Estimation of the Population of Mountain Lions in the Santa Ana Mountains and Comparison of Techniques for Population Estimation and DNA Collection, Wildlife Photo Technology Development, and Development of a Long-term Monitoring Plan and Collaborations for Mountain Lion Populations in Regional NCCPs

NCCP-Local Assistance Grant Agreement No. Q1986006

Final Report

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With reference throughout to a separate report of results of the Cubby-based hair snare study from co-PI Jeff Manning Ph.D, Caren Goldberg Ph.D. (Spatial Ecology and Conservation Genetics Lab), and T. Miles Hopkins, Graduate Student, Washington State University, Pullman WA.

Submitted to:

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Western Riverside County MSHCP Biological Monitoring Program

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Revision of this report submitted on August 24, 2022 after receipt of additional data from the CDFW Genetics Research Lab and organizational changes

Mountain lion relaxing at cubby-type hair snare structure

Female mountain lion with four kittens in the Santa Ana Mountain Range

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Mountain lion approaching alternative hair snare site.

Executive summary

This project addresses the need to develop mountain lion monitoring protocols that will produce unbiased estimates of population size suitable for detecting changes in population status, with special emphasis on the Santa Ana Mountain Range (SAM) mountain lions (*Puma concolor*, cougar, puma). The mountain lion population in the eastern Peninsular Ranges (ePR) is also of concern, thus the goal of the project was to determine what non-invasive genetic and demographic monitoring techniques will most efficiently detect a specified level of population change in either population. This can inform conservation actions to abate population decline and avoid population extirpation. Testing of non-invasive protocols and analytical approaches is the primary method by which data was acquired for evaluation.

Primary funding for the Project came from the NCCP-Local Assistance Grant Program administered by the California Department of Fish and Wildlife (CDFW). Additional funding came from the Institute for Wildlife Studies and private donors. In-kind funding came from The Nature Conservancy (TNC), the San Diego County Association of Governments San Diego Co. Management and Monitoring Program (SDMMP), and the United States Geological Survey (USGS).

Specific Objectives for this project were to:

1. Provide consistent or coordinated information on mountain lion populations and dispersal success in the Western Riverside County (WRC) Multiple Species Habitat Conservation Plan (MSHCP) and other regional NCCPs, by testing methods to assess the size of the current population and its genetic status (inferring dispersal status).

2. Assess local mountain lion population and dispersal into and out of area by helping to develop and test potential camera-based alternatives for population estimation and monitoring.

3. Test a group of non-invasive sampling methods on the SAM mountain lions and recommend methods for future monitoring of the population.

4. Analyze photos from hair snare and other designated cameras for individual, population, and vertebrate community patterns and interactions.

5. If possible create population estimates from one or more data sets (DNA from hair or other methods and photos) and techniques.

6. Develop a long-term monitoring plan for this population that is repeatable, robust, and cost effective taking into account logistical constraints.

Key findings:

1. Genetic material and individual ID's can be derived from scat, hair, swabs of natural structures and prey, and tissues collected from deceased and captured animals.

2. Hair snares contributed only 9 individual identifications despite a very substantial and expensive effort with multiple hair snagging strategies over a total of 9,156 days of site activity.

3. Scat collection (opportunistic) contributed 14 individual identifications.

4. Swabs at hair snare sites contributed only 2 individual identifications.

5. Tissue and blood collection from animals captured during the study period contributed 11 individual identifications. All but 3 were also detected by one or more of the other means.

6. Mortalities that occurred during the study period yielded tissue DNA for 6 animals, only one of which was detected by other means prior to death – but that animal (M318) was also detected by scat, hair, and swab of a prey carcass prior to death.

7. Seventeen animals were identified by only one method, though one of those was also identified on camera multiple times. Twelve animals were identified by 2 to 4 different methods.

8. The CDFW Genetics Research Lab screened all DNA profiles from the samples acquired during the study period (in both the SAM and the ePR) against all other DNA profiles in their database from the entire region and found no matches from outside the Santa Anas, and only one match with an animal that had previously been sampled within the Santa Anas (a collared female F126). Interestingly that animal is the only known surviving offspring of M86, the only mountain lion previously documented as having crossed I-15 from the east that reproduced (Gustafson et al. 2022). This finding tended to confirm the rarity of movement of animals across I-15.

