort, similar to Alldredge et al. (2019), used a 5 x 5 km^2 grid cell approach. Hair capture was attempted with both barbed wire and rollers or tape coated with a sticky substance. Sixty-six of these grids were created across the landscape and hair snare collection sites were constructed therein, though only 58 sites were active for the entire 3 month period of the initial study due to fire and other access issues. Sites were monitored weekly for 8 weeks then every other week. After the initial 3 month period, a subset of sites where mountain lions had been recorded were adapted with other hair snare designs to test efficacy and monitored every 2-3 weeks. Again, sites were monitored by game cameras. Multiple hair snare strategies were used during the effort. Cubby sites (Figure 1), rub stations (Figure 2), and corral sites (Figure 3) were all used to try and obtain hair samples from mountain lions. Each site visited had an average of 2 mountain lion visits per site (range n=1-5). When all hair snaring methods were combined, this effort resulted in 149 mountain lion detections over 9,156 trap nights (0.02 detections per trap night) with no single method having a detection rate > 0.04 per trap night. Suspected mountain lion hair samples were collected on 55 of the 149 occasions (37%); 11 of these 55 samples (20%) yielded adequate mountain lion DNA to derive a unique genotype, with identification of 7 individuals. Thus, the rate of individual identification of a mountain lion via DNA from hair was 0.0012 per trap night. Two additional individuals were identified in 3 hair samples from feeding and daybed sites. Again, the raw number of genotypes derived over the extent of study was too few to allow for modeling of local mountain lion density.

Some things that limited the success of this method include: 1) site disturbance (bait theft) by other species; 2) site maintenance frequency that was required; 3) drying of stick materials making the rollers/tape less effective; 4) apparent interference of adhesive with the SNP processing; and 5) mountain lion interest in a site (i.e., an individual becoming desensitized to any attractants, or overly cautious about using any). This first limitation is simply due to the fact that other animals can visit a site and remove part of the attractant deposit hair prior to visitation by a mountain lion and reduce the stickiness of the adhesive used to collect samples. Given that, throughout their range, mountain lions exist at densities lower than pretty much every other species of wildlife they co-occur with, the likelihood of other animals visiting a site prior to a mountain lion is very high. The second limitation is related to the fact that likelihood of amplification of DNA from a hair sample declines rapidly following deposition of the sample (Vickers et al. 2022). This necessitates sites being monitored on a regular (i.e., at least once a week) basis. Again, given the naturally low density of mountain lions on the landscape, many sites, activated over a prolonged period of time, are needed to increase likelihood of a mountain lion encountering a site and depositing a sample. This in turn increases the number of personnel hours required to monitor and maintain sites. The third limitation is related to the idea that an animal could become desensitized to the attractants (e.g., lure or predator call) and not visit other sites or, if using the passive hair collection method used in Rossettie et al. (2022), animals could become more wary of certain travel corridors. Either scenario results in a decrease in number of recaptures per individual which is the essential aspect of estimating density using genetic capture-recapture methods.

From these three efforts, and some of the limitations discussed, it is clear that much more technique development and improvement are needed before this method can be considered a reliable one for deriving local mountain lion densities. As it stands, it is not feasible to employ this method to obtain meaningful and repeatable results related to mountain lion densities. Further, the quality and robustness of the data collected from this method are limited given the number of overall samples the individual studies were able to collect and the limited number of recaptures (i.e., number of times the same individual was detected at > 1 site) the studies experienced. These results demonstrate that the method is not currently financially and logistically feasible.

As stated, this method does not currently appear capable of helping derive demographic data. Additionally, given the low success in collecting hair from mountain lions, it also seems like this method is currently limited in understanding population genetics and habitat connectivity. Understanding such things does not require multiple samples from the same individual, which is key for demographics, but it does requires a large number of samples from multiple individuals across a large geographic area. Thus, the logistical and financial limitations mentioned above limit the utility of this method for understanding population genetics and habitat connectivity. The hair samples could be used to look at things like stress (i.e., cortisol) and foraging ecology (i.e., stable isotopes) but, given the limited number of hair collected every time a sample is deposited, looking at these things detracts from an already limited sample set.

Scat Sampling

Research involving use of detection dogs to non-invasively collect scat from various carnivore species has been evolving since the early 2000’s (Long et al. 2007b). From the outset the primary use of the method has been for determining presence and deriving density estimates via genetic capture-recapture methods (Long et al. 2007a). Long et al. (2007b) also showed that data from scat detection dogs was more robust than other methods (i.e., hair snares and camera methods) when it came to determining species presence, distribution, and density; albeit the camera methods used in this study are different than that discussed in the following section.

Davidson et al. (2014) surveyed for mountain lion scat in a relatively small area 220 km^2 in size for 1 month in northeastern Oregon, USA using trained scat detection dogs. They collected and analyzed 249 samples, 73 (29%) of which contained sufficient DNA to derive individual genotypes representing 21 mountain lions for a recapture rate of 3.5 detections per individual. Such a sample size and recapture rate allowed them to derive local mountain lion densities. While this effort took place over a relatively small area as relates to mountain lion spatial requirements, it demonstrates the efficacy of the method for determining local densities.

Dellinger et al. (*In Prep*) utilized scat detection dogs at 12 sites across California (Figure 4) for deriving local and statewide mountain lion densities. These sites were each surveyed over a 1-month period of time and on average each survey entailed scat detection dogs and their handlers covering 958 km (708 – 1256 km) of transects (Figure 5). Ideally, surveys took place just prior to the rainy season and at the end of the dry season. This was done given that moisture readily degrades integrity of DNA, so surveying during the driest time of the year potentially meant more viable samples were available for collection. On average, each 1-month survey collected 52 (24 – 83) mountain lion scat with sufficient DNA to derive individual genotypes representing an average of 24 (13 – 37) individuals at each site for an average recapture rate of 2.2 detections per individual. Given that this effort was statewide, such a sample size is sufficient for estimating density and abundance; however, these analyses were bolstered using pre-existing mountain lion radio-collar data to inform the statistical modeling (Murphy et al. 2019). Preliminary results from this work show that the data from the scat, combined with pre-existing radio-collar data, yields density and abundance estimates with a low coefficient of variation (CV; <0.1) which indicates that the estimates are robust (Murphy et al. 2022).

More specifically, one of the 12 efforts mentioned above occurred in the Cleveland National Forest, and other public and conservation lands, in San Diego county in 2020. This effort yielded 61 genetically confirmed mountain lion scat from 28 individuals for a recapture rate of 2.2 and a local density of 1.86 (SD = 0.09) mountain lions per 100 km^2. Given that the area surveyed was 3,028 km^2, the local abundance estimate was 56 (95% CI = 51 – 62) mountain lions. Additionally, there were three radio-collared mountain lions that were known to be alive in the study area at the time of these surveys. This effort detected all three known individuals (i.e., compared DNA from scat to DNA from when these animals were captured). This success detecting known individuals, and the low standard deviation and relatively narrow 95% confidence intervals again suggest this method is viable at regional as well as statewide scales.

However, it should be noted that this method has limitations with respect to estimating mountain lion density and abundance. First, as with hair snaring, this method requires a genetics lab capable of accurately analyzing samples (Buchalski et al. 2021) and finances to cover costs therein. Next, as mentioned above, success of amplifying DNA in scat is readily influenced by environmental conditions (e.g., precipitation). Prior to genetic analyses of scat, it is also necessary to have: 1) access to enough lands (generally >1,200 km^2; Beausoleil et al. 2013) to derive information reflective of the local mountain lion population; and 2) conditions conducive to survey work (i.e., roads and trails that facilitate good coverage of the survey effort; Figure 5). From a data interpretation standpoint, it is important to remember that age of the animal that deposited the scat cannot be derived via any genetic methods. Thus, genetic results from scat do not allow for inferring age structure of the population of interest. Sex ratios are the only relevant demographic, beyond density and abundance, that can be derived. However, if scat surveys were conducted frequently enough (e.g., every 1 – 5 years) in the same area one could derive coarse estimates of survival rates (Broseth et al. 2010). It should be noted that it is likely that non-invasive surveys like scat detection surveys primarily detect more mobile individuals > 6 months of age (Beausoleil et al. 2016). This is because more mobile individuals are depositing scat across a larger area and thus there is more chance for a scat detection dog survey to intersect a scat deposited by an individual.

In addition to using scat collected via scat detection dogs to estimate mountain lion demographics, this method has also been used to look at mountain lion population genetics (Wultsch et al. 2014) which directly relates to and informs understanding of regional habitat connectivity (Dellinger et al. 2020). Additionally, scat can also be used to look at foraging ecology, whether manually via identification of remains in scat (Hass 2009) or genetically via metabarcoding (Monterroso et al. 2019). Next, recent lab analyses have been successful in detecting viruses in mountain lion scat (Payne et al. 2020) which demonstrates the efficacy of scat for also non-invasively monitoring health and disease status of a population. Given the size of a typical mountain lion scat, and analyses involving demographic, population genetics, foraging ecology, and disease monitoring via scat each only requires a small portion of the total scat sample, the different aspects of inquiry are not competing for a limited amount of physical and genetic material in the scat. Thus, scat can serve to inform multiple aspects of mountain lion population ecology and status.

Given that this appears to be a viable method for monitoring various aspects of mountain lion populations, it is necessary to also provide logistical and financial information to guide any future efforts using this method.

1. How large an area needs to be sampled for informative results?

a. Beausoleil et al. (2013) suggested that relevant population level information about mountain lions should involve data collection efforts that cover ≥ 1,200 km^2. All the current scat detection dog efforts to date in California have surpassed this threshold; thus, replicating these past efforts should allow for understanding any population level trends.

b. As such the survey should attempt to gain access to large blocks of habitat.

2. Considerations when applying this technique.

a. As discussed above, it is ideal to collect scat during the driest time of year since wet and humid conditions cause DNA in scat to more rapidly degrade.

b. Surveying features that facilitate mountain lion travel are the most productive for finding and collecting scat. For example, areas with drainages, ridgelines, and saddles are all good spots to survey.

c. In predominately scrub habitat (which is prevalent in southern California) it will be most productive to try and identify areas with more open understory such as areas with conifer and deciduous tree cover. While mountain lions readily use scrub habitat, the thick vegetation is difficult for the scat detection dog and handler to survey.

3. Who can do both the field and lab work?

a. To date all fieldwork has been conducted by Rogue Detection Teams based in Washington state. Scat detection dog work takes specially trained dogs and handlers. It is best if a biologist familiar with a study area coordinates the effort such that the scat detection teams can focus primarily on the fieldwork.

b. To date all genetics work has been conducted by California Department of Fish and Wildlife's (CDFW) Wildlife Genetics Lab (GRL). The San Diego Zoo Wildlife

Alliance also has a genetics lab that could do the work. Buchalski et al. (2021) outlined a SNP panel that works well for deriving individual mountain lion genotypes from scat and should be used when possible.

4. What are costs associated with the technique?

   a. It takes at least one month of coordination for a biologist to work to obtain access to various lands for a survey (e.g., public and conservation lands) and establish survey grids to guide the scat collection efforts. Following these pre-collection coordination efforts, the biologist will also have to coordinate getting genetic samples to a lab and working to analyze and report results. These later steps can take another month at least. Costs associated with this will vary depending on the hourly rate of the biologist doing the work but it should be anticipated that it will cost $65-$100/hour for a total of $20,800-$32,000.

   b. Equipment costs are minimal and generally limited to Ziploc bags, desiccant for drying out scat, and brown sandwich bags. Costs associated with this should not exceed $2,000.

   c. To date CDFW's GRL has not charged for analyzing scat. However, costs would likely be ~$10,000 if average number of scats analyzed for all the previous surveys is any indication as to how many samples would need to be analyzed after a survey effort. However, if deriving information of population structure, diet via metabarcoding, and health, in addition to individual genotypes then lab costs for genetics work will likely be ~$15,000-20,000.

   d. The scat detection dog contractor generally charges $45,000-$50,000 per mountain lion survey effort. This cost has gone up in recent years from $35,000 per effort due to rising gas costs and less ability to stay in government housing during the 1-month survey efforts due to health/disease concerns. If housing is available then costs are less and if the housing is centrally located in the study area, which reduces amount of driving, then costs are also less.

5. What is the best analytic technique with scat and can supplemental data improve inference?

    a. Given the complexity of the data, Bayesian spatial capture-recapture (SCR) modeling (Turek et al. 2021) performed in the R package nimbleSCR (Bischof et al. 2020) is currently the best approach.

    b. Supplemental data such as mountain lion radio-collar data greatly improves model performance and subsequent inference (Mitchell et al. 2021). Thus, using pre-existing radio-collar is beneficial. Camera data is much less informative as it does not directly inform the spatial scale parameter of the Bayesian SCR model which involves aspects of home range size. Telemetry data does not have to be from the exact survey area as long the scat and telemetry data are from the same habitat types with similar amounts of anthropogenic disturbance such that the home range size of the mountain lions represented in the telemetry data is not thought to be overly different from that of mountain lions that occur where the scat surveys took place.

Camera Monitoring

Research involving use of cameras to monitor mountain lion populations has been occurring since game cameras became widely available to wildlife researchers. However, given that mountain lions lack unambiguous natural markings (e.g., spots or stripes) to distinguish individuals in game camera photos, utilizing game cameras alone primarily limits inference to understanding mountain lion presence, distribution, and some demographic information (McClanahan et al. 2017, Dellinger et al. 2019, Vickers et al. 2022). Kelly et al. (2008) estimated mountain lion densities in Latin and South America where mountain lions can develop scars from bot flies and other life events (e.g., fighting) that can help distinguish between individuals. This method does not appear applicable to animals in higher latitudes due to a decrease in parasitic insects that can infect mountain lions and coats composed of longer/thicker hair to help animals endure a wider range of temperatures. Further, even if individuals have natural markings/features to help distinguish them from conspecifics, agreement amongst researchers to accurately differentiate individuals in game camera photos is still limited (Alexander and Gese 2018).

Some research efforts using game cameras have combined them with parallel efforts to capture and mark a subset of the mountain lions in an area to develop density estimates (Rich et al. 2014, Murphy et al. 2019). These efforts can produce statistically robust results but any efforts to capture, mark, and radio-collar mountain lions is inherently costly and not financially sustainable over long periods of time (Cougar Management Guidelines 2005). We readily acknowledge that capturing and radio-collaring mountain lions provides a wealth of information about that individual animal and, if enough animals in a given area are captured and radio-collared, then one can gain in-depth insight on multiple aspects of mountain lion ecology (e.g., demographics, genetics, diet, health, etc.). However, capturing and radio-collaring the requisite number of animals in a population to gain such levels of insight then quickly render game camera data less useful given how coarse it is compared to radio-collar and other data sets collected during and after captures. If survival rates are high enough, one approach could be long-term monitoring collars, which are cheaper than standard GPS radio-collars, on a few animals (that allow for individual identification) paired with game camera arrays and regular intervals (e.g., every 1-5 years). Such an approach could provide some more in-depth insight on aspects of demographics at a reduced cost. Yet, low survival rates in some areas of California could also make this approach difficult to successfully implement (Vickers et al. 2016).

More recently, space-to-event and time-to-event (STE/TTE) models have attempted to derive local mountain lion densities using large-scale game camera monitoring efforts (Loonam et al. 2021). This method does not rely on individual identification of animals but utilizes information on the space between cameras with detections and time between single cameras with multiple detections. This approach has only been used in two locations (i.e., central and southeastern Idaho) for concurrent research and on a small scale (> 700 km^2 at each study area) relative to mountain lion use (Loonam et al. 2021). In the work by Loonam et al. (2021) 64-77 cameras were deployed for 7-9 months each year. During the three years of the study, the number of mountain lion detections in a deployment period varied from 40-81 for TTE and 7-26 for STE modeling for a total detection rate of 0.24 and 0.06 per day, respectively. Confidence intervals for the TTE and STE models were fairly wide for each year of the study in both locations. Time-to-event models in one location estimated mountain lion densities at 6.26 (4.43 – 8.10) per 100 km^2 in the first year of the study and 12.13 (9.41 – 14.46) per 100 km^2 in the third and final year of the study. These same analyses were more consistent in the second location from the first to last year at 6.88 (5.01 – 8.72) per 100 km^2 and 6.20 (4.26 – 8.15) per 100 km^2. Given mountain lions have an intrinsic population growth potential of 0.11-0.14% (Beausoleil et al. 2013), such interannual growth as reported in the first location of this study is not considered biologically possible over a three-year period. Additionally, the range in 95% confidence intervals are concerning in that in some instances the upper confidence interval is almost twice that of the lower confidence interval. For a naturally low-density species like mountain lions, the difference between 4 and 8 individuals per 100 km^2 is dramatic. Along these same lines, the space-to-event models in one location estimated mountain lion densities at 5.07 (1.3 – 8.85) per 100 km2 in the first year of the study and

20.24 (12.48 – 28.00) in the third and final year of the study. Again these same analyses were more consistent in the second location from the first to last year at 7.33 (3 – 11.66) per 100 km^2 and 6.19 (2.14 – 10.28) per 100 km^2. The same concerns mentioned for the TTE models are even more magnified for the STE models. Murphy et al. (2022) reported that both empirical and simulation-based studies have found that TTE and STE models likely produce unreliable density estimates for wide-ranging large carnivores, like mountain lions, that inhabit landscapes at low densities. Murphy et al. (2022) encouraged research to validate mountain lion density estimates produced with these new modeling methods (i.e., having marked radio-collared animals on the landscape).

Another work that used STE models to estimate the density of mountain lions and other sympatric species (moose, black bear, wolf, deer) was performed by McMurry et al. (2022). The data used in the study were originally collected for a moose study. Camera trap data were collected over a period of three years and the cameras were deployed near linear features where target species were likely to travel. Photos were manually classified to determine species presence. Their results offered lower density estimates than those derived from other methods (e.g., DNA, telemetry, camera-based spatial capture mark recapture (SCR), population reconstruction, road, and aerial transects) in the study area. Mountain lion density estimates ranged from 3.8 individuals/100km^2 (southern survey zone) to 4.1 individuals/100 km^2 (northern survey zone), with a combined density of 3.9 ± 0.9 /100 km^2. One of the main disadvantages of using the Space to Event (STE) model for estimating mountain lion densities is the low abundance of mountain lions in the study area. The study suggests that multiple years of data collection may be required to obtain reliable density estimates for low-abundance species like mountain lions. This limitation highlights the challenge of accurately estimating densities for elusive and rare species using camera traps. Additionally, the study acknowledges that violations of model assumptions and limitations in camera viewshed estimation may have affected the density estimates for mountain lions and other species.

Martin et al. (2023) used two noninvasive survey methods to reveal the distribution and density of mountain lions in Yosemite National Park. In their study, they employed a combination of camera-traps and DNA analyses from scats. Specifically, they used integrated spatial capture-recapture (SCR) models which provided a framework for estimating the abundance, distribution, and density of mountain lions. The study was conducted in 2019 and 2020, with a total of 121 genotyped mountain lions scats and 1340 remote camera images of mountain lions. For the integrated SCR model, spatial covariates, such as survey effort, topography, and distance to linear features, were incorporated into the model to estimate detection probability. The model also accounted for the effects of vegetation on the occurrence of mountain lion activity centers. The model estimated a median of 31 mountain lions in Yosemite and 84 mountain lions in the broader state space. The predicted mountain lion density was higher in areas with higher vegetation productivity. The probability of detecting mountain lions was influenced by survey effort, sex, year of survey, distance to trails, roads, and perennial rivers. The detection probability was higher with increased survey effort and in the second year of surveys. Mountain lions were more likely to be detected closer to trails and farther from roads and perennial rivers. Due to the model performance, this study demonstrates the utility of noninvasive survey methods and integrated modeling approaches for studying rare and cryptic species. The main disadvantage of using noninvasive methods, such as DNA from scats and detection-nondetection data from remote cameras, to estimate mountain lion densities is the potential for low detectability. The probability of detecting cougars during detection team surveys was influenced by survey methodology, mountain lion demography, and landscape structure. This means that there are factors that can affect the ability to accurately detect mountain lions using these methods. For example, detection probability was found to be lower for males compared to females, likely due to males exhibiting higher movement rates and larger territories. Additionally, mountain lions were less likely to be detected near roads, indicating that their proximity to human activity can decrease their detectability. These factors can introduce bias and uncertainty in the estimation of mountain lion densities using noninvasive methods.

Overall, it is clear that much more technique development and improvement are needed before some methods (e.g., TTE/STE) can be considered reliable for deriving local mountain lion densities. Southern California would be an area where such methodological development and refinement could be readily achieved. There are already radio-collared mountain lions present in some areas. It is much cheaper to maintain radio-collars in an area compared to deploying radio-collars on new individuals. The telemetry data provided by these collared mountain lions adds precision to density estimates performed by camera-trap data analysis (e.g., spatially mark-resight models -SMR-; Murphy et al. 2019). While using generalized SMR, telemetry data from GPS collars are critical for accurately estimating density and improving parameter estimate precision (Murphy et al. 2019). Further, the isolated nature of the mountain lion populations in southern California, while not ideal from a conservation standpoint, do help ensure assumptions of closed populations during survey periods would not be violated, since population closure is a general assumption for most models used with unmarked or partially marked wildlife populations (e.g., TTE, STE, random encounter model -REM-, SMR. Research involving wolves in Idaho suggests that, if the models are validated and improved, these camera monitoring approaches are cheaper than non-invasive genetic approaches to estimating local large carnivore densities (Ausband et al. 2022).

However, even if these new modeling approaches solely utilizing camera data are improved such that they are a viable and cheaper approach, the method itself is still only capable of providing information on mountain lion density. The primary benefit of this method would be that the data used could be used to estimate densities for multiple species in a given area (e.g., mule deer, bobcats, coyotes, etc.). Information involving genetic population structure, foraging ecology, habitat connectivity, and disease/health of mountain lions would have to be derived in other ways. This would counteract any realized cost-savings as it relates to monitoring a suite of aspects related to local mountain lion population status.

Opportunistic and Less-Intensive Data Collection

The methods mentioned above cover all the current non-invasive methods that have been utilized to some degree or another for monitoring mountain lion populations. However, there are additional lower-cost and easy to implement approaches that could bolster any of the methods mentioned above. It is an unfortunate reality that vehicles are a primary source of mortality for mountain lions in southern California (Vickers et al. 2016). Also, the public and various agencies (e.g., California Department of Transportation) regularly document and/or report roadkill (California Roadkill Observation System (CROS) | Road Ecology Center (ucdavis.edu)) mountain lions in southern California. Carcasses from roadkill animals would provide an opportunity to opportunistically monitor various aspects of a mountain lion population including population genetics via a tissue sample, diet via stomach contents and stable isotope analyses of whisker samples, and health/disease status via analyzing blood and organ tissue. Collecting a carcass and conducting all the analyses mentioned above would cost ~$1,000 per carcass. In southern California (San Diego, Riverside, and Orange counties) one could expect ≤ 10 carcasses a year that would be available for opportunistic sampling.

In addition to opportunistic sampling of carcasses, long-term monitoring collars could be used to maintain a level of understanding about local mountain lion survival and recruitment rates. Long-term collars take 1-2 locations/day and thus can last for five or more years, assuming that the animal lives that long, and are less than half the cost (~$1,200) of regular collars (~$3,000) when accounting for location fees.. This means that recollaring does not have to occur regularly (currently recollaring is something that has to occur every 1-2 years to continue monitoring a given animal) which reduces costs. Further, long-term collars would allow low-intensity monitoring of adult females and attending young. This could take the form of placing a camera at a kill site once/month to check on the number of kittens present which would inform understanding of kitten survival. Having long-term collars on adult animals, including adult females, would also readily allow opportunities to place long-term monitoring collars on subadults just prior to dispersal. In short, if you have the mother radio-collared then you know where the subadults are and one can readily capture them on a kill. This would provide info on recruitment and habitat connectivity to name a few things. Lastly, it is well established that spatial data from radio-collars greatly improves population estimation methods involving non-invasive methods like those mentioned above (Murphy et al. 2019, 2022). Thus, this approach would bolster any of the methods previously mentioned.

The time to deploy and maintain such collars would vary depending on how many collared animals there were on the landscape but per animal the cost would be ~$2,200-3,200 for the collar and personnel time. As an example, given there are currently mountain lions radio-collared in the Santa Ana Mountains, switching to long-term collars on these animals, again assuming they're still alive when the time for recollaring comes, would cost ~$15,400-30,800. This cost would assume that all animals are recollared in the same general timeframe and would not need to be recollared again for many years. The only additional costs would then be if there was interest in radio-collaring subadults for the reasons mentioned above, and monitoring adult females with young. Cost of collaring subadults would not be different than that mentioned above (~$2,200-3,200) and costs of monitoring family groups once per month, which would just involve putting out and retrieving cameras placed on kills, would take two days of work per month per family group and cost ~$600/month/family group assuming cameras and associated equipment are already in-hand.

Other alternatives

a) Tourism and human computers to monitor mountain lion populations

Elbroch et al. (2023) discuss the potential use of tourism and human computers as tools for monitoring mountain lions in Patagonia. The mountain lions in the study area (Torres del Paine) are the most observed population in their range. The authors explore the ability of people, including puma guides and individuals from the USA, to differentiate between individual pumas based on facial recognition. They found that participants performed well in this task, with no significant differences between local guides and US participants or based on years of experience with pumas. The authors then use the data collected by the participants to estimate puma abundance in the Torres del Paine. They built a historic capture-recapture dataset of individual mountain lions observed by local guides during the summer seasons from 2017 to 2020. Abundance estimates were calculated using the Chapman's modification of the Lincoln-Peterson estimator. The results showed overlapping confidence intervals across years, indicating a stable puma population in the area. This research shows an alternative to monitoring mountain lions where they can be observed and photographed. This may be applied, to some extent, to certain peri-urban wilderness parks in southern California where amateur photographers and wildlife enthusiasts have shown the ability of photograph mountain lions.

b) Environmental DNA to improve surveys for rare carnivores

Environmental DNA (eDNA)—DNA shed from an organism in its environment—coupled with quantitative PCR (qPCR) analyses, has become a reliable and extremely sensitive mean for identifying rare species in aquatic systems. Franklin et al. (2019) use this methodology in surveys for Canada lynx, wolverine, and fisher. Samples were collected by means of snow-track testing (snow samples were collected from tracks of Canada lynx, fisher, and wolverine) and

from snow collected at camera stations (snow samples were collected from areas where a rare carnivore was photographed). The authors stated that qPCR-based DNA analyses provide more reliable species identifications, reducing misidentification and missed detection errors. Snow-track surveys can be improved by using qPCR methods, eliminating the need for backtracking and reducing misidentification rates. Snow-columns (i.e., snow collected from a specific location, typically near a bait station) provide a potential sampling tool for detecting multiple species and can be used in areas with limited access.

Snow track eDNA has demonstrated the feasibility of individual genotyping for brown bears, lynx, and wolves by using amplicon sequencing of short tandem repeats (STR) and a sex marker to obtain individual genotypes. Since the genotyping success varied among samples and species, further research is needed to understand the factors affecting genotyping success and to optimize snow track sampling conditions and laboratory protocols (de Barba et al. 2023, *in prep*.).

Although this methodology may be applied to mountain lions' habitat presenting snow cover during the winter months, the validity in the Santa Ana Mountains is limited due to the benign climate and less snow precipitation.

c) Aerial surveys using thermal imagery

Havens and Sharp (1998) discussed the use of thermal imaging technology to provide a method for obtaining complete counts of animals with little risk of bias. The authors conducted a study in southwest Florida using thermal imagery to survey animals. They recorded thermal signatures of deer on video tape during flights along transects. The thermal imagery survey counted 42% more deer compared to standard visual aerial survey methods. The authors successfully located radiocollared panthers using thermal imagery. The detectability of thermal contrast between biological objects and their background was sufficient for species identification

Table 1 below summarizes the information detailed above in a decision matrix.

Table 1: Decision matrix for mountain lion monitoring strategies

	Population Monitoring Approach				
	Hair Sampling	Camera Monitoring	Scat Sampling	Carcass Sampling	Long-Term Collars
PROs	-If method can be refined then can derive density	-If method can be refined then can derive density	-Shown to work in Southern California and beyond	-Allows chance to thoroughly sample an individual carcass	-Can track survival and recruitment long-term for lower cost
		-If method can be refined then it is more cost effective than others for deriving density	-Allows inference on density, genetics, habitat connectivity, and foraging Ecology	-Provides insight on issues with connectivity (e.g., wildlife vehicle collision hotspots) or other sources of mortality	-Intermittent captures allow for collecting samples to monitor genetics, health, and foraging ecology
		-If method can be refined then it can also be used to derive density for other species		-If enough samples are collected can allow for inference on population genetics and habitat connectivity	-Supplements non- invasive data collection efforts
CONs	-Sensitive to site disturbance (e.g., other species visiting the site)	-Unable to allow for inference on genetics, habitat connectivity, foraging ecology or health/disease	-Unable to derive demographic structure of the population (e.g., proportion adult vs. juvenile)	-Requires someone to be able to quickly respond to collect and sample the carcass	-Requires staff to be able to monitor collared animals and carryout intermittent radio- collaring efforts
	-High level of site maintenance requirements (i.e., genetic samples degrade rapidly necessitating frequent checking of equipment)	-Overall Much More Technique Development is Needed	-Requires a genetics lab capable of performing analyses	-Likely small sample size and opportunistic nature makes planning difficult	-Coarse scale monitoring does not allow for readily assessing fine scale habitat connectivity

	-Adhesive material dries out which decreases effectiveness of sample collection			-Method does not allow for deriving density estimates	-Method does not allow for deriving density estimates
	-Adhesive material interferes with genetic analysis			-Method is only supplemental to other data collection methods and not a primary method in and of itself	-Method is primarily supplemental to other data collection methods and not a primary method for long-term population monitoring
	-Low likelihood of regular mountain lion interest in visting a site				
	-Requires a genetics lab capable of performing analyses				
	-Overall much more technique development is needed				
Paired with Another Method	-Radio-collar and other detection data (i.e., any of the other methods mentioned) would likely improve inference	-Radio-collar data would likely improve inference	-Radio-collar data improves inference	-Does not inform modeling efforts of non-invasive methods but does provide intermittent information on other aspects of population status	-Pairs with all non- invasive monitoring efforts
Overall Costs	$150,000/surve y	$150,000/surve y*	$82,800 - $104,000/surve y^	$1,200/carcass	$5,200 - $6,200/collaring effort
Interval	1-3 years	1-3 years	1-3 years	~10 times per year annually	~10 times per year annually

*Equipment such as cameras would be reusable and allow for cheaper costs, roughly half cost, after the initial effort		
^Current and previous work has not had to pay for genetics work so there is potential for cost savings of 15-20k if current collaborations hold		

Discussion:

Mountain lion population monitoring in the ePR, as in the Santa Ana Range, is challenging due to the wide ranging nature of the animals and their relative scarcity on the landscape. However, as this document shows, a number of tools can be deployed individually or in concert with others to provide information that is useful for monitoring populations over time. These tools, by also giving information on habitat use in some cases, can assist on land conservation prioritization. No tool is perfect but we feel that the decision matrix in Table 1, along with the discussion of the various tools above, gives our UCD team and the San Diego Management and Monitoring Plan team adequate resources to develop a good long term monitoring plan in 2023.

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Figures

Figure 1. Photo of a mountain lion entering a cubby site and leaving hair on sticky roller.

Figure 2. Photo of a mountain lion rubbing against scented post at a rub station and leaving hair.

Figure 3. Photo of a mountain lion stepping over wire covered with adhesive and leaving hair at a corral site.

Figure 4. Distribution of survey areas where mountain lion scat was collected for estimating mountain lion density and abundance regionally and statewide in California, USA, 2017-2021. Survey areas are overlaid on a map of the ecoregions that make up the state of California.

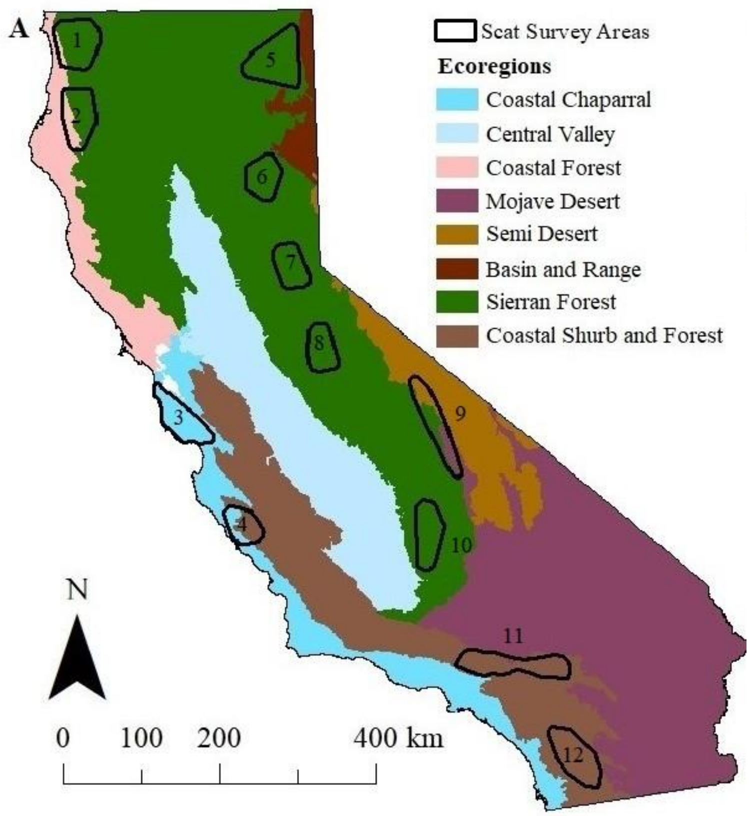
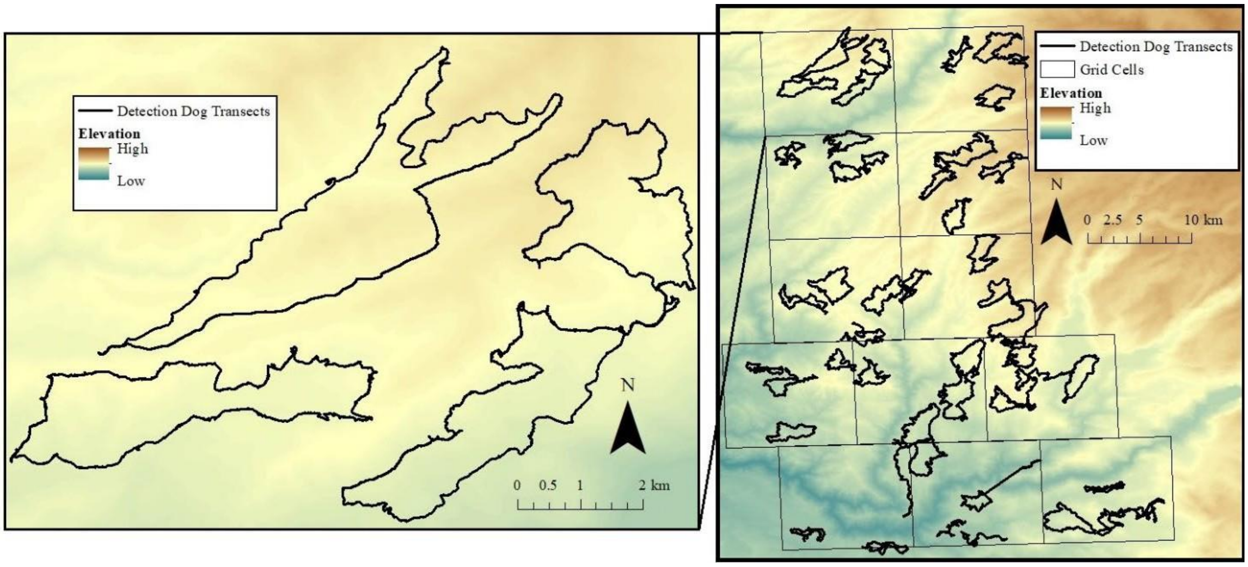


Figure 5. An example of the grid cell design used to help space the detection dog transects for a mountain lion scat survey conducted in the Stanislaus National Forest in California, USA, 2019. The zoomed in panel shows the individual non-overlapping detection dog transects in one grid cell.



APPENDIX B. LONG-TERM MONITORING PLAN FOR THE SAN DIEGO COUNTY MOUNTAIN LION POPULATION.

LONG-TERM MONITORING PLAN FOR THE SAN DIEGO COUNTY MOUNTAIN LION POPULATION

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Executive summary

This document seeks to provide the San Diego Association of Governments (SANDAG) with the UC Davis Wildlife Health Center recommendations on scientific techniques for long-term monitoring of mountain lions in southern California that can be applied under the scope of work of the San Diego Management and Monitoring Program (https://sdmmp.com/metrics/).

For each technique, we have considered the following factors:

1. Applicability to solitary carnivores and specifically to mountain lions, based on relevant past efforts using the technique,
2. Feasibility of employing the technique to obtain meaningful and repeatable results,
3. Quality of data and robustness of results associated with the technique, and
4. Costs and intervals associated with employing the method through time.

Key Findings:

1. From all the techniques discussed in the Literature Review (Task 6, University of California – Davis Agreement A37682 Amendment #2 SANDAG Contract #5005298 Amendment #2 (S890571) as well as in the Southern California Mountain Lion Workshop (October 11th, 2023) up to seven techniques, employed independently or in combination, may serve to generate the required data to inform long term monitoring of the mountain lion population.
2. The two main recommended techniques that can provide the most data (e.g., habitat use, reproduction, recruitment, survival, genetics, health/disease exposure) are: a) the establishment and maintenance of a long-term camera trap database at fixed locations and b) capture, radio-collaring, and telemetry.
3. The capture and radio-collaring of mountain lions provide the highest quality of certain data (e.g., genetics, health/disease exposure, survival-mortality) and it can also be included within the camera trap analysis to estimate population densities through spatial-mark-resight models.
4. Other non-invasive techniques recommended for long-term monitoring include: non-invasive DNA sampling (e.g., hair and scat) and camera-trapping density models. The use of scat DNA via scat detection dogs is recommended at 5-8-year intervals to serve in the estimation of population density. Randomly placed camera-traps can potentially also be used for population density estimation utilizing models that are currently under investigation such as time-to-event and/or space-to-event models.
5. There is potential to include newer techniques in capture-recapture models for population estimation as they emerge, such as using other sources of DNA from hair shafts, since they are viable in the environment for a longer period of time than DNA

in hair follicles, or other capture-recapture techniques based on facial recognition if that becomes more accurate with software advances.

6. As the genetic health of the southern California mountain lion population is severely compromised, we also recommend DNA collection effort via tissue and blood, since isolation of DNA from these types of samples is most reliable. This DNA collection will provide genetic diversity metrics (e.g., allelic richness, heterozygosity, internal relatedness) and will contribute to estimates of population density. Lower quality DNA from hair and scat may also yield this type of information with advances in genetic analysis.

1. Introduction

1.1. Description and ecological role.

Mountain lions (*Puma concolor*) are the largest non-pantherine felid. They are uniformly colored without body markings, typically light to dark tawny-brown with creamy-white underparts. The tail tip and the ear backs are dark brown to black, and the white muzzle is bordered by black (Hunter, 2015).

The mountain lion is the top carnivore in southern California and is important in maintaining the biodiversity and integrity of natural communities. Mountain lions are a key indicator of preserve system connectivity. They have very large territories and juveniles disperse long distances (Beier 1995; Zeller et al. 2017; Dellinger et al. 2020). They may act as an umbrella species since protecting land and improving connectivity for mountain lions could also benefit other species, especially those that are wide-roaming (Zeller et al. 2017). Mountain lions also influence food webs and the flow of energy through natural ecosystems. This species can change community composition and structure by affecting prey population dynamics, which impacts herbivory on plants and competition between herbivores (Ripple and Beschta 2006). In other cases, there may be short-term impacts on prey populations but no change in long-term dynamics (Hurley et al. 2011). The mountain lion is also a charismatic species that sparks public interest and fascination. The facts explained above plus their potential to act as an umbrella species, rank mountain lions among felids of the highest conservation priority (Dickman et al. 2015).

1.2. Taxonomy and phylogeny.

The mountain lion is the only species classified in the genus *Puma*. The jaguarondi (*Herpailurus yaguarondi*) is its closest relative. Both species are also related, although more distantly, to the cheetah (*Acinonyx jubatus*) and all these three species together comprise the *Puma* lineage.

Up to six subspecies in three geographic groups (North, Central, and South America) were formerly suggested based on genetic analysis:

- *Puma concolor cougar* (North America)
- *Puma concolor costaricensis* (Central America)
- *Puma concolor capricornensis* (East South America)
- *Puma concolor concolor* (N South America)
- *Puma concolor cabrerae* (central South America)
- *Puma concolor puma* (S South America).

Currently, only two subspecies are recognized: *Puma concolor concolor* (South America) *and Puma concolor cougar* (North and Central America) (Castello, 2020). The remnant population in Florida, although usually classified as its own subspecies (*Puma concolor coryi),* is an ecologically and geographically distinct population of the North American subspecies *Puma concolor cougar,* since its isolation occurred only in the last 100 years and genetic data indicates its relatedness to other North American populations (Hunter, 2015).

1.3. Conservation status

The mountain lion is a specially protected species in California. The California Wildlife Protection Act of 1990 (Proposition 117) declared the mountain lion a “specially protected mammal under the laws of this state” (Cal. Fish & Game Code § 4800(a)). As a result, hunting of mountain lions is generally prohibited, and there are restrictions on taking, injuring, possessing, transporting, importing, or selling mountain lions (Cal. Fish & Game Code § 4800(b)). However, there are exceptions that allow for the removal or killing of mountain lions if they are perceived to be an imminent threat to public health or safety or pose a threat to the survival of threatened, endangered, candidate, or fully protected sheep species (Cal. Fish & Game Code § 4801).

Furthermore, if a mountain lion damages or destroys livestock or other property, a person may request a permit to “take” the mountain lion (Cal. Fish & Game Code § 4802). The California Department of Fish and Wildlife (CDFW) is responsible for issuing depredation permits, which authorize the removal of mountain lions in such cases. Currently, mountain lions are being evaluated to be listed as threatened under the California Endangered Species Act (CESA). While Proposition 117 already provides protections for mountain lions, listing them under CESA would offer supplementary safeguards and align with the goals of protecting and preserving their habitat and genetic diversity.

Southern California and Central Coast mountain lions would comprise an Evolutionarily Significant Unit (ESA), as is supported by genetic studies (Gustafson et al 2018). An ESA allows for the designation and listing of these populations as endangered under the CESA.

The six recognized subpopulations within the Southern California/Central Coast ESU of mountain lions are as follows:

1. Central Coast North – includes mountain lions in the Santa Cruz Mountains and East Bay.
2. Central Coast Central – represents a genetically distinct mountain lion population in the central coastal area.
3. Central Coast South – includes mountain lions in the Santa Monica Mountains.
4. San Gabriel/San Bernardino - consists of mountain lions in the San Gabriel and San Bernardino Mountains.
5. Santa Ana Mountains – represents a genetically distinct mountain lion population in the Santa Ana Mountains. San Diego County contains a portion of this mountain range, that is west of Interstate 15 and south of the Riverside County line. 6. Eastern Peninsular Range - includes mountain lions in the eastern portion of the Peninsular Range, the majority of which is in San Diego County .

Thus, San Diego County mountain lions exist as part of two of the six subpopulations that have been petitioned for listing under the California Endangered Species Act, and both populations are primarily dependent on gene flow from a third petitioned population, the San Gabriel/San Bernardino population to the north.

1.4. Conservation threats

There are a variety of threats facing mountain lions in southern California. Southern California's human population grew rapidly over the last half century. This rapid human population growth is leading to extensive habitat loss and fragmentation from urban and agricultural development (Vickers et al. 2015). Despite conservation of large blocks of habitat, many mountain lion populations are small and isolated by freeways and surrounded by development (Vickers et al. 2015, 2017; Dellinger et al. 2020). Mountain lions have unusually high mortality rates in southern California, primarily from vehicle strikes and human conflicts (for example, depredation permits) (Vickers et al. 2015). In particular, up to 60% of mortalities in the Santa Ana Mountains were due to vehicle collisions and in the eastern Peninsular Range depredation permits and vehicle collisions were the first and second most common causes of mortality(Vickers et al. 2015). The eastern Peninsular Range population was in the top tier of mortality rates in a statewide mortality analysis assessing all the genetic subpopulations (Benson et al. 2013) and mortalities related to humans were judged to be additive versus compensatory – raising the issue of sustainability of these two populations.

• Climate Vulnerability: Changing climate can increase the frequency, intensity, and duration of droughts and negatively affect lion populations by reducing prey availability (Stoner et al. 2018). Plant productivity in semi-arid regions is correlated with rainfall, and drought limits food availability for prey and causes prey populations to shrink. A reduction in prey availability can lower lion productivity and survival and adversely impact populations (Stoner et al. 2018).