9. The analysis did detect a match of DNA from one road mortality in the ePR in 2021 with scat collected elsewhere in the ePR in late 2020 during the CDFW scat dog survey project there.

10. The analysis also matched DNA from one animal sampled via scat, hair, and saliva in the western SAM in summer 2021 with tissue from a dispersal age male (M318) that was killed on SR60 in Diamond Bar in the spring of 2022. This confirmed that the individual had dispersed across SR 91 and crossed the Chino Hills from south to north to the point where he was killed, only the 3rd animal that we have confirmed crossing SR91, and the first to have been confirmed

conclusively as moving through the Chino Hills during the course of our long term study – though sightings have been occasionally reported there.

11. All the DNA collection efforts combined identified 29 discrete individual mountain lions in the Santa Anas during the course of the study, including those sampled after suffering mortalities.

12. The age as determined by direct exams or cameras of the 29 animals identified by genetics in the Santa Anas (including 5 animals detected only after mortality were:

    Confirmed Adults (over 30 mo, or female with kittens): n=7 (5F, 2M)
    Adults or Subadults (Fully grown animals not with mother but exact age unknown): n=5 (2F, 3M)
    Confirmed Subadults (18-30 mo.): n=5 (1F, 4M)
    Kittens (0-18 mo): n=6 (4F, 2M)
    Age unknown (scat detection only): n=6 (2F, 2M, 2 could not be determined)

13. Density estimation based on analysis of the totality of information available utilizing the methods of Cooley et al. (2009) suggests a density of mountain lions in the Santa Anas of 1.6 per 100 sq. km., resulting in an estimate of 24.48 individuals in the Range (exclusive of the Chino Hills which were not sampled). If the Chino Hills are included then the estimate is 32 animals though there is little evidence of significant use of the Chino Hills by mountain lions when compared to the rest of the SAM. This estimate does not differentiate between demographic classes, thus is not intended to suggest this number of territorial adults are present, and is more properly interpreted as a minimum number.

14. An alternative method of population estimation utilizing extrapolation of findings from a statewide scat DNA-based study (Dellinger et al. in prep) and habitat mapping suggests a somewhat higher density estimate of 2.12 (1.9-2.3) animals per 100 sq. km. This estimate is based on the expectation of minimal detection of scat from animals less than 10-12 months of age, and extrapolation from scat sampling done in 12 different regions of the state. One of those regions however did include the eastern Peninsular Range adjacent to the Santa Anas. This density estimate results in an estimated population of 32.4 (95% CI: 29-35.2) total animals above 10-12 months of age.

15. Benson et al. (2019) estimated the capacity of the range as5-7 adult males and 11-14adult females based on territory sizes and habitat availability (exclusive of the Chino Hills, which were presumed to be unoccupied or minimally occupied). Thus, both estimates, along with our minimal population of 17 adults and subadults, is not

inconsistent with the Benson capacity estimate.

16. These findings suggest that there is value in taking every advantage of opportunities to collect DNA from mountain lions, whether systematic or opportunistic, including taking swabs from animals that mountain lions are found to be feeding on.

17. Cameras are an effective ways to identify demographic characteristics of mountain lions that are or are not at sites where DNA is collected. In cases where DNA may be collected at a camera site, the combination of DNA and photos is much more informative than either one separately.

18. Cameras can identify a subset of animals on a landscape even without other means through subtle markings that close observation can reveal especially with daylight photos. In this study, 4 animals were identifiable with cameras alone, even without any computer assistance, and were photographed repeatedly across the landscape – not only on our cameras but also by numerous hobbyists and land managers with trail cameras in the region.

19. At all hair snares combined, the demographic group (male, female, dependent kitten, adult/subadult) could be ascertained from photos 83% of the time. Photos were taken of mountain lions on 149 occasions. This resulted in photos of 151 adults/subadults and 48 dependent kittens since family groups were present on a significant number of occasions. For adults/subadults, females outnumbered males 67 to 53, with the gender of 31 adults/subadults undetermined.