- Human Use: A growing human population results in less habitat for mountain lions free from human disturbance. There are increasing interactions between lions and humans that can result in safety and livestock protection concerns and in the death of the lions from depredation permits (Vickers et al. 2015).
- Connectivity: Habitat loss and fragmentation are causing increasing risk to mountain lion populations in southern California. Mountain lions are constrained or blocked in moving between small, isolated populations, leading to a loss of genetic diversity, which has been reported from a molecular to a phenotypical level in Southern California (Huffmeyer et al. 2022). Loss of connectivity is leading to a potential extinction vortex in the Santa Ana Mountains population and is likely to similarly affect the Eastern Peninsular Ranges population over time (Ernest et al. 2014; Gustafson et al. 2018; Benson et al. 2019; Dellinger et al. 2020).
- Fire: Increasing frequency of large-scale wildfires in shrublands is leading to conversion of shrublands to invasive, nonnative annual grassland, a habitat infrequently used by mountain lions. Fire in linkages and corridors can be impactful as loss of shrub and tree cover can lead to decreased connectivity between habitat patches (Jennings et al. 2016). In urbanized regions affected by wildfires, mountain lions avoid burned areas and increase activities that elevate their risk of negative interactions with humans and conspecifics (Blakey et al. 2022).
- Urbanization: Loss and fragmentation of habitat are negatively impacting lion populations which require very large, unfragmented natural habitats to persist. Lions bordering urbanized and rural residential areas are at risk of death from vehicle collisions and conflicts with humans (Vickers et al. 2015; Dellinger et al. 2020).

1.5. California mountain lion population monitoring.

Population monitoring (e.g., population density, population trend) is a key element of a carnivore management strategy. Because most carnivores are highly mobile, usually secretive, and occur in relatively low densities, they are difficult to study and require greater and longer-term funding for monitoring compared to other taxa. Nonetheless, population monitoring schemes if planned properly, can provide reliable estimates of changes in population size over time and assess the impact of management actions on populations (Clevenger 1998). Therefore, reliable long-term research into trends is crucial in ecology and conservation studies (Sereno-Cadierno et al. 2023).

Several monitoring methods have been developed for large carnivores including telemetry, capture-mark-recapture, harvest data, sign survey among others (Wilson and Delahay 2001, Barea-Azcón et al. 2007). Noninvasive genetic sampling techniques, which use DNA extracted from animal sign such as hair, scat, saliva, urine, or regurgitates (Waits and Paetkau 2005), have become an effective method for studying wildlife populations and are the preferred monitoring methods for some species and populations (e.g., Rudnick et al. 2005, De Barba et al. 2010, Borthakur et al. 2011).

Monitoring mountain lion density and abundance is typically estimated using two approaches: a minimum count of known animals with associated monitoring data (e.g., radio-collar data and age class data) divided by the amount of habitat in a demarcated study area (Lambert et al. 2006, Cooley et al. 2009), or a spatial capture-recapture (including spatial mark-resight) framework using a variety of methods including, but not limited to, DNA from scat (Davidson et al. 2014), skin biopsies (Proffitt et al. 2015), or hair (Alldredge et al. 2019), or collars and/or cameras (Alexander and Gese 2018, Murphy et al. 2019, Loonam et al. 2021).

The minimum count approach has been used for many decades and has the benefit of generating a lot of detailed site-specific data on mountain lion populations (Beausoleil et al. 2013). However, such an approach is very resource-intensive and difficult to replicate. Murphy et al. (2022) conducted a review of techniques and determined approaches employing a spatial capture-recapture framework could estimate mountain lion populations but precision of estimates, primarily due to size of the study areas, was a concern that should be addressed prior to initiating any data collection efforts. In general, there is uncertainty related to how different data collection and analytical methods relate to one another (Beausoleil et al. 2021, Murphy et al. 2022). Additionally, some of the difficulty in comparing results is simply related to differences in how studies define spatial and temporal scales (Cooley et al. 2009, Dellinger et al. 2018) and what segment of the population (e.g., adults and/or subadults) density is being estimated for (Rinehart et al. 2014, Beausoleil et al. 2016). To this point, most efforts to estimate mountain lion density have been conducted once over a short time period (e.g., < 5 years) equal to or less than 1-2 generations as it relates to mountain lions, and/or most efforts occur at a spatial scale that does not allow extrapolation to large habitat areas or subpopulations; Beausoleil et al. 2013, Murphy et 6 al. 2022). Given that processes influencing mountain lion population viability occur over much longer temporal (van de Kerk et al. 2019) and larger spatial scales (Dellinger et al. 2020), there is a need to standardize efforts across a given jurisdiction (e.g., regional, state, or provincial level; Beausoleil et al. 2013) and replicate efforts regularly (e.g., every

5-10 years given mountain lion generation times; Jenks 2011) to understand population trends.

Mountain lions exist throughout San Diego County and have relatively low overall survival rates (Vickers et al. 2015, Benson et al. 2023). As a result, concerns have been raised about the possibility of long-term overall population decline and the need to monitor the population trajectory over time.

Long-term monitoring of this species is imperative to detect in a timely way any changes that this subpopulation may suffer which might put it on the verge of extinction. Different methodologies have been discussed under Task 6 - Literature Review (University of California – Davis Agreement A37682 Amendment #2 SANDAG Contract #5005298 Amendment #2 (S890571). Moreover, monitoring techniques for mountain lions have also been the focus of the Southern California Mountain Lion Workshop organized by the UC Davis Wildlife Health Center on October 11th, 2023.

2. Monitoring techniques for mountain lion populations with a robust scientific foundation from our literature review.

Our literature review explored and discussed three non-invasive techniques for monitoring mountain lion populations:

2.1) camera monitoring;
2.2) hair sampling;
2.3) and scat sampling.

We also discussed the modifications of more invasive approaches (i.e., radio-collaring) to inform long-term population monitoring of mountain lions in southern California. Finally, we discussed the utility of opportunistic data collection (e.g., sampling carcasses of recently deceased animals) and other alternatives such as the use of environmental DNA and aerial surveys using thermal imagery.

2.1. Camera monitoring.

The use of cameras is a widely utilized method for monitoring mountain lion populations. This method allows to gather valuable data on various aspects of mountain lion populations such as population density, distribution, behavior, activity patterns, and interactions with other species. It helps detect changes in habitat use and movement patterns, as well as interactions with prey or other individuals. This information contributes to a comprehensive understanding of mountain lion ecology and can inform conservation and management efforts.

Validation and improvement of camera monitoring methods are necessary to ensure reliable density estimates. Techniques like density modeling and occupancy analysis can be employed to enhance the accuracy of density estimates derived from camera monitoring. Camera monitoring can be paired with other monitoring techniques, such as radio-collar data, to improve inference and provide a more comprehensive understanding of wildlife populations. More recently, space-to-event and time-to-event (STE/TTE) models have attempted to derive local mountain lion densities using large-scale game camera monitoring efforts (Loonam et al. 2021). This method does not rely on individual identification of animals but utilizes information on the space between cameras with detections and time between single cameras with multiple detections.

In general, camera monitoring is a valuable tool in monitoring mountain lion populations, offering insights into their behavior, distribution, and interactions with the environment. When combined with other monitoring approaches, such as radio-collaring or genetic analysis, it provides a more comprehensive understanding of mountain lion populations.

2.2. Hair sampling.

Hair sampling is a population monitoring approach that involves collecting hair left behind by mountain lions. This method typically uses sticky rollers or wire brushes placed in strategic locations such as trails or marked scent stations. As mountain lions pass by these stations, their hair gets caught on the adhesive material.

The collected hair samples are then carefully removed from the sticky rollers or wire brushes and stored for further analysis. The hair samples are typically sent to a genetics lab capable of performing DNA analysis.

Hair sampling allows for genetic analysis of the collected hair samples, which provides valuable information about the population size, genetic diversity, and even individual identification of mountain lions. This method has been shown to be effective in estimating mountain lion densities, as well as assessing population structure and genetics.

However, hair sampling has some limitations and challenges. It is sensitive to site disturbance, meaning that other animal species visiting the sampling site can interfere with the collected hair samples. Regular site maintenance is required to ensure the effectiveness of the sampling equipment.

Additionally, the technique requires access to a genetics lab for DNA analysis, which can add to the overall costs of implementing this method. Coordinating and planning hair sampling efforts can be complex due to the opportunistic nature of collecting samples and the small sample sizes often obtained.

Broadly speaking, hair sampling is a non-invasive and informative method for monitoring mountain lion populations, but further refinement and validation of the technique are necessary for more accurate and reliable results.

2.3. Scat sampling.

Scat sampling is a non-invasive method that involves collecting feces left behind by mountain lions in their natural habitat. Scat samples are collected from various locations, such as trails, marking sites, or areas where mountain lions are known to frequent.

Once collected, the scat samples are preserved for further analysis. DNA analysis allows researchers to extract genetic information, such as identifying individual mountain lions, estimating population size, and assessing genetic diversity.

Scat sampling has been successfully used in Southern California and other regions to estimate mountain lion densities. It provides valuable insights into population dynamics, including changes in population size over time and the identification of individuals within the population. Moreover, scat sampling allows for assessing genetic diversity and understanding the genetic health of the population.

One of the advantages of scat sampling is that it is relatively non-invasive, minimizing disturbance to the animals and their habitat. It is also a cost-effective method compared to other techniques like camera monitoring or long-term collaring. However, scat sampling does require access to a genetics lab capable of performing DNA analysis, which adds to the associated costs.

Overall, scat sampling is a valuable tool for monitoring mountain lion populations, providing information about population size, genetic diversity, and individual identification. It complements other monitoring methods and contributes to a comprehensive understanding of mountain lion populations.

2.4. Capture and radio-collaring mountain lions.

Some research efforts using game cameras have combined them with parallel efforts to capture and mark a subset of the mountain lions in an area to develop density estimates (Rich et al. 2014, Murphy et al. 2019). These efforts can produce statistically robust results but any efforts to capture, mark, and radio-collar mountain lions is inherently costly. Capturing and radio-collaring mountain lions provides a wealth of information about that individual animal and, if enough animals in a given area are captured and radio-collared, then one can gain in-depth insight on multiple aspects of mountain lion ecology (e.g., demographics, genetics, diet, health, etc.). However, capturing and radio-collaring the requisite number of animals in a population to gain such levels of insight then quickly render game camera data less useful given how coarse it is compared to radio-collar and other data sets collected during and after captures. If survival rates are high enough, one approach could be long-term monitoring collars, which are cheaper than standard GPS radio-collars, on a few animals (that allow for individual identification) paired with game camera arrays and regular intervals (e.g., every 1-5 years). Such an approach could provide some more in-depth insight on aspects of demographics at a reduced cost.

There are already radio-collared mountain lions present in some areas within southern California. It is much cheaper to maintain radio-collars in an area compared to deploying radio-collars on new individuals. The telemetry data provided by these collared mountain lions adds precision to density estimates performed by camera-trap data analysis (e.g., spatially mark-resight models -SMR-; Murphy et al. 2019). While using generalized SMR, telemetry data from GPS collars are critical for accurately estimating density and improving parameter estimate precision (Murphy et al. 2019). Further, the isolated nature of the mountain lion populations in southern California, while not ideal from a conservation standpoint, do help ensure assumptions of closed populations during survey periods would not be violated, since population closure is a general assumption for most models used with unmarked or partially marked wildlife populations.

A less intensive variation of this technique is the use of long-term collars. Long-term monitoring collars could be used to maintain a level of understanding about local mountain lion survival and recruitment rates. Long-term collars take 1-2 locations/day and thus can last for five or more years, assuming that the animal lives that long, and are less than half the cost of regular collars when accounting for location fees. This means that recollaring does not have to occur regularly (currently recollaring is something that has to occur every 1-2 years to continue monitoring a given animal) which reduces costs. Further, long-term collars would allow low-intensity monitoring of adult females and attending young. This could take the form of placing a camera at a kill site once/month to check on the number of kittens present which would inform understanding of kitten survival. Having long-term collars on adult animals, including adult females, would also readily allow opportunities to place long-term monitoring collars on subadults just prior to dispersal. In short, if you have the mother radio-collared then you know where the subadults are and one can readily capture them on a kill. This would provide information on recruitment and habitat connectivity to name a few things. Lastly, it is well established that spatial data from radio-collars greatly improves population estimation methods involving non-invasive methods like those mentioned above (Murphy et al. 2019, 2022). Thus, this approach would bolster any of the methods previously mentioned.

2.5. Opportunistic data collection.

Carcasses from roadkill animals would provide an opportunity to opportunistically monitor various aspects of a mountain lion population including population genetics via a tissue sample, diet via stomach contents and stable isotope analyses of whisker samples, and health/disease status via analyzing blood and organ tissue.

2.6. Environmental DNA (eDNA).

Environmental DNA (eDNA)—DNA shed from an organism in its environment—coupled with quantitative PCR (qPCR) analyses, has become a reliable and extremely sensitive mean for identifying rare species in aquatic systems. Franklin et al. (2019) use this methodology in surveys for Canada lynx, wolverine, and fisher. Samples were collected by means of snow-track testing (snow samples were collected from tracks of Canada lynx, fisher, and wolverine) and from snow collected at camera stations (snow samples were collected from areas where a rare carnivore was photographed). The authors stated that qPCR-based DNA analyses provide more reliable species identifications, reducing misidentification and missed detection errors.
Although this methodology may be applied to mountain lions' habitat presenting snow cover during the winter months, the usefulness in San Diego County would be limited due to the rarity of snow except in the highest elevations. This technique is also labor intensive in that it requires extensive time spent searching for tracks of this animal that is relatively rare on the landscape.

2.7. Aerial surveys using thermal imagery.

Havens and Sharp (1998) discussed the use of thermal imaging technology to provide a method for obtaining complete counts of animals with little risk of bias. The authors conducted a study in southwest Florida using thermal imagery to survey animals. They recorded thermal signatures of deer on video tape during flights along transects. The thermal imagery survey counted 42% more deer compared to standard visual aerial survey methods. The authors successfully located radio-collared Florida panthers using thermal imagery. The detectability of thermal contrast between biological objects and their background was sufficient for species identification. Due to the environmental temperature and habitat characteristics in southern California generally and in San Diego County specifically, obtaining enough thermal contrast to distinguish fauna may be constricted to a very few months out of the year, making this technique more challenging to apply successfully than in other areas.

3. Techniques recommended for the long-term monitoring of the San Diego County mountain lion population from literature review and workshop.

Our literature review and the discussions/round table at the Southern California Mountain Lion Workshop revealed what techniques are the most appropriate to perform long-term monitoring for the population of mountain lions in San Diego County. Since this is an evolving field, this document is focused on monitoring techniques as of November 2023. This is a living document, and our UC Davis mountain lion team will keep collecting new techniques applicable to mountain lions as they emerge from the scientific literature and could be included in the long-term monitoring strategy for San Diego County. Applicability of techniques and strategies will also depend on the factors mentioned above in the Executive Summary (e.g., applicability to southern California mountain lions, feasibility, replicability, data quality, data robustness, cost).

Herein we propose different techniques in order of relevance to our study site and characteristics of the mountain lion population.

3.1. Long-term fixed-location camera trap database.

Camera traps serve as a research tool for the study of the occurrence of elusive carnivores such as mountain lions. Researchers from UC Davis Wildlife Health Center Mountain Lion Project have been using this non-invasive methodology to detect mountain lions in the landscape for +20 years. Our team has secured collaboration with numerous other entities in the region (e.g., Irvine Ranch Conservancy, Orange County Parks, The Nature Conservancy, California State Parks, and potentially USGS and others) that include camera arrays within their wildlife monitoring techniques. These collaborations, when paired with new cameras that the UC Davis team anticipates placing in unmonitored areas can ensure the monitoring of a vast area of suitable habitat for mountain lions. These long-term placed cameras are very valuable in providing data regarding:

- Occurrence data
- Demographic metrics (e.g., kitten production over time)
- Genetic traits (e.g., kinked tails)

The analysis that can be included using this type of long-term placed cameras include but are not limited to:

- Photographic rates

  Photographic rates measured as an index of abundance (i.e., relative abundance index -RAI-) present some challenges to provide accuracy since photographic rates are influenced by the species abundance, movement patterns, camera trap setup, or habitat to name a few (Sollman 2018). Nevertheless, photographic rates should be interpreted as an index of activity, where activity of a species at a site can increase because a higher number of individuals use that site and/or because individuals use that site more frequently (Sollman 2018).

- Presence-only data

  Presence-only data, where there is no information on locations where the species is absent, are common in wildlife studies. Effective models are needed to explore species distribution or species use of habitat using presence-only data and the study design is critical in such models where only presence can be identified (Pearce and Boyce 2006). For modelling presence-only data, four approaches can be used:

  A) Describing the distribution of the presence-only records: This approach involves characterizing the spatial distribution of the species based on the recorded presence locations. It provides a descriptive analysis of the data without explicitly modeling the underlying environmental factors.

B) Contrasting the distribution of presence records with pseudo-absences: In this approach, random background or pseudo-absence locations are generated to contrast with the presence locations. The environmental attributes of the presence and pseudo-absence locations are compared to identify the factors associated with the species' presence.

C) Contrasting the distributions of presence records and available sites: Similar to the pseudo-absences approach, this method focuses on contrasting the environmental attributes of the presence locations with the available sites in the study area. The available sites can be selected randomly or based on certain criteria, such as a defined buffer around the presence locations.

D) Modeling abundance when abundance given presence is known: This approach is used when information on species abundance is available, in addition to presence records. Statistical models are developed to estimate the relative abundance or density of the species based on the presence data and other relevant covariates.

The choice of approach depends on the quality of the presence-only data and the research objectives since different approaches may provide different insights into the species distribution and habitat use.

- Occupancy

Occupancy models describe spatial patterns of animal occurrence (Sollmann 2018). Occupancy has been proposed as a proxy for abundance (Noon et al. 2012). In theory, occupancy and abundance share a predictable relationship. As population size increases, the number of sites occupied by members of that population should also increase (until all sites are occupied); likewise, a decrease in population size should lead to a decrease in the number of sites used (Gaston et al. 2000, Royle and Dorazio 2008). This is called an occupancy-abundance relationship, and therefore occupancy can be used as an index of abundance (Clarke et al. 2023).

- Habitat selection

Habitat is a key determinant of the distribution and abundance of wildlife. Based on habitat use and occupancy data, resource selection functions (RSFs) and related models can be used to estimate abundance (Boyce et al. 2016). RSFs and occupancy models can be used to estimate abundance by analyzing habitat selection and use patterns of animals. RSFs estimate the probability of habitat use based on the distribution of attributes associated with used resource units compared to available resource units. This information can then be used to calculate the relative probability of selection.

Occupancy models, on the other hand, estimate the presence or absence of animals in a given area. By combining occupancy data with habitat use information, abundance can be predicted. These models consider factors such as detection probability and can be adjusted to account for false absences or difficulties in detecting animals.

- Density

  Estimates of population density are essential for mountain lion management and conservation in southern California. Although estimation of population density in wildlife populations without individual recognition is more challenging than estimates in naturally or artificially marked wildlife populations, recently, certain camera trapping techniques make feasible to attain precise and accurate estimates of population density. Two of these techniques are highly recommended for mountain lions (see recommendation #4) but we may use our long-term place camera trap database to explore density with other techniques that have also used for other carnivores. Within the techniques employed to estimate density in unmarked wildlife populations we may consider the following ones to apply to San Diego County and other southern California mountain lions:

  A) Distance Sampling with Camera Traps (or Camera Trap Distance Sampling; CT-DS).

     Distance Sampling model was developed to estimate density from line- or point-transect surveys, and it has been adapted for use with camera trap data (Howe et al 2017). This model can be applied to low-density populations (Palencia et al. 2021).

  B) Random Encounter Model (REM).

     This model treats animals like ideal gas particles (i.e., randomly moving entities which are neither attracted to nor repelled by one another or landscape features (Rowcliffe et al. 2008; Gilbert et al. 2021). If animals behave like ideal gas particles, the rate at which they are detected by camera traps is a function of population density, animal movement, and the area within which cameras detect animals (Nakashima et al. 2018).

  C) The random encounter and staying time (REST).

     The random encounter and staying time model is an extension of the random encounter model. The REST treats animals like ideal gas particles but without requiring measures of animal movement speed. Instead, the model uses the time animals spend in the camera viewshed (i.e.,“staying time”) as a proxy for animal movement speed, since the two measures are inversely proportional (Nakashima et al. 2018).

These three models assume that camera traps are placed randomly with respect to animal movement, the study population is closed, animal movement and behavior are not affected by the camera trap, and the observations are considered independent events (Palencia et al. 2021). The general recommendations for the use of these three models to estimate density of unmarked populations are: REST could be recommended in scenarios of high abundance, CT-DS in those of low abundance while REM can be recommended when camera trap performance is not optimal, as it can be applied with less risk of bias (Palencia et al. 2021).

Due to the compromised genetic health of the southern California mountain lion subpopulation, long-term placed cameras may reveal further deterioration on the genetic status of the population by recording a decrease on kitten productivity over time or presence of morphological traits like kinked tails that have been associated with inbreeding in mountain lions.

3.2. Capture and radio-collaring mountain lions and spatial-mark -resight models.

Capture and radio-collaring can significantly enhance population estimation and monitoring efforts for mountain lions through their continuous tracking capabilities. Radio-collaring mountain lions provide a wealth of knowledge in regards to 1) demographics (e.g., survival, mortality, productivity, recruitment); 2) population genetics; 3) habitat connectivity and habitat use; 4) health/disease exposure; 5) foraging ecology; 6) behavior and population interactions; 7) human-wildlife conflict; and 8) response to environmental change (e.g., as habitat loss, climate change, or human disturbances).

Radio-collaring provides a vital source of detailed data on mountain lion populations, helping make informed decisions about population monitoring, management, and conservation efforts. By collaring a proportion of the mountain lion population other population density analyses can also be incorporated. When only a fraction of a population carries marks (i.e., radio-collars, ear tags), the spatial mark-resight (SMR) models can be implemented to estimate population density.

Mark-resight models need only a subset of the population to be marked (either naturally or from a single trapping-and-tagging event; Sollmann et al. 2013). The entire population is then resighted using a non-invasive survey technique (i.e., a camera trap survey; Sollmann et al. 2013). Incorporating this analysis for future density estimates adds value to our capture efforts and also supports the financial investment of the capture operations.

3.3. Time-to-event and space-to-event models.

The time-to-event (TTE) and space-to-event (STE) models represent more recent camera-based analysis that attempt to derive local mountain lion densities using large-scale camera trap monitoring efforts (Loonam et al. 2021, Clarke et al, 2023).
Due to our vast experience working with cameras these techniques may well be viable tools for population estimation in San Diego County and elsewhere in southern California using our own cameras but also investigating the potential value of other cameras placed by collaborative entities.

Some of the advantages of the TTE model include (Clarke et al. 2023):
The model requires less image processing time/effort than other models. Researchers need only "take" images until an animal is first in frame; any further images do not need to be processed (Moeller et al. 2018). The TTE model relies on whether an individual was within frame during each sampling period (Moeller et al. 2018). The TTE can account for spatial variation in density (Moeller et al. 2018). A model extension is available which compares densities at different camera stations as a result of habitat covariates (Loonam et al. 2021b). TTE studies are simple to scale up or down, since the number of cameras determines the precision of estimates, the precision is not determined by camera density or coverage (Loonam et al. 2021a). A hundred cameras can be used to estimate density in a large area just as effectively as a small area (Loonam et al. 2021a).

On the other hand, some of the advantages found while using STE models include (Clarke et al. 2023):
The STE model does not require measurements of animal movement speed (Moeller et al. 2018). Instead, sampling occasions are folded into instants in time using camera traps' time-lapse function. The STE is not biased by animal movement speed (i.e., animals travelling slow vs fast). This differs with the TTE model, which is sensitive to animal movement speed (i.e., more reliable when animals move fast; Moeller et al. 2018). The STE model, as the TTE, does not rely on counts of animals in images; researchers need only record whether any animals were in the viewshed at each camera during a sampling occasion (Moeller et al. 2018). Consequently, the model is unaffected by weather, travelling and explorative behaviour, obstructions, camera malfunctions and other factors that make accurately counting the number of individuals challenging (Moeller et al. 2018). The STE uses time-lapse images, therefore there is essentially no uncertainty in detection probability when using time-lapse

images (Moeller et al. 2018). STE studies are simple to scale up or down. Just the number of cameras determines the precision of estimates (Loonam et al. 2021a).

Conversely, some restrictions apply while using these models. One of the limitations of the TTE model is its sensitivity to movement speed. This model requires accurate measures of animal movement speed to set the sampling period (Moeller et al. 2018, Loonam et al. 2021a, Morin et al. 2022). Incorrect values of movement speed bias density estimates (Loonam et al. 2021b). Randomly-placed cameras may not collect sufficient images of elusive/rare species, therefore, this model is best suited to relatively common, high-density species (Moeller et al. 2018, Morin et al. 2022). The STE model does not apply well to very rare species, as detections of animals from randomly-placed cameras in time-lapse images can be too few to draw significant conclusions (Moeller et al. 2018, Loonam et al. 2021b), which may obligate to deploy more cameras to increase the number of detections (Moeller et al. 2018, Loonam et al. 2021a). Because animal movement speed is not an input, the STE is less precise than the TTE (Moeller et al. 2018, Loonam et al. 2021a). Depending on the time-lapse interval chosen, the STE can produce a high number of images (Morin et al. 2022).

Both models will also allow the analysis of population density of other species ecologically related to mountain lions such as deer, which increases the value of these techniques.

3.4. DNA analysis: hair sampling

Hair snares are more time consuming and financially challenging than other methods such as scat dog surveys for acquiring DNA for population estimation in southern California. However, the ability to use cameras at hair snares, and the information that they provide, may make the extra investment worthwhile if protocols are refined and if more effective snaring methods can be developed.

3.5. New DNA technique: hair shafts as new source of DNA.

We are currently investigating the potential use of hair shafts as source of DNA. Hair sequencing has been used in humans and it has potential to work in mountain lions. The California Conservation Genomics Program already has about 500 lion genomes from across the state. If this technique is proven to work, it would easily increase this database size, and allow identifying almost any lion's pedigree through its genome.

3.6. Scat DNA

Collecting fecal excrement (scat) deposited by animals can be used to examine a variety of information on wildlife species. Using molecular techniques to extract and analyze DNA that may be present on the surface of the scat sample provides data from as basic as presence/absence of a species in a specific area to identifying specific individuals along with their sex. Specially trained "scat dogs" are used to locate the fecal samples and by associating the GPS location of the sample with an individual animal it can allow for analysis of distribution, relative abundance, density and sex ratios. This technique may also provide genetic health metrics such as heterozygosity. Since this metric does not change in a short period of time (< 5 years), conducting a grid-based scat collection survey across the county conserved lands would not be advised to occur in the next year(s). This future sampling effort will compare to density/abundance estimate to the previous estimate generated in 2023 from the 2020 sampling effort. Opportunistically collected scat that is found by field personnel can also add to the total DNA detections in an area and be incorporated in models that use multiple sources of data (Vickers et al. 2022).

3.7. Facial recognition in camera trap surveys

The UCD-WHC team is currently evaluating trail camera photographs taken at hair snare stations used for DNA collection as a method for population estimation in the Santa Ana Mountain Range. Photographs taken at those stations, along with large numbers of other photographs of known mountain lions from UCD-WHC's previous and ongoing studies in San Diego County will be utilized for software development and testing. This software development is aimed at identifying animals to species level, and mountain lions to the individual level, from trail camera photographs.

This work will help the collaborating researchers to fully develop the potential of trail camera arrays to assist in the long-term monitoring of the San Diego County mountain lion populations.

4. Time-frame

Technique	2024	2025	2026	2027
Long-term placement of cameras and documentation of photos from all source cameras in a database	X	x	x	X*
Capture and radio-collaring	x	x	x	X*
DNA sampling from captures and mortalities, and health monitoring via necropsies of deceased animals	x	x	x	X*
TTE-STE model trial in Santa Ana Range and deployment in eastern Peninsular Range if viable		X**	X**	X**
Hair shaft DNA extraction investigation and further hair snare trials	x	?	?	
Scat dog survey				X*
Mountain lion facial recognition software development	x	x	x	

*If funding is secured/ongoing

**If trial in Santa Ana Range is successful and funding becomes available

5. Budget

This budget expresses approximate costs based on previous efforts.

Technique	2024 Cost	2025 Cost	2026 Cost	2027 Cost
Long-term placement of cameras and documentation of photos from all source cameras in a database	$47,095	$47,095	$47,095	$47,095*
Capture and radio-collaring	$35,730	$35,730	$35,730	$35,730*
DNA sampling from captures and mortalities, and health monitoring via necropsies of deceased animals	$48,425	$48,425	$48,425	$48,425*
TTE-STE model trial in Santa Ana Range and deployment in eastern Peninsular Range if viable	Other funding		$175,000**	
Hair shaft DNA extraction investigation and further hair snare trials	No cost	$175,000**		
Scat dog survey				$175,000**
Mountain lion facial recognition software development	Dependent on entities conducting analyses	Dependent on entities conducting analyses	Dependent on entities conducting analyses	

*****Funding not secured

******DNA extraction and processing costs may not be included

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APPENDIX C. I-15 Wildlife Crossing Design Experts Report







INTERSTATE 15 WILDLIFE CROSSINGS: DESIGN CONSIDERATIONS FOR FOCAL WILDLIFE SPECIES

Santa Ana-Palomar Mountains Linkage
Southern California

Prepared by:
The Nature Conservancy
California Department of Transportation
US Geological Survey
UC Davis Wildlife Health Center
San Diego State University
LSA Associates
Wildspring Ecology

September 2023

INTERSTATE 15 WILDLIFE CROSSINGS: DESIGN CONSIDERATIONS FOR FOCAL WILDLIFE SPECIES

Santa Ana-Palomar Mountains Linkage
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September 2023

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Acknowledgements

This report is the product of an experts workshop convened by The Nature Conservancy and California Department of Transportation. The workshop, held in February 2022, brought together over 50 wildlife experts from throughout California and draws from state and federal agencies, non-profit conservation organizations, and research institutions. We are thankful to the many wildlife experts and the organizations they represent (listed in Appendix A) who participated in the crossing design workshop. In addition to the authors, several individuals helped plan the workshop, including Sally Brown (US Fish and Wildlife Service), Michelle Mariscal (Puente-Chino Hills Habitat Authority), Kristeen Penrod (SC Wildlands), Gordon Pratt (UC Riverside), and John Taylor (US Fish and Wildlife Service). We thank Dudek for providing workshop participants access to drone footage of the proposed Interstate 15 crossing locations. We thank Lance Vallery for providing conceptual cross-sections and perspectives for the vegetated overcrossing and wildlife culvert based on the input from wildlife experts. We thank Carlton Rochester, Jonathan Richmond, and Diane Elam from the U.S. Geological Survey for reviewing the manuscript. Finally, we thank the State of California Wildlife Conservation Board, California Department of Fish and Wildlife, and The Nature Conservancy donors who have provided funding to the planning of these wildlife crossings.

Executive Summary

The Nature Conservancy (TNC) and the California Department of Transportation (Caltrans), along with landowners including San Diego State University, California Department of Fish and Wildlife, Western Riverside Regional Conservation Authority and Riverside County Flood Control District are developing wildlife crossing infrastructure projects along a 3-mile stretch of Interstate 15 (I-15) in the Santa Ana-Palomar Mountains Linkage (hereafter 'Linkage') in southern California. These crossings will provide a critical missing link that will help reconnect wildlife in the coastal Santa Ana Mountains west of I-15 with those in the interior Palomar and Eastern Peninsular ranges to the east of I-15. The Linkage supports intact and diverse habitats including coastal sage scrub, grasslands, chaparral, and oak and riparian woodlands, and has been a focus of regional conservation efforts for the last 30 years.

The three wildlife crossing infrastructure projects include enhancement of the existing Temecula Creek I-15 Bridge, construction of a new vegetated wildlife overcrossing, and construction of a new stand-alone wildlife culvert.

Given the challenges and level of financial investment required to secure wildlife crossings for I-15 in the Linkage, TNC and Caltrans proposed that planning efforts would benefit from input by taxonomic experts on design concepts that meet the needs of the broadest range of wildlife. While wildlife crossings are becoming more common, optimal designs that meet the needs of a variety of wildlife species are largely unknown and can be site specific. To address this challenge, we held a workshop in February 2022 that brought together over 50 wildlife experts to brainstorm and identify specific design considerations for various focal wildlife species groups (medium/large mammals, small animals, birds, bats, plants, and invertebrates) that might use the identified I-15 wildlife crossings (The Nature Conservancy 2022).

Lead experts for each focal species group worked together to identify specific wildlife crossing features or attributes for each of the three proposed wildlife crossings.

Specific attributes evaluated by experts for each crossing type and species group included, at a minimum:

- Crossing Structure Attributes:
    - Habitat features (cover, habitat structure, substrate, moisture, light and noise mitigation)
- Crossing Approach Area Features:
    - Habitat features (cover type/density, substrate, water, light and noise mitigation)
- Barrier design to reduce roadkill and/or to funnel wildlife to the crossing
- Additional research to resolve uncertainties related to crossing design

Based on the design considerations for each potential crossing type, the experts then weighed in on the suitability of the existing location and probability of use by their focal species or groups of species. With proposed design features, Temecula Creek Bridge has moderate or high probability of use by 27 of the 36 focal wildlife species assessed, while the vegetated overcrossing could meet the needs of 26 of the 36 species. When combined, Temecula Creek Bridge and the vegetated overcrossing have a moderate or high probability of use for 34 of the 36 species. The wildlife culvert has a moderate or high level of expected use by 10 of the 36 focal species and could serve connectivity needs for representative species from all but the bird and plant species groups.

1. Introduction

The Nature Conservancy (TNC) and the California Department of Transportation (Caltrans), along with landowners including San Diego State University, California Department of Fish and Wildlife, Western Riverside County Regional Conservation Authority and Riverside County Water Conservation and Flood Control District are planning two proposed wildlife crossing infrastructure projects along a 3-mile stretch of Interstate 15 (I-15) in the Santa Ana-Palomar Mountains Linkage (Linkage) in southern California (Luke et al. 2004). These crossings will provide a critical missing link that will help reconnect wildlife in the coastal Santa Ana Mountains west of I-15 with those in the interior Palomar and Eastern Peninsular ranges to the east of I-15 (Figure 1). The Linkage supports intact and diverse habitats including coastal sage scrub, grasslands, mixed chaparral, and oak and riparian woodlands, and has been a focus of regional conservation efforts for the last 30 years.

As stated in the Federal Highway Administration's Wildlife Crossing Structure Handbook (Clevenger and Huijser 2011): "There is currently an urgent need to provide transportation and other stakeholder agencies with technical guidance and best management practices on the planning and design of wildlife crossing mitigation measures. While wildlife crossings are becoming more common, the design of crossings to meet the needs of a variety of wildlife species is largely unknown and can be site specific".

Figure 1: Regional Location Map, I-15 Wildlife Crossing Project Locations

Given the challenges and financial investment required to secure wildlife crossings for I-15 in the Linkage, TNC and Caltrans proposed that planning efforts would benefit from input by taxonomic experts on design concepts that would meet the needs of the broadest range of wildlife. A workshop was held, bringing together over 50 wildlife experts to identify specific design considerations for various focal wildlife that might use the identified I-15 wildlife crossings.

This report presents the results of that effort, and includes background on the Linkage, focal wildlife species, wildlife crossing planning efforts to date, methods on how focal species experts evaluated wildlife crossing designs, and summary results for each focal species group and crossing type that was evaluated.

1.1 Workshop Objective

The objective of the workshop was to provide information to TNC and Caltrans for the development of new or improved wildlife crossings across I-15.

1.2 Background

The Santa Ana Mountains represent the largest block of coastal open space in the South Coast Ecoregion and support intact communities of coastal sage scrub, native grasslands, Engelmann oak woodlands, chaparral, and riparian woodland habitats as well as the Santa Margarita River, the longest free flowing river in Southern California (The Nature Conservancy 1992). This landscape supports many of southern California's rarest wildlife species, including southwestern arroyo toad (*Anaxyrus californicus*), least Bell's vireo (*Vireo bellii pusillus*), cactus wren (*Campylorhyncus brunneicapillus*), California gnatcatcher (*Polioptila californica*), and Tecate cypress (*Cupressus forbesii*). This landscape also sits at the intersection of three regional planning areas for the State of California's Natural Communities Conservation Plan in Orange, Western Riverside, and San Diego Counties (R.J. Meade Consulting, Inc. 1996, Riverside County 2003, Ogden Environmental and Energy Services 1996).

Although large, at roughly 500,000 acres, the Santa Ana Mountains and its biological diversity are at risk of degradation because it is not large enough to sustain populations of wildlife that inhabit the area nor ecosystem processes. Dr. Paul Beier, who conducted the first regional mountain lion (*Puma concolor*) study in the late 1980s, identified the need for landscape-scale connections to other southern California mountain ranges. Dr. Beier's research revealed that I-15 was a nearly impenetrable barrier for mountain lions, and that if connections across I-15 to the eastern Peninsular ranges were not restored, mountain lions of the Santa Ana Mountains would be extirpated within the next 100 years due to effects of small population size and associated inbreeding (Beier and Barrett 1993). This research has been validated by more recent studies (Benson et al. 2019) that identify an 11–21% risk of extirpation in the next 50 years due to demographic, stochastic, and environmental factors, and a probable extirpation within a median time of 12 years if inbreeding depression should occur.

The Linkage is one of 15 South Coast Missing Linkages identified by SC Wildlands that are widely considered the backbone of a regional conservation strategy for southern California, stitching together over 18 million acres of existing conservation lands. The missing linkages are critical for maintaining connected wildlife populations from Baja California Norte, Mexico to the southern Sierra Nevada and from the beaches of Marine Corps Base Camp Pendleton eastward to the deserts of Anza-Borrego Desert State Park. The 15 linkages were designed based on the habitat and movement needs of 109 focal species across the 15 priority linkages, including 26 plants, 25 insects, 4 fish, 5 amphibians, 12 reptiles, 20 birds and 17 mammals. These focal species cover a broad range of habitat and movement requirements such that planning linkages adequate for their needs is expected to cover connectivity needs for the ecosystems they represent (SC Wildlands 2008).

The Linkage is the only viable landscape connection that would link the Santa Ana Mountains and coast to the inland mountain chain that includes the Palomar, Laguna, Santa Rosa, and San Jacinto ranges. The Linkage sits at the San Diego-Riverside County line and supports a band of habitat roughly 16 miles long from west to east and 4 miles wide at its narrowest point where it intersects I-15. The Linkage extends from Marine Corps Base Camp Pendleton, Fallbrook Naval Weapons Station, and the Trabuco Ranger District of the Cleveland National Forest (CNF) in the west to the Palomar Ranger District of the CNF and Agua Tibia Wilderness in the east and encompasses riparian and upland habitats (Luke et al. 2004). The Linkage design was informed by a suite of focal species that represented the widest range of movement needs and ecosystem function. Focal species represent diverse taxonomic groups and include both common and rare species, with the goal of maintaining common species and enhancing the resilience of rare species (SC Wildlands 2008). Table 1 lists the focal species originally identified by the SC Wildlands linkage design in 2004 as well as those added by species experts in 2022. Appendix B provides a list of the focal plant and wildlife species with special federal or state regulatory status.

Common Name	Scientific Name
Medium/Large Mammals	
American badger	*Taxidea taxus*
Bobcat	*Lynx rufus**
Coyote	*Canis latrans**
Gray fox	*Urocyon cinereoargenteus**
Mountain lion	*Puma concolor*
Ringtail	*Bassariscus astutus**
Southern mule deer	*Odocoileus hemionus fuliginatus**
Small Animals	
Arroyo toad	*Anaxyrus californicus**
Big-eared woodrat	*Neotoma macrotis*
Blainville's horned lizard	*Phrynosoma blainvillii**
California tree frog	*Pseudacris cadaverina*
Red diamond rattlesnake	*Crotalus ruber*
Western gray squirrel	*Sciurus griseus**
Western pond turtle	*Actinemys pallida*
Western spadefoot toad	*Spea hammondii**
Western toad	*Anaxyrus boreas halophilus*
Bats	
Big brown bat	*Eptesicus fuscus**
Pallid bat	*Antrozous pallidus**
Birds	
California gnatcatcher	*Polioptila californica**
Cactus wren	*Campylorhyncus brunneicapillus**
California quail	*Callipepla californica*
California thrasher	*Toxostoma redivivum**
Greater roadrunner	*Geococcyx californianus**
Hawks/Owls	*Accipitridae/Strigidae**
Least Bell's vireo	*Vireo bellii pusillus*
Oak titmouse	*Baeolophus inornatus**
Wrentit	*Chamaea fasciata**
Yellow warbler	*Setophaga petechia*
Plants and Invertebrates	
California sister	*Adelpha californica*
Chaparral yucca	*Hesperoyucca whipplei*
Comstock's fritillary	*Speyeria callippe comstocki*
Engelmann oak	*Quercus engelmannii*
Jerusalem cricket	*Ammopelmatus* spp.*
Monarch butterfly	*Danaus plexippus**
Pale swallowtail	*Papilio eurymedon*
Rainbow manzanita	*Arctostaphylos rainbowensis*
Timema walking stick	*Timema podura**

Table 1: Santa Ana-Palomar Linkage Focal Species Assessed during February 2022 Workshop process. Focal species added by species experts are asterisked (*).

1.3 Wildlife Crossing Infrastructure Planning Efforts

Wildlife connectivity needs within the Linkage have been addressed by stakeholders through significant research and planning, including strategic land protection and the identification of locations and concepts for wildlife crossing structures for I-15.

Numerous studies have been conducted to evaluate the best location along I-15 for a wildlife crossing structure (Gibbons 2008, Tracey et al. 2011, Zeller et al. 2016, Riley et al. 2018). In addition, camera monitoring studies conducted at existing drainage culverts along I-15 (Stricker 2015, Regional Conservation Authority 2013, Vickers et al. 2020) have documented numerous wildlife species on both sides of I-15, including bobcat (*Lynx rufus*), coyote (*Canis latrans*), gray fox (*Urocyon cinereoargenteus*), ringtail (*Bassariscus astutus*), spotted skunk (*Spilogale gracilus*), striped skunk (*Mephitis mephitis*) and mountain lion, with only a few instances of wildlife using existing culverts to get to the other side of the highway. Stricker (2015) identified ringtail moving through a 4-foot culvert, while Vickers (unpubl. data) documented at least three separate instances of a three-legged coyote moving through a 4-foot culvert approximately 1.5 miles south of the Temecula Creek Bridge. In October of 2021, and again in October 2022, camera traps set up along Temecula and Pechanga Creeks documented a mountain lion successfully moving eastward under the Temecula Creek Bridge, with the October 2021 lion documented further to the east in Pechanga Creek (Vickers 2022).

Thus, while mountain lions and other wildlife have been documented on both sides of the highway, existing crossing structures appear to be inadequate to allow regular safe passage. While regular accounting of wildlife mortalities is not conducted for I-15, four known fatalities of mountain lion by vehicle strikes were documented along a 6-mile stretch of I-15 between 2017 and 2020 (Vickers et al. 2020).

In response to wildlife mortality from vehicle strikes, Caltrans (District 8) installed wildlife exclusion fencing along both sides of I-15 from the Temecula Creek Bridge southward to the San Diego County border as part of a highway pavement repair project in 2020. The installation of wildlife exclusion fencing has increased the urgency to plan, secure funding for, and implement needed wildlife crossing structures.

Data from studies as well as expert opinion have prioritized three locations for enhanced or new wildlife crossing structures: one at the north end of the Linkage to enhance the function of the existing Temecula Creek Bridge, and two locations 1.3 miles to the south, for a new wildlife undercrossing and a new vegetated wildlife overcrossing (Riley et al. 2018).

In 2019, UC Davis received a planning grant from the California Department of Fish and Wildlife to work with student teams and faculty from Cal Poly Pomona School of Civil Engineering and Caltrans to develop concepts and feasibility studies for these three prioritized wildlife crossing locations along I-15 (Vickers et al. 2020).

Below are summaries of the design concepts developed by Cal Poly Pomona, depicted in Figures 2 and 3 (Cal Poly Pomona 2019). Riley et al. (2018) recommended that a minimum of two wildlife crossings, including Temecula Creek Bridge and either a new culvert or vegetated overcrossing, be pursued to meet the needs of the broadest range of wildlife species. To this end, TNC is developing design specifications for Temecula Creek Bridge, while Caltrans is developing design plans and assessing alternatives for either a new culvert or overcrossing. All crossing structures may include installation of barriers along the highway to shield noise and light from the highway, which are known issues at each location (Shilling et al. 2022).