20. Other cameras deployed for other studies during the project period recorded mountain lions on an additional 459 occasions, with 565 animals photographed. Photos were taken of 476 adults/subadults and 86 kittens on these occasions, with the age class of 3 animals undetermined. Of the adults/subadults, 157 were female, 110 male, and 209 undetermined.

21. Thus overall during the study, the ratio of adults/subadults to kittens was approximately 4:1, and the ratios of females to males (when sex could be determined) was approximately 3:2.

22. Isolation of DNA from samples and identification of individuals with the CDFW Genetics Research Lab's SNP assay was most reliable in the following order (from most reliable to least):
    a. Tissue/blood – 100%
    b. Scat – 44%
    c. Swab* – 31%

d. Hair – 29%
e. *Includes swabs from ePR at collared mountain lion kill sites. Swabs of surfaces for saliva were poorly effective, swabs of prey animal skin at wound sites was much more effective

23. Mountain lions can be attracted to sites with predator calls, and utilizing both predator calls and other unique sounds these devices can assist in getting large numbers of photos of individuals, as well as collecting DNA from hair.

24. Dr. Holly Ernest at the U. of Wyoming recently processed and characterized 34 DNA samples from the region (Santa Anas and the eastern Peninsular Range) via a large SNP array that have been also processed through Dr. Mike Buchalski's SNP process at CDFW. In total, 179 samples from the region will be analyzed by Dr. Kyle Gustafson to update the pedigree for the regional mountain lions, assess genetic diversity and connectivity, and test the ability of the CDFW genetics lab SNP array to ascertain close genetic relationships. Existing data from the Dr. Vickers and the UCD project will be used to ground truth the results via known mother-offspring and sibling relationships.

25. Samples from the region will be incorporated in broader genome assessments being conducted at UC Santa Cruz, and will provide wider insight into many aspects of the regional mountain lion genetics.

26. GPS collaring during the study period contributed significantly to the population density estimation via having marked animals on the landscape, defining female home ranges, and understanding to a greater degree the distinctions between animals – ie actual ages, movement patterns suggesting territorial (adult) or dispersal (subadult) movements, etc.

27. Ten mountain lions were captured and GPS collared during the study period in the Santa Anas (6 M, 4 F), and 1 (a female) captured and collared in the ePR. Two of those SAM individuals successfully crossed I-15 from the SAM into the ePR – one in Murrietta and one at the northern edge of Escondido , but returned within days to weeks to the Santa Anas. One additional collared individual left the Santa Anas by crossing the 91 Freeway into the edge of the Chino Hills, but also returned within 2 weeks. One male that was detected with its mother via hair, scat, and saliva in the western Santa Anas was killed on the 60 freeway by a vehicle after crossing the 91 freeway and traversing the Chino Hills south to north. All 4 of these individuals were dispersal age individuals. Two other uncollared animals were detected by cameras while crossing these freeways – 1 animal crossed SR91 south to north through a

culvert then returned within hours, and 1 animal crossed I-15 west to east at the Temecula Creek Bridge and has not been recorded as returning. These events illustrate further that road mortality, roads as barriers, and inhospitable factors in territory new to dispersers can all add up to result in isolation of the Santa Anas population.

28. A scat detection dog survey is planned for the Santa Anas in November 2022, also funded by the NCCP-Local Assistance Grant program. It is hoped that the results of that survey when combined with these results will give us a more complete picture of the mountain lion population in the Santa Anas and when combined with previous genetic results from the eastern Peninsular Range, inform connectivity between all the NCCP's in the region.

29. The experts workshop held as part of this project came to no specific conclusions or recommendations regarding long term monitoring of mountain populations in the region, though there was general agreement that the strategies for the SA and ePR ranges may need to be different. The SAM range may benefit more from having marked animals (collared/otherwise sampled and marked) on the landscape to assist in mark-recapture based assessments. The small size of the population means that detecting changes of significance at the population level is much more difficult. Changes may not be detectable until quite pronounced without significant ongoing collaring (perhaps with long term collars) and marking. Camera and scat surveys on a regular basis can complement this, as well as opportunistic DNA acquisition. The ePR could potentially be effectively monitored with similar techniques.