<u>Temecula Creek Bridge</u>

Tributaries to the Santa Margarita River, Temecula Creek and Pechanga Creek, are currently the only viable connections between the Santa Ana and Palomar Mountains and are the only riparian connection in the Linkage that could serve the needs of both aquatic and terrestrial wildlife. Temecula Creek is in the upper watershed of the Santa Margarita River and supports a diverse riparian habitat composed of emergent wetland, willow (*Salix* spp.), cottonwood (*Populus fremontii*), and coast live oak (*Quercus agrifolia*) woodlands typical of the region (ICF 2023). To the east of I-15, Pechanga Creek, which flows out of the Palomar Mountains, connects to Temecula Creek. The I-15 Temecula Creek Bridge spans Temecula Creek downstream of its confluence with Pechanga Creek and before its confluence with Murrieta Creek, which creates the Santa Margarita River. The bridge system is composed of two separate bridges for north and south-bound traffic lanes. Each individual bridge has four travel lanes with right and left shoulders, spans approximately 65 feet wide, and has 50-foot open-median baton-bridge decks. The bridge length is approximately 310 feet, and the height is approximately 50 feet (ICF 2023). Land ownership at this crossing location consists of California Department of Fish and Wildlife, San Diego State University, Caltrans, Western Riverside County Regional Conservation Authority, and Riverside County Flood Control and Water Conservation District.

Because riparian areas are natural dispersal corridors that can span elevational gradients, Temecula Creek may facilitate wildlife movement in response to a warming climate (Jennings et al. 2020). Rojas et al. (2021) identified Temecula Creek as an important linkage between refugia from multiple climate and non-climate stressors. Despite its values, Temecula Creek currently functions poorly as a wildlife corridor due to several issues, the biggest of which is frequent human presence. Human activity can deter wildlife use of natural areas and crossing structures (Murphy-Mariscal et al. 2015, Larson et al. 2016, Longcore et al. 2018) while urban runoff from upstream development can degrade water quality by the addition of chemicals, pollutants (e.g., nitrogen and phosphates from fertilizers), trash, and other debris (Larry Walker and Associates 2018). In addition, Temecula and Pechanga Creeks are inhabited by invasive exotic plants such as pampas grass (*Cortaderia selloana*), which can be inhospitable to wildlife and choke water flows. Light and traffic noise associated with I-15 are also likely deterring wildlife use of this crossing (Shilling et al. 2022).

To address these challenges at Temecula Creek, Cal Poly Pomona student engineers developed concept plans to enhance the function of a 58-acre area of Temecula and Pechanga Creeks, including under Temecula Creek Bridge, as a wildlife corridor (Cal Poly Pomona 2019). Design concepts for the crossing included fencing and signage at the urban interface to deter trespass, exotic plant control for pampas grass and other weeds that have displaced native riparian habitat, restoration of riparian woodland, and recommendations for measures to reduce traffic noise associated with I-15.

TNC received a grant from the State of California Wildlife Conservation Board to conduct baseline vegetation, wildlife, and noise studies for Temecula and Pechanga Creeks, and to use the Cal Poly Pomona Engineering School design concepts to produce detailed plans and complete the permitting requirements for a shovel-ready project by the end of 2023.

<u>Wildlife Culvert</u>

The proposed location evaluated for a new wildlife culvert intersects small drainages on both sides of the highway. Camera data from these drainages indicate activity by a wide range of wildlife species (Vickers et al. 2020). The new structure would be located close to, but would not replace, an existing culvert structure and would be designed primarily for wildlife use. To the east of I-15, the proposed undercrossing location supports coastal sage scrub, chaparral and a riparian/oak woodland drainage that flows to the north. West of I-15, the proposed crossing location supports a small drainage with intact oak woodland, chaparral, and rock outcrops. The culvert designed by Cal Poly Pomona students included a 12-foot by 12-foot box culvert or an 8-foot high by 11-foot-wide arched corrugated pipe, both with soil on the bottom, which would reduce the overall height but would promote wildlife usage. The Cal Poly designs would include a concrete lined drain on the side of the structure to facilitate water flows. The structure would be slightly elevated on the west side to prevent inundation. Students also investigated the potential for a larger arched culvert with a natural bottom at this location, but this was determined to be infeasible due to traffic-related construction constraints. Caltrans is currently considering a 15 x 15-foot-wide wildlife culvert design. Land ownership at the proposed wildlife culvert location consists of San Diego State University, Western Riverside County Regional Conservation Authority, Caltrans, and The Nature Conservancy.

Figure 2: Wildlife Culvert Concept Design. Cal Poly Pomona. 2019.

Vegetated Wildlife Overcrossing

Just 0.1 mile south of the proposed wildlife culvert location is the proposed site for a vegetated overcrossing. Wildlife crossing experts suggested that this is the best location for an overcrossing because there are elevated benches on either side of the highway that could serve as anchor points for support (Gibbons 2008, Riley et al. 2018).

Figure 3: Vegetated Wildlife Overcrossing Design, Cal Poly Pomona. 2019.

Cal Poly Pomona produced engineering feasibility and preliminary design concepts for the vegetated overcrossing, which would span 10 lanes of highway (235 feet) and would be up to 165 feet wide, similar to the Wallis Annenberg Wildlife Crossing currently under construction, which will span Highway 101 in northern Los Angeles County. The overcrossing would include roughly 4 feet of engineered soil on top of the structure, which would require that plantings be shallow rooted. The student design also included berms on the edges of the crossing structure to buffer sound and light. Land ownership at the proposed vegetated wildlife overcrossing location consists of San Diego State University, Western Riverside County Regional Conservation Authority, Caltrans, and The Nature Conservancy.

Caltrans will prepare an alternatives analysis, technical studies, and environmental document for the wildlife culvert and vegetated overcrossing. Additional alternatives for the overcrossing and culvert have since been developed. Final engineering and construction documents for the preferred alternative (vegetated overcrossing or wildlife culvert) will follow and are expected to be completed by the end of 2026, pending funding availability.

2. Methods

For the workshop, focal species were broadly classified into 5 groupings for assessment by species experts: medium/large mammals, birds, bats, small animals (smaller bodied mammals, herpetofauna) and plants/invertebrates. Lead experts for each group were enlisted to develop the workshop format, solicit additional experts to participate in the workshop process for their focal species group, and facilitate, gather, and summarize expert input as part of the workshop process. Table 2 provides a listing of experts and facilitators for each species group. Appendix A provides a full listing of the species experts and their professional affiliations.

Workshop planners and lead experts gathered background data, maps, literature, reports, and drone footage of the crossing locations (Dudek 2021) and provided the information to workshop participants in advance of the workshop.

Lead experts worked together to identify specific attributes for evaluation by species groups for each of the three proposed wildlife crossings (Temecula Creek Bridge, vegetated overcrossing, wildlife culvert).

Attributes for each crossing type and species group included, at a minimum:

- Crossing Structure Attributes
    - Habitat features (cover type/density, habitat structure, substrate, moisture, light and noise mitigation, etc.)
- Crossing Approach Area Features
    - Habitat features (cover type/density, substrate, water, light and noise mitigation, etc.)
- Barrier design to reduce roadkill and/or to funnel wildlife to the crossing
- Other features critical to promote wildlife use
- Additional research to resolve uncertainties related to crossing design

Based on the input provided by the species experts for each potential crossing type, the groups then weighed in on the suitability of the existing location and probability of use by their focal species or group of species.

Medium/Large Mammals	Birds	Bats	Small Animals	Plants/Invertebrates
Megan Jennings (Co-Lead)	Barbara Kus (Lead)	Jill Carpenter (Lead)	Cheryl Brehme (Lead)	Spring Strahm (Lead)
Winston Vickers (Co-Lead)	John Taylor (Facilitator)	Trish Smith (Facilitator)	Sally Brown (Facilitator)	Kristeen Penrod (Facilitator)
Michelle Mariscal (Facilitator)	Melody Aimar	Alisha Curtis	Nancy Frost (Facilitator)	Allison Anderson
Eric Abelson	Peter Beck	Tim Dillingham	Adam Backlin	Greg Ballmer
Devin Adsit-Morris	Robb Hamilton	Stephanie Remington	Denise Clark	Pablo Bryant
Paul Beier	Gjon Hazard	Drew Stokes	Rulon Clark	Lauren Jonker
Kevin Crooks	Melanie Madden	Greg Tatarian	Katy Delaney	David Lipson
Justin Dellinger	Nicholas Peterson		Liz Fairbank	Dan Marschalek
Calvin Duncan	Kris Preston		Robert Fisher	Eric Porter
Julie King	Phil Unitt		Steve Montgomery	Gordon Pratt
Barry Martin			Will Miller	Zach Principe
JP Montagne			Debra Shier	Jessie Vinje
Brock Ortega			Scott Tremor	Susan Wynn
Dustin Pearce				
Seth Riley				
Carlton Rochester				
Fraser Shilling				
Jessica Sanchez				

Table 2: Lead Experts, Facilitators and Participating Species Experts for each Focal Wildlife Species Group

3. Results

3.1 Medium and Large Mammals

<u>Importance of Wildlife Crossings for Medium and Large Mammals</u>

Medium and large mammals are important to include when planning for connectivity because they tend to be wide-ranging, are sensitive to fragmentation by roads, and normal movements often result in encounters with infrastructure, particularly roads. Large and medium mammalian carnivores function as primary predators that affect prey populations ranging from small rodents to large herbivores and other carnivores, and the effects of roads interfering with carnivore movement behaviors can reverberate through the food web.

<u>Focal Medium and Large Mammal Species</u>

Medium and large mammals identified in the SC Wildlands report included mountain lion and American badger (*Taxidea taxus*). The mountain lion has guided much of the connectivity research in the Linkage since the early 1990s, as its population in the Santa Ana Mountains is known to be at risk of extirpation unless connectivity is restored to the eastern Peninsular Ranges (Beier and Barrett 1993). Based on known occurrences in the area and to ensure that crossing structures facilitate movement for a range of medium and large carnivores, experts added the following focal species: ringtail, coyote, gray fox, and bobcat. The southern mule deer (*Odocoileus hemionus fuliginatus*) was also added due to its importance as mountain lion prey and to increase its own regional connectivity (Mitelberg et al. 2019).

<u>Probability of Use of Crossing Type by Focal Medium and Large Mammals</u>

When considering wildlife crossing use by medium and large mammals, it is important to evaluate designs that support connectivity for daily movements, seasonal movements, dispersal, gene flow, and range shifts due to threats such as climate change or wildfire.

Experts evaluated the probability of use by focal medium/large mammals of all crossing types with the planned improvements based on occurrence information in the vicinity of the crossings (documented by available scat, track and camera trap data), the likelihood of a species encountering the structures based on habitat associations, and prior knowledge of species' responses to crossing structure designs elsewhere.

Based on the medium and large mammal expert group evaluation, the **Temecula Creek Bridge** plan has a high probability of use by mountain lion, mule deer, bobcat and coyote, and a moderate probability of use by gray fox, ringtail, and badger. Although mule deer have not been documented in Temecula Creek, they have been documented on lands farther to the east and west of I-15 and the large, open design of the bridge is well-suited for this species. Camera and radio collar data show that mountain lions approach Temecula Creek Bridge from the west fairly frequently, and recently a mountain lion used the I-15 underpass to reach Pechanga Creek. Coyotes are regularly documented at camera traps in Temecula Creek, as are bobcats and gray fox, although less frequently than coyotes (Vickers 2022).

Ringtail have not been documented on camera traps in Temecula Creek, though they have been documented to the south along I-15 between Temecula Creek and the proposed sites for a new wildlife crossing. The absence of ringtail in Temecula Creek itself is likely due to the lack of continuous tree canopy in the creek and the lack of boulders or crevices for hiding. Badgers have also not been confirmed in the vicinity of I-15 or Temecula Creek, although one workshop participant noted that they had seen badger sign just west of the I-15 Temecula Creek Bridge in 2007. Badgers have occasionally been documented using riparian corridors for long distance movements. In addition, the friable, loamy substrate in Temecula Creek and adjacent grasslands provides suitable habitat for burrowing.

The **wildlife culvert** design has a high probability of use for all species except mule deer and badger. Mountain lion, coyote, bobcat, and gray fox are all expected to use the wildlife culvert if there are natural travel routes leading to the entrances, such as along paths or drainages. Ringtails are also expected to use the wildlife culvert, as they have been documented using an existing 4-foot culvert along I-15 just north of the proposed wildlife culvert location (Stricker 2015). For ringtail, the presence of boulders and oaks in the vicinity of the crossing entrance may increase the probability of use. There is a question as to the suitability of habitat for deer in the area near the proposed culvert. Current use of the area by deer appears to be very low. Similarly, there are no records of badger in the vicinity of the proposed wildlife culvert location. Given the unsuitable soil conditions (compacted decomposed granite) in the approach areas, experts determined there was only a moderate probability of use of the culvert by badgers, although the structure itself may facilitate badger passage.

The proposed **vegetated overcrossing** has a high probability of use by coyote, gray fox and bobcat, and a moderate probability of use by mountain lion, mule deer, and ringtail. Noise and light impacts on the approach slopes are the most limiting factors for mountain lions. If deer use in the vicinity of the proposed overcrossing increases, individuals would likely approach and use the structure due to its open character. More research is needed to understand crossing design features for ringtail. Cover on the overcrossing itself will also be a factor in balancing the crossing needs of mountain lion, gray fox, and coyote. Badgers are unlikely to use the crossing, due to a lack of occurrence records and poor soil conditions in the immediate area.

Species	Temecula Creek Bridge	Wildlife Culvert	Vegetated Overcrossing
American badger	M	M	L
Bobcat	H	H	H
Coyote	H	H	H
Gray fox	M	H	H
Mountain lion	H	H	M
Ringtail	M	H	M
Southern mule deer	H	L	M

Table 3: Probability of use of each crossing type by medium and large focal mammals: High (H), Moderate (M), Low (L). Scientific names are in Table 1.

<u>Crossing Design Features to Encourage Use by Medium and Large Mammals</u>

In assessing design elements of each crossing and approach area for medium and large animals, experts considered species' needs related to vegetation type and cover, movement behaviors, prey species, and the impacts of human activity and disturbance from the roadway.

Temecula Creek Bridge

To increase the likelihood of use of Temecula Creek by mountain lion, mule deer, bobcat, gray fox, and coyote, experts proposed measures to deter human use of the area by erecting security fencing at the perimeter of developed areas, controlling invasive plants, and managing understory vegetation to improve visibility and access to the crossing. Improving visibility through the crossing is particularly important for deer, which typically avoid high cover areas where they may be susceptible to predation. In addition, experts proposed measures to reduce lighting and noise associated with I-15 and adjacent residential uses. Some experts identified that the water treatment plant just west of the Temecula Creek

Bridge could be a potential deterrent due to lights and odors and may require management. Because the creek can have periods of water inundation, experts proposed incorporating dry ledges under the bridge to accommodate terrestrial wildlife movement. Maintaining smaller prey under and around the bridge will also be important for focal medium and large carnivores. To enhance the function of Temecula Creek for ringtail, suggestions included the removal of invasive plants, and adding oaks and other trees, boulders, logs or other structures. To accommodate the differing needs of species preferring more open or closed vegetation, different segments of the bridge span could be managed to achieve different levels of cover. For example, one section could be managed to be more open to enhance visibility for wildlife while the other section could be managed to retain more vegetation to achieve a closed canopy. In addition, the smooth wire on the cantilevered section of the I-15 exclusion fencing could be replaced with fine mesh to prevent ringtail and gray fox from climbing over into the highway. Also, it would be prudent to conduct regular inspections of the lower section of the I-15 exclusion fencing to ensure that wildlife are not able to burrow under it.

Wildlife Culvert

To encourage use of the wildlife culvert by mountain lion, coyote, gray fox, and bobcat, experts proposed that natural pathways leading to the crossing structure be maintained or created. In addition to the above design elements, a culvert of at least 15 x 15 feet would maximize natural light in the culvert and increase the likelihood of use by mule deer. For ringtail, it will be important to locate the culvert entrances in areas with adjacent boulders and trees and to replace any of these features if they are removed by construction. In addition, incorporating water features (proposed for other focal species) in the approach areas and within the culvert itself may attract ringtails. While lights have been recommended in other locations to increase visibility within the culvert for some species, ringtails prefer a dark environment. It was also proposed that the existing nearby drainage culvert be maintained to carry the bulk of existing water flows, allowing the wildlife culvert to function solely for wildlife passage. The wildlife culvert would be most effective if it is slightly elevated above the drainage inlet on the west side so that it does not become inundated during heavy rainfall events. It was suggested that the wildlife culvert incorporate a natural soil bottom with a small channel on one side to carry low flows. Finally, sound dampening treatments within the culvert as well as noise and light barriers along the highway at the crossing would increase probability of use.

Vegetated Overcrossing

Features to encourage use of the overcrossing by the widest range of focal medium and large mammal species include creating areas of varying cover where feasible, including small and large shrubs, occasional trees where the root systems can be accommodated, boulders (artificial boulders are fine if weight is an issue) and logs to provide shelter as well as habitat for prey species. It will be important to determine the right balance of cover that will shelter more cryptic species such as gray fox but will also attract other taxa that prefer more open habitat. As with the undercrossing, creating and maintaining pathways to the crossing are proposed to guide wildlife to the crossing. Barrier walls at least 10 feet in height are proposed for both along I-15 in the approach area and on the edges of the structure to prevent climbing, noise, and light.

<u>Areas For Additional Study</u>

- Evaluate the need for creating game trails to the crossings to attract large/medium animals. Would such paths only serve to attract human trespass? Is it possible to modify the habitat somewhat to facilitate access by medium and large mammals without creating trails? Could the

creation of game trails be implemented as an adaptive management strategy after crossings are in place?
- Research potential movement pathways for badger by locating nearest suitable habitat east and west of I-15.
- Expand existing regional modelling for mule deer to better understand connectivity between the Santa Ana and Eastern Peninsular Ranges.
- Conduct research to better understand ideal crossing features for ringtail for all crossing types.
- Develop methodology to monitor human presence and its effect on wildlife crossing use by focal species.

3.2 Small Animals

Importance of Wildlife Crossings for Small Animals

Major highways are barriers to most terrestrial small animal species, because they often move shorter distances and are slower moving, making them susceptible to roadkill. These factors require that highway crossings for small animals incorporate both shelter and foraging habitat.

A connected and diverse small animal community assemblage is an important part of ecosystem function and resilience, and it is likely that if small animals use a crossing structure, their predators and larger fauna will use the crossing as well.

Focal Small Animal Species

A wide variety of small animal focal species was identified in the SC Wildlands report including reptiles, amphibians, and small mammals with varying conservation status and representing both terrestrial and semi aquatic species. Focal small animal species were categorized as either terrestrial or semi aquatic for wildlife crossing planning purposes (Table 4). Focal small animal species that were added by stakeholders or small animal experts to the list developed by SC Wildlands include arroyo toad, Blainville's horned lizard (*Phrynosoma blainvillii*), western spadefoot toad (*Spea hammondii*), and western gray squirrel (*Sciurus griseus*).

Experts identified that focal species could serve as umbrella species for a wider suite of small animals. For example, the red diamond rattlesnake (*Crotalus ruber*) could serve as an umbrella species for other snake species and/or for other small terrestrial animals that prefer open habitat.

All terrestrial and semi aquatic species have different habitat requirements that were considered when assessing their connectivity needs. For example, some terrestrial and semi aquatic species prefer open habitat, while others prefer more closed habitat.

Terrestrial species included specialists that require open habitat for thermoregulation and foraging (Blainville's horned lizard, red diamond rattlesnake) and those that require large shrubs and/or tree cover for their general habitat and dietary needs (big-eared woodrat [*Neotoma macrotus*], gray squirrel). Semi-aquatic species, which include the western spadefoot toad, arroyo toad, western toad, California tree frog (*Pseudacris cadaverina*), and western pond turtle (*Actinemys pallida*), use both aquatic and terrestrial habitats for their life history needs. For instance, western spadefoot toads require upland sage scrub and chaparral habitat and breed in upland pools, such as vernal pools (Stebbins 2003). Arroyo and western toads (*Anaxyrus boreas halophilus*) typically require low-flow shallow water (streams, creeks, rivers) for breeding as well as open sandy upland natural habitats for foraging and overwintering (Griffin et al. 1999). California tree frogs are specialists that require small pools and boulder habitats. Finally, western pond turtles can use streams for dispersal, but require deeper pools and basking habitat (logs, boulders) for breeding and other life history needs, as well as natural upland habitat for egg laying (Bury and Germano 2008, Rathbun et al. 1992).

Probability of Use of Crossing Type by Focal Small Animals

Not all focal small animal species were evaluated for each passage location due to differences in habitat composition and whether water is present. Terrestrial species were considered for all crossings, while semi aquatic species were only considered for the Temecula Creek Bridge where water is present year-round. In addition, the gray squirrel was only considered for locations where oaks are present on either side of the crossing, which is limited to the proposed wildlife culvert location and Temecula Creek Bridge.

In evaluating the use of each of the crossing types by focal small animals, experts assessed connectivity objectives and other factors. For example, is infrequent passage for genetic connectivity a sufficient goal for the species or is frequent use of the passage for living and breeding habitat more important for long term population viability?

All three crossing types would function for a subset of identified small animal focal species (Table 4). The **vegetated overcrossing** would function for all the terrestrial species, excluding the western gray squirrel, and would also function for the spadefoot toad, a semi aquatic species, if the designs included some sort of water feature in the approach area. The **wildlife culvert** would likely have a high probability of use for the red diamond rattlesnake and big-eared woodrat, with a lower probability of use for gray squirrel, spadefoot toad, and western toad. Finally, the **Temecula Creek Bridge** has some probability of use by all the small animal focal species, with the California tree frog and spadefoot toad having the lowest probability of use. Of all the focal small animals, both the red diamond rattlesnake and big-eared woodrat have high probability of use of all three crossing types, while California tree frog has only a low probability of use for one of the crossing types, the Temecula Creek Bridge.

Common Name	Terrestrial	Semi-Aquatic	Temecula Creek Bridge	Wildlife Culvert	Vegetated Overcrossing
Arroyo toad		X	M	-	-
Big-eared woodrat	X		H	H	H
Blainville's horned lizard	X		M	L	H
California tree frog		X	L	-	-
Red diamond rattlesnake	X		H	H	H
Western gray squirrel	X		M	L	-
Western pond turtle		X	M	-	-
Western spadefoot toad		X	L	L	H
Western toad		X	H	L	L

Table 4: Classifications for planning purposes and probability of use of each crossing type small animal species: High (H), Moderate (M), Low (L). Crossing type that is improbable for use is indicated using "-". Scientific names are in Table 1.

Crossing Design Features to Encourage Use by Small Animals

In assessing design elements of each crossing and approach area for small animals, experts considered species needs related to soils and vegetation structure, aquatic habitat, time of activity, prey availability, temperature, and cover.

Temecula Creek Bridge

Experts felt that after planned invasive plant control and native plant restoration are implemented for the Temecula Creek Bridge crossing, natural flood events will help create natural channels with a variety of depths and flows that would benefit semi-aquatic species. Experts did not suggest adding boulders or deep pools for the pond turtle or California tree frog, as they believe Temecula Creek in this location provides transitional habitat for these species to move through, using the habitat under the bridge during flood events.

To enhance connectivity for terrestrial species at this location, experts proposed the creation of ledges along the bridge abutments to facilitate small animal movement, particularly during winter months when seasonal inundation may occur. The installation of rope bridges between trees was also suggested to facilitate movement of the gray squirrel (a tree specialist) under the bridge where there are large gaps between trees (Timmermans 2018). Gray squirrels were documented in Pechanga Creek in 2022 (Martin 2022); however, the presence of gray squirrels west of I-15 warrants future investigation.

While Temecula Creek could potentially be suitable habitat for the arroyo toad and western toad, invasive predators, particularly bullfrogs (*Lithobates catesbeianus*) and large predatory fish, are a major threat to native herpetofauna in this location (Fisher et al. 2001). Collaboration with groups such as Cal Trout on invasive predator control could be important for connectivity and persistence of native aquatic species both at this location and within the larger scale planning area.

Experts proposed that any fencing designed to keep humans out of Temecula Creek extend as far upstream as possibe to reduce all known potential access points, as human presence and associated poaching, hunting, fishing, dogs, trampling, and waste, which are all a major concern for focal small animals. Fencing designs that incorporate fine or solid mesh fabric on the lower 4 feet of the fence would preclude small animals from inadvertently entering human development. In addition, the feasibility of constructing low barrier fencing along the border of the Temecula Creek Inn Golf Course could be explored to prevent turtles and toads from entering the golf course. Such low fencing would not preclude passage by larger wildlife species.

Wildlife Culvert

The proposed wildlife culvert poses design challenges for small animals, as such structures can be cold, dark, dry, and musty. Natural soils, grates and skylights, and designs that allow some passage of water can make underpasses more like their surrounding environment. Grates and skylights installed in the median and on road shoulders of the highway could provide light and air flow to the crossing (Langton and Clevenger 2020). These grate and skylight structures could be slightly elevated above grade so as not to create pitfall traps or drains into the culvert.

Cover, such as rocks, logs, or cinder blocks, placed throughout the interior of the culvert have been shown to provide hiding places for smaller species (Tracey et al. 2014). Cover structures are most effective if less than 2 feet in height. Incorporating ledges into the design of the culvert could provide elevated movement through the passage and a dry crossing for small animals when the culvert is inundated. Small wildlife ledges are typically 8 to 12 inches wide and approximately 4 feet in height and include ramps at the culvert entrances to facilitate access (Langton and Clevenger 2020, Clevenger and Huijser 2011).

The current culvert design includes a concrete channel for water flow. Experts proposed that the culvert be designed instead with a natural soil drainage channel that flows during rainfall events. High velocity flows could be directed to the existing adjacent culvert structure to reduce excessive flows in the wildlife culvert.

Sound absorbing materials could be added to the interior walls of the culvert to increase sound attenuation and reduce vibration, both of which could deter small animal use of the structure, particularly if a metal culvert is selected as the design.

Small animals can be funneled to the crossing with short barrier fencing that extends from the wing walls. The barrier fencing should be at least 2 feet high, and composed of solid or fine mesh material with overhangs, as necessary, to prevent climbing (Langton and Clevenger 2020).

Vegetated Overcrossing

For the vegetated overcrossing, tall walls or earthen berms over 6 feet in height are needed along the edge of the structure to provide a visual barrier and to shield small wildlife from light and noise from the highway. Tall barrier walls that incorporate the ledges discussed for the wildlife culvert could provide elevated passage for small animals.

Vegetation on the overpass and approach area would best include both dense and sparse shrub plantings to accommodate both open- and closed-habitat specialists as well as generalists. Dense habitat for closed-habitat specialists could consist of near continuous or overlapping shrub cover. Sparse habitat for open-habitat specialists could consist of small areas of bare ground, large patches of native forbs and grasses, and scattered shrubs. Other types of cover, such as logs or brush piles, could also be included in both the approach area and on the crossing.

In addition, small seasonal ponds could be created in the approach area on either side of the crossing for the spadefoot toad. USGS has had success in creating seasonal ponds for spadefoot toad, fed by rainwater, in Orange County, California (Baumberger et al. 2020). Experts felt that such seasonal pools would benefit many other species groups as well.

Any barrier fencing incorporated into the design for the approach area to funnel wildlife to the crossing would best include design considerations for small animals, as described above for the wildlife culvert.

Areas for Additonal study

- Conduct surveys to identify small animal species present or near crossing sites to validate or modify focal species.
- Test methods and feasibility for the establishment of ephemeral pools in the approach area for the proposed vegetated overcrossing.
- Deveop and incorporate design considerations for arroyo chub (*Gila orcuttii*) and southern steelhead (*Oncorhynchus mykiss*) to develop and incorporate into the planning for the Temecula Creek Bridge crossing.

3.3 Bats

Importance of Wildlife Crossings for Bats

It is a common misconception that because they are capable of flight, bats are not negatively affected by road development. However, the development and expansion of roads often results in the destruction of roosting habitat (e.g., mature trees, rock outcrops) and foraging habitat for bats. While transportation structures (e.g., bridges and culverts) in some cases can provide replacement roosting habitat, particularly when these structures span or are situated along natural flyways, these structures cannot provide suitable roosting habitat for all bat species that are affected by loss of roosting habitat related to road construction. In addition, there is increasing evidence that roads may alter bat movements related to both seasonal roost switching and nightly foraging behavior, and that bat populations may in fact be vulnerable to the barrier effects of roads. For instance, bats can be negatively affected by roadway noise (e.g., Schaub et al. 2018; Bennett and Zurcher 2013), lighting along roadways can disrupt commuting corridors for bats (e.g., Stone et al. 2009; Seewagen and Adams 2021), and bats can be killed when crossing roads, particularly when these roads are located at natural crossing points between areas of good habitat (e.g., Russell et al. 2009; Berthinussen and Altringham 2012; Medinas et al. 2013). Given the low reproductive turnover of bat species coupled with collision mortality estimates of up to 5 percent for local populations as calculated in some studies (e.g., Russell et al. 2009; Altringham and Kerth 2016), roadway mortality could present yet another conservation risk for bat species that are already declining from other factors. Therefore, when considering how roadways affect bats and how wildlife crossings can be created or modified to minimize those impacts, it is important to consider both the creation and/or enhancement of roosting habitat as well as methods of facilitating movement across roadways that will minimize the potential for barrier effects and collision mortality.

<u>Focal Bat Species</u>

No bat species were included in the list of focal species in the original SC Wildlands report, so experts added the following focal bat species for wildlife crossing consideration, based on their observed population declines in the region and their potential occurrence in the proposed crossing areas.

The pallid bat (*Antrozous pallidus*) is a large-bodied and broad-winged species that consumes ground-dwelling arthropods in addition to flying prey (e.g., moths). This species roosts in various types of crevices and cavities, and in southern California is associated with open scrub and grassland habitats, oak savannah, and relatively open sycamore riparian and oak riparian habitats. There is evidence that bat species that hunt by listening passively for prey-produced sounds, as pallid bats do, often avoid approaching or foraging near highways with high levels of noise (e.g., Bennett and Zurcher 2013), and it is hypothesized that roadway noise could mask the sounds that these bats rely on for hunting because some of these sounds are within the same range as roadway noise (California Department of Transportation 2016).

The big brown bat (*Eptesicus fuscus*) is a relatively large-bodied and broad-winged species that primarily consumes flying beetles, but also consumes a variety of other flying insects. Although it is considered a forest-dwelling species in other parts of its range, in southern California the big brown bat is often associated with a variety of habitats including scrub, chaparral, and riparian habitats, and this species can be found foraging over a variety of vegetation types, at ponds, and along riparian corridors. While their propensity to roost in human-made structures may suggest that this species can adapt to the anthropogenic environment, colonies have been disturbed or illegally exterminated and these structures may serve as habitat sinks. Recent studies in North America (Seewagen and Adams 2021) and in other *Eptesicus* species in Europe (e.g., Voigt et al. 2018) suggest that while big brown bats are known to opportunistically forage around streetlights, this species may be highly sensitive to lighting along commuting corridors. Consequently, roadway lighting may create additional barriers and fragment habitat for a species that is already sensitive to the effects of habitat fragmentation.

Both species are well adapted to roosting in human-made structures, which can put them at risk for vandalism or illegal extermination. However, this behavior also creates opportunities for creation or enhancement of roosting habitat for these species at bridges or culverts, where they can be better protected from these threats. Bat movement on the landscape can be broadly categorized as nightly movements (e.g., commuting between roost sites and foraging areas) and seasonal movements (e.g., autumn/spring dispersal or summer aggregation at maternity sites for colonial species). Although research on the effects of roadways on bats has predominantly been conducted in Europe and limited data are available specific to the focal bat species, the experts were able to hypothesize how the focal bat species might cross roadways based upon their foraging ecology and flight behavior to evaluate whether wildlife crossings could be beneficial to improve movement across Interstate 15. Available data on the barrier effect of roads indicate that this effect is likely species-specific and is primarily influenced by factors including wing shape and foraging ecology (Altringham and Kerth 2016; Møller et al. 2016). While the specific flight and foraging behavior of pallid bats compared to big brown bats is distinct, both focal bat species have broad wings and relatively slow flight, and studies indicate species with these attributes are more likely to be affected by the barrier effects of roads (Norberg and Rayner 1987). Encouragingly, in addition to using culverts and bridges beneath roadways for day and night roosting, both species have also been observed using large culverts to cross beneath freeways (J. Carpenter, unpublished data, D. Stokes pers. comm., S. Remington pers. comm.).

Summary of Probability of Use of Crossings by Focal Bat Species

When assessing the probability of use of the three crossing types being analyzed, the **Temecula Creek Bridge** has the highest probability of use for both roosting and movement/connectivity, while the **wildlife culvert** has a moderate-to-high probability of use for roosting and a low-to-moderate probability of use for movement depending on the species. The use of wildlife overpasses by bats has not been well studied; however, some recent data from Europe suggest that vegetated wildlife crossings have a lot of potential to facilitate bat movement (e.g., Claireau et al. 2021; Martinez-Medina et al. 2022); therefore, if the best available knowledge to encourage use of this type of structure for bats is implemented, the **vegetated overcrossing** has a moderate to high potential for use as a flyway for bats to safely cross the highway. With regard to use of the vegetated overcrossing for roosting, because known successful roost designs are placed on the undersides of bridges, it would be generally incompatible with a vegetated overcrossing structure spanning a busy freeway. Thus, specific strategies for the creation of roosting habitat for the overcrossing were not pursued by the experts at this time.

It should be noted that efforts to make the wildlife crossings suitable for use by the focal bat species will likely also benefit other bat species, but the experts focused on identifying specific elements that will optimize the characteristics of each type of crossing structure for the focal bat species.

Species	**Temecula Creek Bridge**	**Wildlife Culvert**	**Vegetated Overcrossing**
Usage Type: Connectivity/Movement			
Pallid bat	H	L	M
Big brown bat	H	M	M
Usage Type: Roosting			
Pallid bat	M	M	-
Big brown bat	H	H	-

Table 5: Probability of use of each crossing type by focal bat species and usage type: High (H), Moderate (M), Low (L). Crossing type that is currently not being pursued for use is indicated using "-"; however, it should be noted that this categorization is specific to this crossing location and to current data limitations for the successful creation of freestanding roosting habitat. Current research is underway, and it is expected that these issues will be resolved so that this option can be pursued at other overcrossing locations. Scientific names are in Table 1.

Crossing Design Features to Encourage Use by Bats

In assessing design elements for each crossing and approach area to promote roosting by bats, experts considered species needs related to the design, potential placement locations, and access for roosting habitat. Both day- and night-roosting habitat features were evaluated. With regards to movement, experts considered design elements for each crossing and approach based upon knowledge of the focal species' foraging ecology and flight behavior.

Temecula Creek Bridge

Crevice features suitable for use by day-roosting bats can be added to the Temecula Creek Bridge by bolting on structures made from concrete or a material with similar thermal properties. Placement of these structures in different areas of the bridge to provide bats with more thermal options was proposed by the experts and is consistent with studies showing that varied placement locations increase the probability of use by bats across seasons (e.g., Carpenter 2017). While these crevices could also be used by night-roosting bats, including other types of roost structures that create cavity spaces to encourage night roosting was proposed. The pallid bat in particular favors cavity spaces for night roosting. The experts agreed that it is possible to create structures that incorporate both types of habitats in one modular design, and that the roosting surfaces of all roost structures would be most effective if they have a roughened texture. The design and placement of these roost structures would best be overseen by a bat biologist with documented experience and success designing and implementing bat roosts on bridge and culvert structures.

The experts discussed various attributes that would encourage the movement of bats beneath the Temecula Creek Bridge as bats move along the drainage course, rather than over the bridge where there is risk for collision mortality. The experts expressed concerns that the existing area beneath the bridge might be too cluttered and lack clear flyways to promote bat movement beneath the bridge. The experts considered thinning of some of the shrubs and trees along the approach to the bridge and beneath the bridge itself to create flyways that allow movement of bats below the height of the bridge; however, the trimming and removal of vegetation could conflict with needs of other species that require more vegetative cover and may require maintenance. Ultimately, this strategy was not proposed due to those factors and the likelihood that the removal of invasive vegetation will create some flyways. In addition, at least two species of bats, one of which is the focal species big brown bat, were documented crossing beneath the bridge during recent mist netting surveys (Carpenter pers. comm.), suggesting that the vegetation is not currently inhibiting movement beneath the bridge. It is possible that sound walls or flight elevating structures installed to force birds to fly across the bridge at a higher level and not at vehicle height could also be used by bats depending upon the design of those structures. Fencing and other measures that are effective in keeping humans out of Temecula Creek are proposed by the experts because the presence of humans is expected to cause disturbance that would reduce the potential for bats to both move through and roost at the bridge. Traffic noise could also be a deterrent, particularly for pallid bat, so measures to reduce vehicle noise in the vicinity of this crossing could benefit bats and aid in their use of the crossing. Finally, native plant restoration at the Temecula Creek Bridge crossing is expected to increase the quality of this area for foraging for the focal bat species by attracting a wider diversity and abundance of native insect prey.

Wildlife Culvert

To encourage use of the wildlife culvert by roosting bats, the experts agreed that using a design that creates higher structural complexity within the culvert structure would provide more thermal variation and options for roosting. This complexity could include adding one or more short side tunnels along the length of the culvert and/or the creation of domed recesses in the ceiling to trap heat and create areas sheltered from wind and ambient light. These recessed areas are particularly important if other taxa require more airflow or ambient light in the culvert because features installed to provide those conditions, such as grates or skylights, could be expected to lower the probability of use by bats unless the proposed enhancements described here are implemented. For example, siting grates or skylights far away from bat roosting habitat would minimize their deterrence on bat use. Providing roosting habitat in recessed areas in the ceiling, which are effective in creating areas of darkness and trapping heat, is proposed if skylights or grates are used to increase light and airflow. The experts agreed that the creation of multiple size options for the ceiling recesses would be beneficial and suggested 2 feet by 2 feet wide (or 4 feet square) and at least 2 feet deep would likely be suitable. Although bats often night-roost along the walls of concrete culverts, ceiling recesses would provide even higher quality night-roosting habitat. The experts noted that all roosting surfaces would be most effective if they have a roughened texture. Crevices that provide day-roosting habitat can be built into the culvert structure or bolted on as proposed for the Temecula Creek Bridge. These roost structures can be incorporated into recessed areas, along the tops of the culvert walls, or on the culvert headwalls.

The proposed 15 x 15-foot culvert is expected to be sufficient for use by the focal bat species, particularly given that most of the experts have observed one or both focal bat species roosting and/or passing through concrete culverts of this size. Maintenance of the culvert entrances to keep them open and free of obstruction from vegetation would allow bats to readily access the culvert interior for roosting and/or movement.

If a metal culvert design is selected, roosting habitat can still be provided for bats by installing vertical pipes or other recessed areas in the ceiling to trap heat. Given that some research indicates that corrugated metal culverts produce patterns of echoes to which some bats are averse (Simmons et al. 2020), if a metal culvert design is chosen, sound-absorbing materials could be added to the interior walls of the culvert to reduce these echoes and increase the probability of use by bats. Spraying the culvert interior with gunite or a similar material is likely to provide both sound-dampening effects and a textured surface that will also increase the probability that bats will roost within the metal culvert.

Vegetated Overcrossing

The experts agreed that the most important attribute to encourage bat use of the overcrossing was to place trees and/or large shrubs along the edges of the bridge to create an “edge” habitat for the bats to follow, thereby creating a flyway that will direct the movement of bats across the overcrossing. The placement of trees and large shrubs along the edges of the structure would also serve to shield bats and other wildlife on top of the overcrossing from noise and light along the roadway below.

Roosting habitat for bats is often created successfully on the undersides of bridge structures, where it is possible to take advantage of shelter from the elements as well as the structure’s thermal capacity to maintain stable roost temperatures. However, in the case of an overcrossing, the experts were concerned about potential for increased risk of collision mortality for bats emerging from roosts on the underside of the overcrossing at or near active traffic lanes. Some possibilities that were discussed to minimize or eliminate this risk included the creation of a pier wall between the roadway and the abutment where bats could roost away from the active lanes; this physical barrier would discourage exit toward the roadway while also serving as a barrier for disturbance (e.g., noise and light) from the roadway. The experts also discussed potential placement options for roosting habitat away from the roadway (e.g., on top of the overcrossing or near its approaches) to avoid this risk; however, these structures would need to be free-standing, and this type of roost structure is not typically successful in southern California due to the difficulty of providing adequate thermal stability required for occupancy by bats. Given that any roosting habitat included in the design of the overcrossing would be experimental, coupled with the fact that large rock outcrops with suitable day-roosting habitat are present in close proximity to the proposed overcrossing location, the experts ultimately decided that it would be best to focus funding and resources on adding elements to the overcrossing that are effective in creating a pathway across the road until more data are collected with regard to attributes of successful free-standing roosts in California. It is important to note that the suggestion not to include roosting habitat on the overcrossing is very specific to this Linkage site, and that at other wildlife crossings, it may be possible or even necessary to include roosting habitat for bats on the overcrossing structure.

Crossing Approach Features to Encourage Use by Bats

For all the crossing structures, experts agreed that creating or enhancing flyways to guide bats toward the structure was very important for both roosting and for movement across or through that structure. At the Temecula Creek Bridge and the wildlife culvert, the experts propose creating or enhancing approach flyways by trimming vegetation or otherwise decreasing cover enough to create pathways for bats to fly toward and beneath the bridge or into the culvert. On the other hand, at the vegetated overcrossing, the experts proposed planting large shrubs or trees along the edges of the overcrossing to provide a natural flight path for bats that also provides cover. The experts also proposed creating continuity between the rows of trees and large shrubs along the edges of the overcrossing and rows of trees and shrubs on each side of the crossing to form a flyway that will funnel bats across the overcrossing. Consistent with suggestions for other taxa, the experts strongly suggest implementing measures to reduce or eliminate light and trespass from adjacent highway and residential uses to increase the probability that bats will approach and use the crossings.

Areas for Additional Study

- Perform acoustic and mist-netting studies to gather data on existing use of the proposed crossing sites by focal bat species.
- Perform acoustic studies to identify where, if anywhere, bats are crossing I-15 as was done for European crossing studies.
- Monitor roadkills of bats in the area now and in future to evaluate success of crossings in reducing collision mortality.
- Monitor added roost features to evaluate effectiveness/use by bats for roosting.
- Monitor added crossing attributes to evaluate effectiveness/use by bats for movement/connectivity.

3.4 Birds

Importance of Wildlife Crossings for Birds

While it is known that birds can fly over highways, reports to the California Roadkill Observation System (2022) identify that, between 2009 and 2014, approximately 7,800 bird mortalities were reported, representing 234 species. While this is not a report of all bird mortalities, these reported bird mortalities made up 12% of all reports across all taxa. In addition, research conducted for the wrentit (*Chamaea fasciata*) in the Santa Monica Mountains in southern California has shown that highways have altered genetic connectivity for this species (Delaney et al. 2010).

When considering how barriers might affect movement of birds in the landscape, it is important to consider both movement type and migration status. Movement can either be a one-time dispersal event when a bird leaves its natal area or can be associated with movement within a territory or home range. Movement within a territory/home range may require repeated crossings if the territory spans the roadway. In terms of migratory status, migratory species are typically less impacted by highways than resident species. Migratory species can travel long distances and at higher altitudes as they seek suitable habitat, making them much less susceptible to roadway impacts. This is in contrast to resident birds, which tend to stay close to the ground and typically don't make long flight journeys.

Focal Bird Species

In addition to the focal species outlined in the original SC Wildlands report, experts added the following additional focal bird species for wildlife crossing consideration: California gnatcatcher, cactus wren, wrentit, California thrasher (*Toxostoma redivivum*), and greater roadrunner (*Geococcyx californianus*). In addition to golden eagle (*Aquila chrysaetos*), raptors (hawks and owls) were added as a group of species that need focus in the Linkage, not related to connectivity across the highway but related to reducing collision mortality associated with hunting for prey in the roadway.

Focal bird species were arranged into the following groupings for purposes of planning: 1) migratory and woodland species, 2) resident species, 3) ground-dwelling species, and 4) raptors (Table 6). Least Bell's vireo and yellow warbler (*Setophaga petechia*) are two migratory species that require connected riparian habitat, whereas the oak titmouse (*Baeolophus inornatus*) requires oak and riparian woodland. Resident species include California gnatcatcher and cactus wren, which are both coastal sage scrub-dependent species whose habitat is highly fragmented across the region, as well as wrentit and California thrasher which are both chaparral dependent species. Ground dwelling focal birds include California quail (*Callipepla californica*) and greater roadrunner, which are both sensitive to habitat fragmentation. The raptor group includes the golden eagle, which requires large contiguous blocks of intact habitat as well as barn owl (*Tyto alba*), great horned owl (*Bubo virginianus*), red-tailed hawk (*Buteo jamaicensis*), red-shouldered hawk (*B. lineatus*), western screech-owl (*Megascops kennicottii*), and other raptors that are highly susceptible to collision mortality.

Common Name	**Migratory/ Woodland**	**Resident**	**Ground Dwellers**	**Raptors**
California gnatcatcher		X		
California quail				
California thrasher			X	
Cactus wren		X		
Golden eagle				X
Greater roadrunner			X	
Hawks/Owls				X
Least Bell's vireo	X			
Oak titmouse	X			
Wrentit		X		
Yellow warbler	X			

Table 6: Focal Bird Species and Categories. Scientific names are in Table 1.