30. This study suggests that 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 based on these findings and if more effective snaring methods can be developed.

31. Camera array – based methods have not yet been fully tested for population monitoring in this study area, but may hold some promise due to newer analytical techniques. In addition, long term radiocollars may have a role to play in the long term monitoring of this population. We hope to test some of those approaches in the future.

Introduction

Natural Communities Conservation Plans (NCCPs) are the backbone of conservation of key wildlife species in California. In the southern California region, numerous NCCPs and other conservation plans name the mountain lion (*Puma concolor*, puma, cougars) as a covered or connectivity evaluation species because of their wide ranging nature. Because they use extensive areas for territorial activities and dispersal, multiple NCCP areas and other conserved lands may be used by any one mountain lion or subpopulation. Physical and genetic connectivity within and between Orange, San Diego and Western Riverside NCCP areas (Figure 1) and other conserved lands is critical for the long-term health and persistence of mountain lions and other wide-ranging species in the region, and for full ecological reserve function.

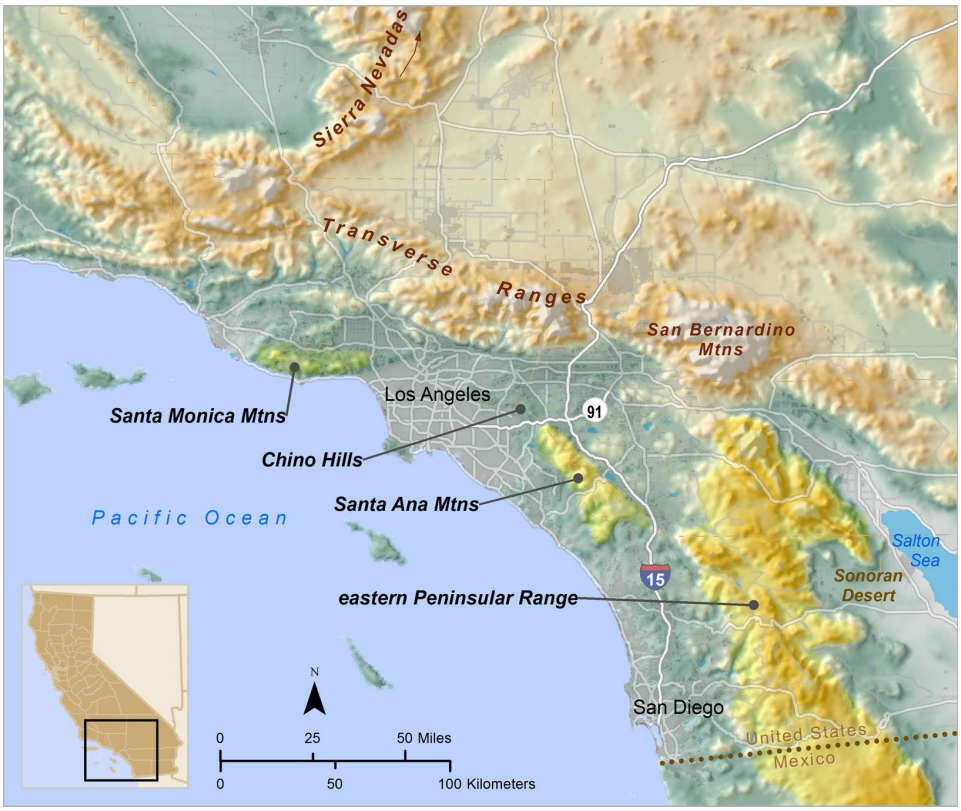


Figure 1. Southern California and the Santa Ana Mountains and eastern Peninsular Range (Benson et al. 2019)

Like other large carnivores around the world, the mountain lion populations in California are challenged by rapid urbanization. California mountain lions have recently been characterized genetically as being divided into ten subpopulations, with six of the ten existing in the southern and central coastal regions of the state (Gustafson et al. 2022), and due to concerns about inbreeding and population decline have been petitioned for listing under the California Endangered Species Act.

Mountain lions in the Santa Ana Mountains (SAM) and eastern Peninsular Ranges (ePR) depend on multiple functioning regional NCCPs, the Western Riverside County NCCP (Western Riverside County Multiple Species Habitat Conservation Plan: WRC MSHCP), the extensive conserved lands of the Cleveland National Forest, other conserved lands, and a functioning linkage between the SAM and ePR (herein SAM-ePR linkage) for their continued persistence (Figure 1). Conversely multiple NCCPs in the region depend on mountain lion persistence for their proper ecological functioning. The level of genetic fragmentation of the California mountain lion population also suggests that fragmentation of certain other wildlife populations is likely occurring, potentially affecting NCCP functions.

Our UC Davis Wildlife Health Center (UCD-WHC) mountain lion study team and collaborators have shown that the mountain lion populations of the SAM and ePR of Orange, San Diego and Western Riverside counties are suffering from serious genetic restriction due to barrier effects exerted by Interstate 15 (I-15), development, and other barriers, with migration across that barrier being rare (Gustafson et al. 2017). As a result, the SAM mountain lion population has the least genetic diversity in the state and is second in the nation only to the federally endangered Florida panther (Gustafson et al. 2018,2022). It has been petitioned for listing under the California Endangered Species Act as Threatened or Endangered along with five other coastal subpopulations including the population in the ePR. Habitat loss and elevated mortality from vehicle strikes, depredation permits, poaching, and wildfire are other major factors affecting population stability (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 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). Recent collaboration between our UCD-WHC team, the National Park Service, and UCLA reproductive and genetics researchers has indicated that inbreeding depression is likely beginning to occur (Huffmeyer et al. 2022). The risks to the SAM population are real, as is the threat to proper NCCP function in the region should substantial population decline or extirpation of mountain lions occur.

Monitoring of mountain lions is difficult, and current monitoring efforts are not providing reliable estimates of the mountain lion population in regional NCCPs or areas with populations at risk such as the Santa Ana Mountain Range. Currently no coordinated mechanisms are in place for detection of dispersal or population change in mountain lion populations in regional NCCPs. Both the SAM and ePR populations are subject to population change and dispersal restriction, thus should be monitored on a long-term basis.

This project worked to address the need to develop mountain lion monitoring protocols that will be both effective and sustainable, so as to detect changes in population status and dispersal, and allow detection of population decline quickly enough for agencies to act to avoid population extirpation.

This project brought together a collaborative group of researchers from UC Davis, California Department of Fish and Wildlife, the University of Wyoming, Washington State University (WSU), The Nature Conservancy (TNC), U.S. Geological Survey (USGS), the San Diego County Management and Monitoring Program (SDMMP), Arkansas State University, and management and monitoring personnel from multiple regional entities that control conserved lands. Its aim was to test the effectiveness of various non-invasive field sampling and analytical approaches, and incorporation of opportunistic tissue and blood sampling, that can inform the development of an integrated long-term population monitoring plan for mountain lions in the Santa Ana Mountains, eastern Peninsular Range, and regional NCCP's. It also advanced collaborations and protocols that incorporate datasets already in existence from ongoing monitoring efforts.

Our results will also help guide future monitoring and management of mountain lion populations in other NCCP areas in the state where mountain lion population fragmentation has been documented (Gustafson et al. 2022). The results presented here will contribute to knowledge that is useful to CDFW when considering land use proposals in the region, and assist CDFW and Caltrans in meeting the goals laid out in the California Essential Habitat Connectivity Project and State Wildlife Action Plan. By helping to guide efforts to monitor populations and their genetics, as well as monitor connectivity for mountain lions and other wildlife between and within NCCPs and other conserved lands, outcomes of this project will assist efforts by CDFW, USFWS, and others to provide more adaptive options for species affected by future development, drought, or climate change.