Experts identified that resident and ground dwelling birds likely require wildlife crossings to ensure connectivity across the highway. As for the California gnatcatcher, it has been documented that they are highly genetically connected across the region (Vandergast et al. 2019); however, we do not know how freeways affect gnatcatcher movement and distribution. To the extent that they do move across a highway, some sort of crossing would likely benefit California gnatcatchers.

In terms of reducing mortality due to roadkill, all species except raptors would benefit from a crossing. Wildlife crossings are not going to solve the issue of mortality of raptors on highways.

Summary of Probability of Use of Crossings by Focal Birds

Least Bell's vireo, oak titmouse, and yellow warbler are expected to have a high likelihood of use of the **Temecula Creek Bridge** crossing, as they are found on both sides of I-15. Quail and greater roadrunner are also likely to use the Temecula Creek Bridge to cross the highway. Experts felt that that there would only be moderate use of the Temecula Creek bridge by California thrasher, California gnatcatcher, and wrentit, as these species require more continuous low shrub cover than currently exists or can be supported in this location. Additionally, California thrasher is more associated with chaparral than the riparian habitat that is found under the bridge, although that does not entirely rule out that thrashers might move under the bridge. As for the cactus wren, experts were concerned about the lack of cactus elements in the surrounding environment for both Temecula Creek Bridge and the vegetated overcrossing location. While cactus wrens do not require cactus in their movement corridors, it is expected that there is only a moderate likelihood that cactus wrens would use Temecula Creek Bridge crossing.

When assessing probability of use of the three crossing types analyzed, the proposed **wildlife culvert** has the lowest probability of use, for reasons relating to lack of light and visibility through the crossing, as well as a lack of habitat needed to promote movement through the culvert structure.

Regarding the **vegetated overcrossing**, the migratory and woodland species would be unlikely to use the crossing due to lack of suitable continuous woodland habitat on or in the vicinity of the proposed crossing. Raptors would also be unlikely to use the crossing for passage across the highway but might use the overcrossing for hunting. There is a high likelihood of use of the vegetated overcrossing by resident and ground-dwelling bird species.

Species	Temecula Creek Bridge	Wildlife Culvert	Vegetated Overcrossing
California gnatcatcher	H	-	H
Cactus wren	M	-	H
California quail	H	-	H
California thrasher	M	-	H
Golden eagle	-	-	-
Greater roadrunner	H	-	H
Hawks/Owls	-	-	-
Least Bell's vireo	H	-	-
Oak titmouse	H	-	-
Wrentit	M	-	H
Yellow warbler	H	-	-

Table 7: Probability of Use of Crossing Type by Focal Birds: High (H), Moderate (M), Low (L). Crossing type that is improbable for use is indicated using "-".

Crossing Design Features to Encourage Use by Birds

The Temecula Creek Bridge and vegetated overcrossing structure were evaluated by experts in terms of specific design features such as substrate and vegetation composition and height that would be needed to encourage use by the focal species.

Temecula Creek Bridge

For the Temecula Creek bridge, attributes considered important for migratory and woodland bird species include natural substrate, tree and shrub cover, and light to support tree and shrub cover under the bridge. Maintaining natural substrate provides the soil, nutrient, and hydrologic conditions required by riparian vegetation. Native tree and shrub cover including various willow species, mulefat, cottonwoods, and coast live oaks are important to encourage focal birds to move towards and under the bridge structure. Maintaining the opening between bridge spans will be critical to ensuring the persistence of tree canopy under the bridge structure.

For ground dwelling bird species, shrub cover in the approach and crossing area would best be arranged such that there are openings/pathways between shrubs to allow movement. Pathways for ground dwelling birds, like the small animal group, would best avoid inundated areas. Elevated passages under the structure that include native substrate could function for ground dwelling birds during inundation events.

To avoid roadkill of birds flying above Temecula Creek Bridge, experts proposed the placement of “flight elevators” (fences or other tall structures) on the edges of the bridge to force birds to fly under or up and over the highway bridge and traffic. Flight elevators could also serve as noise and light barriers, although avoiding walls of clear glass would reduce the risk of bird collision. Adding flight elevators on the bridge structure and maintaining adjacent existing tall trees should help encourage birds to fly above traffic or under the bridge (Kociolek et al. 2015). Additionally, flight elevators could be designed to artfully represent the focal species. Experts suggested that bridge signage identify the waterway and its role as a wildlife corridor (e.g., Temecula Creek Bridge Wildlife Crossing).

Measures focused on preventing human trespass, controlling invasive plants, and abating noise and light from adjacent highway and residential uses will improve focal bird species use of the crossing. Any future reduction of the opening between bridge spans (e.g., highway widening) would cut down on light that currently allows the growth of trees and shrubs under the bridge structure and would negatively impact focal bird species use of the crossing. The vegetation cover currently under the bridge and between the spans is very important for birds in this crossing.

Vegetated Overcrossing

The vegetated overcrossing structure is anticipated to be used primarily by terrestrial species. Important attributes for the overcrossing include a natural substrate and vegetation cover composed primarily of coastal sage scrub and chaparral plant species. Taller shrubs or trees are proposed for placement in the middle of the structure to attract birds to cross. Taller shrubs and trees might also be considered along the edges to shield noise and light. For ground dwelling bird species, experts thought it important to arrange vegetation such that it creates paths or tunnels that provide cover but also accommodate movement.

The substrate of the overcrossing is a concern to experts, as they were unsure if the identified 4 feet of soil proposed is deep enough to support the level of shrub cover needed to promote wildlife use over the long term. Perhaps in locations where taller vegetation is required, such as along the edges of bridge where light/noise shield is desired, raised planters or deeper soil pockets can be installed to support trees and taller shrubs.

Given the limited substrate on the overcrossing, long term maintenance of the vegetation on the overcrossing was also a concern raised by the experts. Periodic plant replacement or supplemental irrigation may be required to maintain the desired habitat conditions required to promote wildlife use of the overcrossing structure. Maintaining sufficient vegetative cover on the overcrossing for prey species could deter use of the overpass as a hunting area for raptors.

Crossing Approach Features to Encourage Use by Birds

For both the Temecula Creek Bridge and vegetated overcrossing, experts proposed that the habitat in the crossing match as closely as possible with the habitat in the approach areas, with some modifications to attract habitat specialists. For example, experts proposed that cactus be added in the approach area for both the vegetated overcrossing and Temecula Creek Bridge to attract the cactus wren to use the crossing structure. For the Temecula Creek Bridge crossing, planners would best look for opportunities to add cactus in upland areas adjacent to the crossing, as cactus likely cannot be supported within the riparian area.

For the vegetated overcrossing, experts proposed planting taller vegetation on both ends of the overcrossing and in the middle of the crossing to both attract and elevate birds as they fly across the structure.

Water features were proposed for placement in the approach area for the vegetated overcrossing to both attract and create regular travel routes to the crossing for quail and roadrunner. Otherwise, trails could be created for ground dwelling birds to lead them to the crossing.

In terms of identifying measures to reduce raptor mortality on the highway, efforts would best focus on reducing raptor prey in the highway. Existing exclusion fencing may already be reducing raptor prey in the highway, but possible upgrades to the existing fencing, such as fine mesh barrier fencing on the lower 2-4 feet of the fence, may further reduce small animal roadkill and associated raptor hunting in the highway. Additionally, opportunities to reduce roadside raptor perches could also be evaluated, particularly perches that are at road level. Raptor hunting on the vegetated overpass could be discouraged by providing and maintaining adequate cover for prey species.

Areas for Additional Study

- Examine the level of genetic fragmentation of focal birds on either side of highway and monitor over time.
- Monitor roadkill of birds now and in future to evaluate effectiveness of crossings.

3.5 Plants and Invertebrates

Importance of Wildlife Crossings for Plants and Invertebrates

Plants and invertebrates are foundational elements of terrestrial ecosystems and a crucial consideration for the design of wildlife crossings for I-15. Plants and invertebrates are food resources for other taxa, and many are species of concern themselves. Plants and insects coevolved as pollinators and hosts, so much so that considering these taxa independently is nearly impossible.

Plants provide habitat structure. The density and stature of plants in the vegetated overcrossing and Temecula Creek will determine in large part which animal taxa will be most inclined to use them. Different planting and management schemes will determine if the habitat in the crossing is open or closed, how much cover is present to obstruct anthropogenic features and provide hiding spots for animals, and which food resources are available in what quantity.

Plants and invertebrates are key food resources for and gain benefits from animals. For example, synzoochory is the “intentional” dispersal of seeds by seed-caching animals such as scatter-hoarding mammals and birds that create caches. In this mutualism, the plant provides food and the animal acts as both seed predator and seed disperser. The same is true for endozoochory, except seeds are transported in the animal's gut unintentionally after being consumed as food. Coyotes and bobcats which consume fruit and acorns are prime examples of endozoochory (Luke et al. 2004).

Finally, plants and arthropods represent some of the most and least mobile species that the proposed crossings could accommodate, and addressing their connectivity needs involves additional considerations to the crossing design, ultimately serving a broader suite of species.

Focal Plant and Invertebrate Species

The focal plant and invertebrate species can be thought of in three general categories based on their dispersal mechanisms: plants (passive dispersal via wind, gravity, and animals), walking insects, and flying insects/butterflies (Table 8). Focal species share the need to disperse for various reasons, including genetic mixing, mating, migration and shifting ranges due to climate change.

Focal plant species utilize several modes of passive dispersal, including abiotic forces like gravity, wind, and water as well as transport via animals. Genetic mixing is (in general) achieved via pollination by insect pollinators, wind, and other means. Pollination keeps the species genetically viable but does not allow for range shifts. Walking insects (Jerusalem cricket [*Ammopelmatus* spp.] and Timema walking stick [*Timema podura*]) are likely the most dispersal-limited focal species under consideration (taking endozoochory and synzoochory into account). Some authors report that walking sticks may only move a few feet within their lifetime (Sandoval 2000) while informal observations by local experts suggest walking sticks may have somewhat greater dispersal ability and may move dozens of feet at a time in some scenarios (Strahm et al. 2022).

Jerusalem crickets are ground dwelling insects that disperse via walking (Vandergast et al. 2009). Local experts estimate movement distances of approximately 50 feet a day (Strahm et al. 2022). Jerusalem crickets require connectivity to find mates and forage (Strahm et al. 2022). Only the Mahogany Jerusalem cricket is known to exist on both sides of the I-15, but there are likely others (Strahm et al. 2022). Jerusalem crickets are a favored prey item for bats (Strahm et al. 2022).

Butterflies are prone to collisions with vehicles (Skórka et al. 2013). For some this is a function of their flight height and behavior. Host and nectar plants on the edge of roads may gain a benefit from water sheeting off the asphalt and collecting in brow ditches (Strahm et al. 2022), which draws butterflies to the edges of roads, increasing the chances of collision. Even species that fly relatively high (e.g., migrating monarch [*Danaus plexippus*] and painted ladies [*Vanessa cardui*]) are subject to collisions, and can benefit from a crossing. The degree of benefit to any one species of butterfly may vary by species and the location of the crossing.

Common name	**Dispersal**
Chaparral yucca	Gravity
	Pollination
	Synzoochory
Engelmann oak	Gravity
	Wind
	Synzoochory
	Endozoochory
Rainbow manzanita	Gravity
	Pollination
	Endozoochory
	Synzoochory
California sister	Flying (high)
Comstock's fritillary	Flying (low)
Jerusalem cricket	Walking
Monarch butterfly	Flying (high)
Pale swallowtail	Flying (high)
Timema walking stick	Walking

Table 8: Focal plant and invertebrate species and dispersal mechanisms. Scientific names are in Table 1.

Summary of Probability of Use of Crossings by Focal Plants and Invertebrates

Experts agreed that most plant and invertebrate focal species will do best with a **vegetated overcrossing** (Table 9). The **wildlife culvert**, if it incorporates a natural substrate, is only appropriate for the Jerusalem cricket because it does not require host plants and is primarily nocturnal. The riparian woodland at the **Temecula Creek Bridge** is inappropriate for most of our focal species, although the upland habitats nearby may be suitable. Upland plants reliant on endozoochory and synzoochory may benefit from out-plantings near but not within the Temecula Creek crossing.

Species	Temecula Creek Bridge	Wildlife Culvert	Vegetated Overcrossing
California sister	-	-	H
Chaparral yucca	-	-	H
Comstock's fritillary	-	-	H
Engelmann oak	M	-	H
Jerusalem cricket	L	H	H
Monarch butterfly	-	-	H
Pale swallowtail	-	-	H
Rainbow manzanita	M	-	H
Timema walking stick	-	-	H

Table 9: Probability of use of each crossing type by plant and invertebrate species: High (H), Moderate (M), Low (L). Crossing type that is improbable for use is indicated using "-"

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Crossing Design Features for Plants and Invertebrates

The following crossing design features proposed for plants and invertebrates are geared toward a vegetated overcrossing as this is the best option for most of the focal species. The vegetation community on both sides of the highway is chamise chaparral at the crossing location, with mixed scrub oak nearby. This, along with the type (and depth) of substrate on the crossing, will exert ultimate control over which plants flourish on the span**.**

The experts stated that transitioning smoothly from habitat leading to the crossing across the span in either direction would prevent abrupt changes in the configuration of habitat that can divert butterflies. This includes the composition, vertical profile, and density of shrubs, the amount of bare ground and rock, etc.

If Timema walking sticks are found in the area, experts suggest creating strips of host plant shrubs down the sides of the overcrossing to act as a "highway" while leaving an open pathway for other taxa in the center. The Timema highway does not need to be dense, but it does need to be relatively contiguous, with some host plants overlapping but not at a density significantly different from surrounding vegetation. Local Timema walking sticks are likely to prefer chamise (*Adenostoma fasciculatum*) over *Ceanothus spp.* as host plants, but this should be confirmed. The Timema highway can be located on either one or both sides of the crossing, provided there are scattered host plants to make a pathway to the crossing.

It is anticipated that the overcrossing cannot support the soil depth necessary for Engelmann oaks (*Quercus engelmannii*) or Rainbow manzanita (*Arctostaphylos rainbowensis*), making both species better suited for planting in the crossing approach area. If pockets of deeper soil can be incorporated in the crossing, it may be possible to incorporate scattered Engelmann oaks, but the depth still may be limiting and result in smaller stature trees. Like Rainbow manzanita and Engelmann oak, chaparral yucca (*Hesperoyucca whipplei*) can be used as part of an upland planting palette in the approach to the overcrossing, and likely will be able to take hold on the span of the crossing as well, at a spacing of not more than 60 feet apart to encourage its pollinator, the chaparral yucca moth (*Tegeticula maculata*), to use the crossing. California buckwheat (*Eriogonum fasciculatum*) is another shrub species that likely will do well on the overcrossing and is attractive to many butterfly species.

Experts proposed out-planting all appropriate butterfly host plants up to and across the crossing structure if soil substrate type and depth allow. Host plants for focal species include:

- Chamise (*Adenostoma fascicularis*)
- Violets (*Viola pedunculata*)
- Oaks (*Quercus* species with large leaves)
- Hollyleaf cherry (*Prunus ilicifolia*), chaparral whitethorn (*Ceanothus leucodermis*) or other members of the Rosaceaeae, Rhamnaceae, and Betulaceae families
- Milkweed (*Asclepias*) species including *A. fascicularis*, *A. erosa*, *A. eriocarpa*

A diverse palette of nectar plants will draw focal butterflies and other pollinators to and over the crossing. California buckwheat as well as native thistles and wallflowers are especially attractive to many butterfly species (Marschalek et al. 2008). Providing flowers year-round would be most effective at attracting focal butterflies and other pollinators.

Plants on the span of the overcrossing will not have access to ground water and may need supplemental water during times of drought. A permanent irrigation system may be required to both establish and maintain plantings on the overcrossing.

Wood and rock piles are attractive cover for Jerusalem crickets and many other species. These would best be positioned within 50 feet (or less) of one another on the overcrossing, while not creating obstacles for other taxa.

A healthy soil community is essential for soil retention and nutrient cycling. In addition, many plant species rely on mutualisms with bacteria and fungi to absorb those nutrients. The top layer of soil (called the “A horizon”) is generally the richest in soil flora (Hartemink et al. 2020). Any topsoil displaced during wildlife crossing construction could be salvaged and re-applied to the crossing structure and approach areas after construction. Using native topsoil will encourage the establishment of beneficial soil biota, including, but not limited to, mycorrhizae.

Jerusalem crickets drum their abdomens on the ground to find mates (Vandergast 2009). Each species has its own unique song. Because vibration and noise from the highway could drown out this drumming, noise barriers on the edges of the vegetated overcrossing may benefit the species.

It is unclear if a windbreak and/or light shield at the edges of the overcrossing is necessary for focal arthropods. Blocking direct light at night is important to the recruitment of chaparral yucca moths. Artificial light at night is detrimental to nocturnal insects in general (Wilson et al. 2021). The extent to which wind and vortices from vehicles influence butterfly flight should be investigated (Damschen et al. 2014).

To maximize their effectiveness, wind or light barriers at the edge of the crossing structure would:

1. Not be climbable by Timema walking sticks;
2. Not block daylight from vegetation and arthropods on the overcrossing; and
3. Obscure direct artificial light sources at night.

Crossing Approach Features to Promote Use by Plants and Invertebrates

It may not be necessary to place Rainbow manzanita or Engelmann oak directly on or in the vegetated overcrossing to encourage dispersal. If the crossing is designed to facilitate the dispersal of animals that transport acorns (e.g., birds, squirrels, coyotes, and other mammals), adequate dispersal may occur. Rainbow manzanita and Engelmann oak can be considered for placement in the approach areas for all crossing types, if conditions are appropriate, to increase the chance that animals will move their propagules from one side to the other.

The host and nectar plants identified for establishment on the overcrossing could also be considered for planting in the overcrossing approach areas if conditions are appropriate. Violets and some milkweed species may not do well in a chamise chaparral environment in the approach areas, but it may be worth planting a small number of test plantings in the approach area in advance of crossing construction to confirm this.

Areas for Additional Study

- Conduct baseline arthropod studies to document the presence of focal invertebrates and their host plants in proximity of the proposed crossings. Specific data are needed for Comstock's fritillary (*Speyeria callippe comstocki*) and its host plant, *Viola pedunculata*, Timema walking stick, Jerusalem cricket, and yucca moth.
- Examine the rooting depth of Rainbow manzanita and Engelmann oak to determine whether the overcrossing can be designed to incorporate the rooting depth needed to support these and other deep-rooted host plants such as *Ceanothus* and *Prunus* species.
- Conduct research to determine how wind and vortices from vehicles are likely to affect butterflies (and other taxa) attempting to fly over I-15, including:
    a. What is the strength and direction of the prevailing wind?
    b. Are windbreaks necessary?
    c. How far above the highway does an overcrossing need to be to avoid vortices coming off the freeway?

4. Summary and Discussion

A summary of the probability of crossing use by focal species and crossing type is provided in Table 10. Of the three crossing types, the **vegetated overcrossing** has a high or moderate probability of use by 26 of the 36 species assessed, while **Temecula Creek Bridge** has high or moderate probability of use by 27. The vegetated overcrossing could serve a subset of taxa from all species groups, while Temecula Creek Bridge was suitable for a subset of taxa from all species groups but invertebrates. When combined, Temecula Creek Bridge and the vegetated overcrossing have a moderate or high probability of use for 34 of the 36 species. The **wildlife culvert** has a moderate or high level of expected use by 10 of the 36 focal species and could serve connectivity needs for representative species from all but the bird and plant species groups. The wildlife culvert was only suitable for one invertebrate species, the Jerusalem cricket. Existing culverts may also be suitable for occasional coyotes, gray fox, and ringtail as shown by camera trap data, but more information is needed. The tree frog had the lowest likelihood of use of any of the crossing types.

Species	Temecula Creek Bridge	Wildlife Culvert	Vegetated Overcrossing
Mammals			
American badger	M	M	L
Bobcat	H	H	H
Coyote	H	H	H
Gray fox	M	H	H
Mountain lion	H	H	M
Ringtail	M	H	M
Southern mule deer	H	L	M
Small Animals			
Big-eared woodrat	H	H	H
Western gray squirrel	M	L	-
Arroyo toad	M	-	-
Blainville's horned lizard	M	L	H
California tree frog	L	-	-
Red diamond rattlesnake	H	H	H
Western pond turtle	M	-	-
Western spadefoot toad	L	L	H
Western toad	H	L	L
Bats			
Big brown bat	H	M	M
Pallid bat	H	L	M
Birds			
California gnatcatcher	H	-	H
Cactus wren	M	-	H
California quail	H	-	H
California thrasher	M	-	H
Greater roadrunner	H	-	H
Hawks/Owls	-	-	-
Least Bell's vireo	H	-	-
Oak titmouse	H	-	-
Wrentit	M	-	H
Yellow warbler	H	-	-
Plants and Invertebrates			
California sister	-	-	H
Chaparral yucca	-	-	H
Comstock's fritillary	-	-	H
Engelmann oak	M	-	H
Jerusalem cricket	L	H	H
Pale swallowtail	-	-	H
Rainbow manzanita	M	-	H
Timema walking stick	-	-	H

Table 10: Summary of probability of use of each crossing type for all focal species assessed by experts. High (H), Moderate (M), Low (L).

4.1 Summary of Proposed Design Features for Crossing Structures

The following summarizes design features identified as compatible for all focal species for each of the proposed crossing structures. Potential conflicts are also identified, with proposals on how to resolve such conflicts.

Temecula Creek Bridge

Experts agreed trespass management would best be the focus of any restoration of the creek corridor. Management of trespass through the construction of walls or durable fencing at least 8 feet in height is proposed along the northern edge of Temecula Creek where it abuts urban land uses. The barrier could extend eastward beyond Pechanga Parkway approximately 0.5 mile where commercial land uses are directly adjacent to the creek. If fencing is installed, it could incorporate fine mesh barriers on the lower 2-4 feet to prevent small wildlife from entering urban areas.

Experts also agreed that maintaining trees and associated native understory under the bridge and between spans will be important to encourage wildlife passage, particularly bird species. Removal of invasive plants in both the approach and structure areas was proposed as the primary vegetation management action to improve visibility and facilitate movement through the structure. The proposal by bat and large mammal experts to thin riparian trees around the bridge structure would best be undertaken only if future bat surveys identify that the trees inhibit bat movement under the bridge. Vegetation within different spans of the bridge could be managed to meet different species needs. For example, the vegetation within the southern span could be managed for high cover, while the vegetation in the more northerly span could be managed for high visibility.

Where conditions are appropriate, experts proposed the incorporation of upland plant species in the approach area, including cactus (*Opuntia oricola*) to attract cactus wren to the crossing, as well as upland focal plant species such as Engelmann oak and Rainbow manzanita.

The bridge structure would best incorporate elevated areas along north and south edges to allow passage for terrestrial wildlife when the undercrossing is inundated with water.

Experts proposed that the addition of flight elevators to the exterior of the bridge structure be investigated to direct birds and bats to fly up and over the structure to reduce potential roadkill. Crevice and cavity features suitable for use by day-roosting and night-roosting bats can be added to the Temecula Creek Bridge by bolting on structures made from concrete or a material with similar thermal properties.

Vegetated Overcrossing

In terms of native plant cover for the vegetated overcrossing, a combination of sparse and dense cover types would serve the needs of the widest range of focal species. A continuous band of *Adenostoma* for a portion of the bridge would be needed to encourage use of the structure by Timema walking sticks. Elsewhere, cover that is representative of natural conditions, e.g., dense habitat with openings of bare ground and sparse habitat would benefit most other species.

Soil depth is the limiting factor for the establishment of most plants, and the proposed 4 feet of topsoil on the overcrossing will likely only be able to support shallower rooted plant species found in nearby chaparral and coastal sage scrub, including California buckwheat, black sage (*Salvia mellifera*), chamise, *Opuntia*, and chaparral yucca. If larger pockets of soil can be accommodated, such as raised planters, taller native shrubs and trees could be planted in strategic locations on the bridge.

While the created habitat on the overcrossing and approach area would best mimic adjacent intact native habitat as much as possible, slight modifications could be included to attract habitat specialists. For example, planting taller species such as Rainbow manzanita and Engelmann oaks in the middle and approach areas of the structure could both attract birds to use and elevate their flight across the crossing, while small pockets of native forbs and grasses could be established to attract pollinators, if conditions allow.

Rock piles as well as downed wood/logs and brush piles spaced throughout the overcrossing and approach area will provide cover for ground dwelling species and may create moister microclimates. Artificial rock structures that are lighter in weight may suffice to reduce additional weight burden on the structure.

Barriers, including walls and native plantings of at least 10 feet in height on the edges of the overcrossing and parallel to the highway in the approach area would shield wildlife from artificial night lighting and noise and could help funnel birds and bats across the structure. Walls on the overcrossing structure could include ledges to provide elevated passage for small wildlife.

The approach areas could be studied for the placement of seasonal pools, which would attract and benefit many of the focal species. Pools would best be a minimum of 700 square feet, incorporate a liner covered with native soil to hold water, and be designed so that they are filled by natural rainfall. Figures 4 and 5 present an illustration of the various design elements proposed by species experts for the vegetated wildlife overcrossing.

Wildlife Culvert

The wildlife culvert design will require several added features to improve its use by a wide array of wildlife species. If possible, the structure would best measure at least 15 x 15 feet to enhance visibility through the structure for medium and large mammals. In addition, a natural soil bottom is preferred for all or at least half of the longitudinal portion of the structure. The structure would best be designed to prevent inundation or major flows during rainfall events, possibly by retaining a nearby drainage culvert to handle storm flows and elevating the wildlife culvert on the west side of the highway to prevent regular water flows into the culvert. Keeping water flows separate may eliminate the need for riprap as an energy dissipater at the culvert exit, as riprap can inhibit small animal access to the crossing. To facilitate both aquatic and terrestrial wildlife passage, culverts would best be designed to allow for some dry ground or a ledge at least 4 feet in height and 8 to 12 inches wide inside the culvert on one or both sides.

All species expected to use the wildlife culvert would benefit from the incorporation of sound dampening treatments on the interior of the structure as well as noise and light barriers where the culvert adjoins the highway to reduce noise, vibration, and light associated with vehicle traffic.

The placement of skylights or grates to improve light and moisture conditions within the culvert for small animals will need to be carefully examined in relation to the placement of bat roosts to eliminate any conflicts in light conditions. Providing roosting habitat in recessed areas in the ceiling, which would be effective in creating areas of darkness and trapping heat, may help reduce conflict if skylights or grates are used to increase light and airflow. Evaluation of potential noise issues related to the incorporation of skylights and grates will also be beneficial. Grates will likely increase vehicle noise transmission from the roadway into the culvert, thus skylights with a transparent sound-absorbing covering may be preferred.

As with the vegetated overcrossing, the approach areas to the wildlife culvert would best incorporate logs, rock piles and seasonal pools to attract wildlife to the structure. In addition, the construction of the culvert is anticipated to result in significant vegetation removal and disturbance related to boring and grading activities. Habitat restoration of areas disturbed by grading could incorporate large native trees and boulders to mimic pre-existing conditions as much as possible. Large trees, boulders, brush piles and native topsoil removed during construction could be salvaged and used in post-construction habitat restoration.

While native boulders and rock piles would best be incorporated in the approach area, riprap is best not used in front of or on the slopes adjacent to the wildlife culvert entrances, as it is difficult for wildlife to traverse. If riprap is required, then it is best buried, backfilled with topsoil, and planted with native vegetation.

Finally, adding structure to the interior of the culvert in the form of rocks, brush piles, or logs will benefit small animals and their medium/large mammal predators by providing needed cover. Ongoing maintenance of added structure will be necessary to clear debris and sediment build-up that could impact water drainage or openness. Figures 6 and 7 present an illustration of the various design elements proposed by species experts for the wildlife culvert.

4.2 Proposed Upgrades to Existing Exclusion Fencing

The existing 8-foot exclusion fencing installed along a 3-mile stretch of I-15, while a much-needed improvement, would benefit from some upgrades to increase its utility in reducing roadkill of focal species.

Currently, the overhangs that were incorporated into the exclusion fencing to prevent wildlife from climbing over the fence are composed of 3-strand smooth wire. The smooth wire would best be replaced with fine mesh to prevent wildlife from breaching the fence. Fine mesh barrier fencing with a small overhang could also be attached to the lower 2-4 feet of the fence to guide small animals to the crossings and prevent roadkill. This feature would also reduce roadkill of hawks and owls that hunt for small animals in the roadway. If the entire fence alignment cannot be lined with the shorter barrier fencing (e.g., due to cost, maintenance or other Caltrans prohibitions), small barrier fencing could extend for at least 0.6 mile beyond the crossing structure in either direction, with small barrier fence ends angled away from the road.

Trees or large shrubs that are adjacent to the exclusion fencing (but not in the approach areas to the crossings) that could facilitate an animal climbing over the fence are best minimized, where possible, by modifying vegetation or adjusting fencing alignment. Once the wildlife crossings and associated noise/light barriers are constructed, the exclusion fencing would best be tied into the barrier walls.

4.3 Adaptive Management Considerations

Long-term monitoring and management will be key to the successful function of any wildlife crossing. Monitoring the effectiveness of the various design features in enhancing wildlife use of the crossing structures will be critical to informing management. Regular inspections of design features for crossings, approach areas and barrier fencing would also be beneficial. Experts agreed that funding for long-term management and monitoring in the form of an endowment would best be prioritized and incorporated into project implementation cost or acquired, perhaps through a separate fundraising effort.

Experts agreed that preventing unauthorized human access to the crossing structures and approach areas will ensure wildlife use of the crossing structures over the long-term. Several studies conducted in

California have documented that human and domestic dog presence can deter wildlife use of wildlife crossings and protected areas, with species such as bobcat, coyote, gray fox, and mule deer avoiding areas with recreational use (Murphy-Mariscal et al. 2015, Reed and Merenlender 2008, Jennings and Lewison 2013, George and Crooks 2006). An integrated trespass monitoring and management program could detect and manage trespass and would best include measures such as fencing and signage at likely human access points, camera trap monitoring, and frequent patrols and public outreach by landowners.

Experts were concerned that it may be difficult to maintain sufficient native cover on the vegetated wildlife overcrossing to promote wildlife use, given proposed shallow soil conditions. A permanent irrigation system was proposed for plantings on both the crossing and in the approach areas, with the irrigation system only operated during extended drought periods or to supplement natural rainfall. The water source could also be used to fill the proposed seasonal pools during drought periods to attract wildlife to the crossing.

Experts also proposed implementing a stepwise approach to some design features to ascertain the need prior to implementation. For example, medium/large animal experts identified the potential need for the creation of wildlife trails to the vegetated overcrossing and wildlife culvert to ensure that wildlife can more easily access the crossing. This treatment raised the concern that it might attract human trespass. It was suggested that the treatment could be delayed until after monitoring wildlife use for a certain period following construction to determine if needed. Prior to full implementation, the creation of game trails to the crossing could be performed on one side of the highway to evaluate if it is enhancing wildlife approach to the crossing prior to full implementation on both sides of the highway. Experts also theorized that seasonal pools, if incorporated on either side of the highway for the overcrossing or wildlife culvert, might provide an additional benefit by promoting the creation of natural trails to the crossing structure.

Thinning of native vegetation around Temecula Creek Bridge to enhance movement of focal species such as deer and bats would best only be undertaken after other enhancement measures (invasive plant control, fencing, noise abatement measures) and monitoring are in place to determine if the thinning is warranted. Some experts suggested that invasive plant control may be enough to improve the openness conditions of Temecula Creek Bridge for wildlife, but monitoring could be in place to evaluate if there is improved passage. In addition, experts agreed that it is important to understand patterns of wildlife approach to the crossings when designing and implementing fencing or other barriers to abate human trespass.

5.0 Conclusions

Design, construction, and long-term management of the wildlife crossings as envisioned here presents a new concept in protected area management that will require innovation, experimentation and monitoring to evaluate effectiveness, management of threats (e.g., human trespass), and refinement of design elements over time. While emphasis has been placed on the design and construction of wildlife crossing structures, more information is needed regarding how animals use these structures and how to maximize their effectiveness over the long term. The success of this effort will be dependent on securing public and private funds for wildlife crossing construction and for the implementation of a long-term adaptive management framework. Success will also depend on landowner engagement and participation in all stages of the process, from helping to develop cooperative adaptive management strategies, to identifying short- and long-term goals and objectives, to engaging in public outreach and education to develop community support for wildlife crossing construction and management.

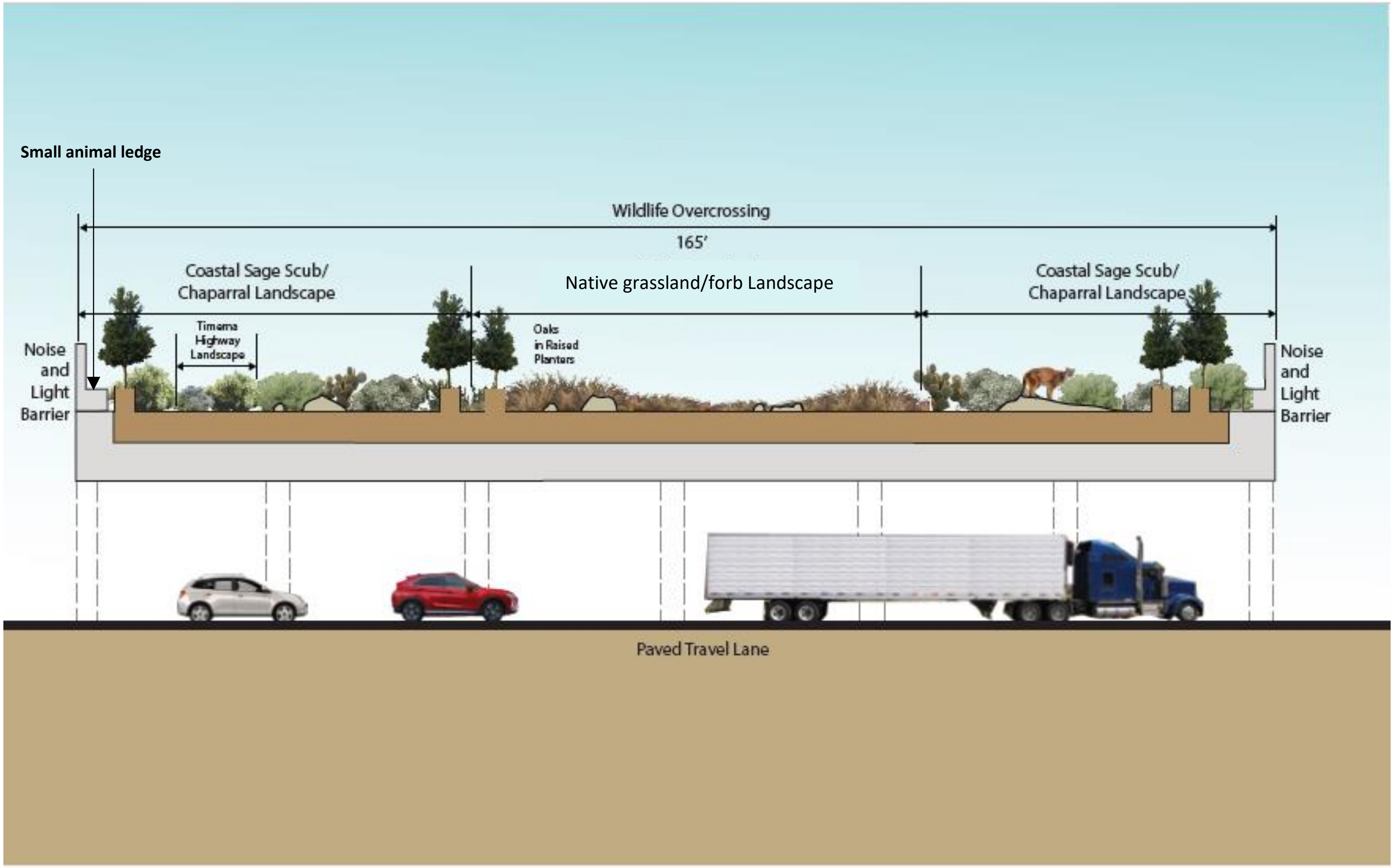

Figure 4: Cross-Section of Vegetated Overcrossing Concept (Lance Vallery).

Figure 5: Illustration of Vegetated Wildlife Overcrossing Design Concept (Lance Vallery).

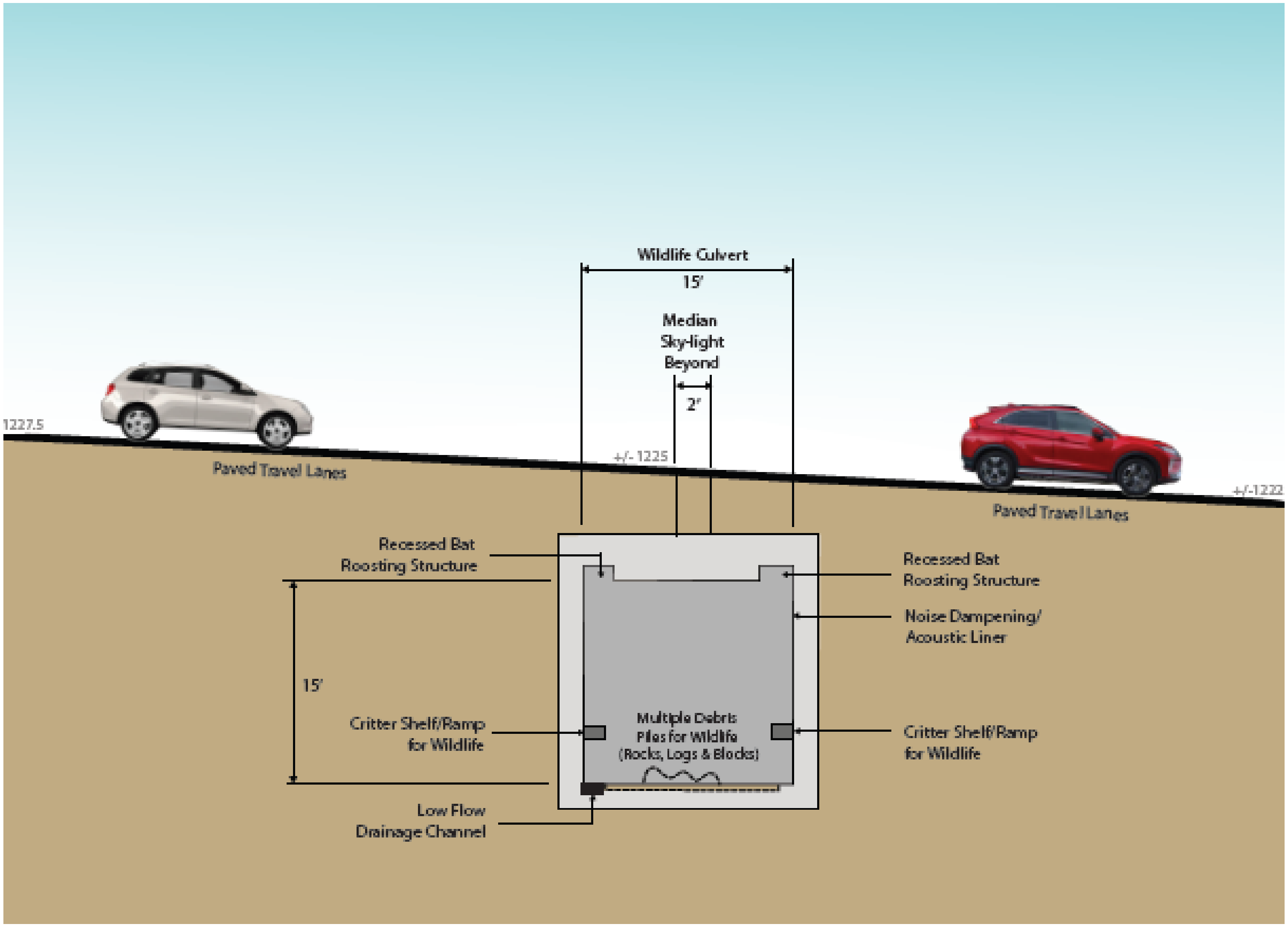

Figure 6: Cross-Section of Wildlife Culvert Design Concept (Lance Vallery).

Figure 7: Illustration of Wildlife Culvert Design Concept (Lance Vallery). Adapted from concept drawing by Cheryl Brehme.

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APPENDIX A
Species Experts and Organizational Affiliations

Name	Affiliation
Adam Backlin	U.S. Geological Survey
Alisha Curtis	Caltrans
Allison Anderson	U.S. Fish and Wildlife Service
Barbara Kus	U.S. Geological Survey
Barry Martin	Wildlife Tracking Inc.
Brock Ortega	DUDEK
Calvin Duncan	UC Davis Wildlife Health Center
Carlton Rochester	U.S. Geological Survey
Cheryl Brehme	U.S. Geological Survey
Dan Marschalek	University of Central Missouri
David Lipson	San Diego State University
Debra Shier	San Diego Zoo
Denise Clark	U.S. Geological Survey
Devin Adsit-Morris	U.S. Geological Survey
Drew Stokes	San Diego Natural History Museum
Dustin Pearce	California Department of Fish and Wildlife
Eric Abelson	University of Texas
Eric Porter	U.S. Fish and Wildlife Service
Fraser Shilling	UC Davis Road Ecology Center
Gjon Hazard	U.S. Fish and Wildlife Service
Gordon Pratt	UC Riverside
Greg Ballmer	UC Riverside
Greg Tatarian	Independent Biologist
Jessica Sanchez	San Diego Zoo
Jessie Vinje	Conservation Biology Institute
Jill Carpenter	LSA Associates
John Taylor	U.S. Fish and Wildlife Service
JP Montagne	San Diego Zoo
Julie King	Santa Clara Valley Habitat Agency
Justin Dellinger	California Dept of Fish and Wildlife
Katy Delaney	U.S. Geological Survey
Kevin Crooks	Colorado State University
Kris Preston	U.S. Geological Survey
Kristeen Penrod	Center for Large Landscape Conservation
Lauren Jonker	SDSU SMER
Liz Fairbank	Center for Large Landscape Conservation
Megan Jennings	San Diego State University
Melanie Madden	U.S. Navy
Melody Aimar	Santa Ana Watershed Association
Michelle Mariscal	Puente-Chino Hills Habitat Authority
Nancy Frost	Caltrans

Species Experts and Organizational Affiliation (continued)

Name	Affiliation
Nicholas Peterson	California Department of Fish and Wildlife
Pablo Bryant	San Diego State University
Paul Beier	Northern Arizona University
Peter Beck	U.S. Fish and Wildlife Service
Phil Unitt	San Diego Natural History Museum
Robb Hamilton	Hamilton Biological
Robert Fisher	U.S. Geological Survey
Rulon Clark	San Diego State University
Sally Brown	U.S. Fish and Wildlife Service
Scott Tremor	San Diego Natural History Museum
Seth Riley	National Park Service
Spring Strahm	Wildspring Ecology
Stephanie Remington	Independent Biologist
Steve Montgomery	Independent Biologist
Susan Wynn	U.S. Fish and Wildlife Service
Tim Dillingham	California Dept of Fish and Wildlife
Trish Smith	The Nature Conservancy
Will Miller	U.S. Fish and Wildlife Service
Winston Vickers	UC Davis Wildlife Health Center
Zach Principe	The Nature Conservancy

APPENDIX B
Special Status Focal Wildlife and Plant Species
Santa Ana-Palomar Mountains Linkage

Common Name	Scientific Name	Federal	State	CNPS
Medium/Large Mammals				
American badger	*Taxidea taxus*		SSC	
Mountain lion	*Puma concolor*		C	
Ringtail	*Bassariscus astutus*		FP	
Small Animals				
Arroyo toad	*Anaxyrus californicus*	E		
Blainville's horned lizard	*Phrynosoma blainvillii*		SSC	
Red diamond rattlesnake	*Crotalus ruber*		SSC	
Western pond turtle	*Actinemys pallida*		SSC	
Western spadefoot toad	*Spea hammondii*		SSC	
Bats				
Pallid bat	*Antrozous pallidus*		SSC	
Birds				
California gnatcatcher	*Polioptila californica*	T	SSC	
Cactus wren	*Campylorhyncus brunneicapillus*		SSC	
Golden eagle	*Aquila chrysaetos*	BGEPA	FP	
Least Bell's vireo	*Vireo bellii pusillus*	E	E	
Yellow warbler	*Setophaga petechia*		SSC	
Invertebrates				
Monarch butterfly	*Danaus plexippus*	C		
Plants				
Engelmann oak	*Quercus engelmannii*			4.2
Rainbow manzanita	*Arctostaphylos rainbowensis*			1B.1

Sources: USFWS 2022; CDFW 2022; CNPS 2022.

Status Key
BGEPA = Federal Bald and Golden Eagle Protection Act
C = Candidate for Listing (Threatened or Endangered)
E = Endangered
FP = California Fully Protected Species
SSC = California Species of Special Concern
T = Threatened

CNPS California Rare Plant Rank
1B – Plants rare or endangered in California or elsewhere
.1 – Plants seriously endangered in California
.2 – Plants common elsewhere, fairly endangered in California
.3 – Plants not very threatened in California

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