This project tested methods to assess the size of the current population and its genetic status (inferring dispersal status), helping to develop and test potential camera-based alternatives for population estimation and monitoring, and comparing population characteristics derived by different methods - hair capture, scat collection, and photo based.

Project Area

This project was focused on the Santa Ana Mountains (SAM), a coastal mountain range in Orange, Riverside, and San Diego Counties, and portions of the eastern Peninsular Ranges (ePR) that are contained primarily within San Diego and Riverside Counties (Figure 1). The Santa Ana Mountains contain approximately 1,530 sq. km. of mountain lion habitat (Benson et al. 2019) exclusive of the Chino Hills north of SR 91, and are bordered by the Pacific Ocean and Interstate 5 on the west, Interstate 15 to the east, SR 91 to the north, and several cities including the City of Oceanside and SR 76 to the south. The Chino Hills were excluded from this study due to minimal evidence of regular mountain lion use since 2005 when the UCD study commenced in the SAM.

The range is "interdigitated by drainages that support year-round and ephemeral rivers and creeks. Dominant vegetation types include chaparral, coastal sage scrub, oak woodlands, conifer forest, willow and cottonwood riparian, non-native and native grasslands, vernal pools, and agriculture."(WSU team report). Owners/managers of conserved lands include the US Forest

Service, California Department of Fish and Wildlife and State Parks, Orange, Riverside, and San Diego County lands, the US Navy/Marines, Bureau of Land Management, water and utility districts, and several non-profit conservation organizations.

"High fire frequency exists in this fire-adapted vegetation system, with the fire season expanding earlier in spring/winter and later into fall/winter. During high fire risk (red-flag warning periods), restrictions to access occurred."WSU team report)

The eastern Peninsular Range contains approximately7,700 sq km of habitat, and extends from I-15 in the west to the Salton Sea in the east, the Mexican border in the south, and I-10 in the north.
From WSU team report: "Mountain lions in the SAM and ePR co-occur with other carnivores, including bobcats (*Felis rufus*), coyotes (*Canis latrans*), grey foxes (*Urocyon cinereoargenteus*), skunks (*Mephitis* and *Spilogale* spp), raccoons (*Procyon lotor*), and ringtails (*Bassariscus astutus*). Primary prey is mule deer (*Odocoileus hemionus*), along with mesocarnivores and other species."(WSU team report)

The most common causes of death in both the SAM and ePR for GPS-collared mountain lions are vehicle strikes and being killed secondary to domestic animal depredations (Vickers et al. 2015).

The SAM and ePR ranges are located within the WRC MSHCP area, but also parts of the proposed North County MSCP plan area in San Diego County and adjacent NCCPs. Approved NCCP plans whose reserve designs are affected by the presence and connectivity of mountain lions are: 1) the WRC MSHCP; 2) SD MSCP (multiple approved NCCPs); 3) SDG&E NCCP/HCP; 4) San Diego County Water Authority NCCP/HCP; and 5) Orange County Transportation Authority (OCTA) NCCP/HCP, as well as the Orange County Southern Subregion Habitat Conservation Plan (HCP only). The Orange County Southern Subregion HCP also contributes to conservation of habitat for the mountain lion within the Santa Ana Mountains and operates an adaptive management program that includes a goal of maintaining connectivity within and between adjoining NCCP subregions. Implementing Agreement dates for these plans range between 1997 and 2011.

Task 1. Project Administration and Management

Dr. Vickers of UCD-WHC led the overall project, and the administration and management, and UCD-WHC administrative staff tracked expenditures, overall monetary status, and provided project oversight. As noted previously in this project's quarterly reports, staff time, vehicle requirements, and travel costs were all increased over original projections due to restrictions dictated by precautions against transmission of the novel coronavirus between staff members, e.g., each individual had to travel in a separate vehicle to field sites, etc. Changes in schedule, and acquisition of supplementary funding from other sources, allowed completion of the field component of the cubby-based project and additional sample acquisition experimentation described here.

Subtask 1.a. – Data Management. The following information is associated with samples and photos taken during the course of this study is accumulated in various excel sheets, workbooks, hard copies of field data collection forms, and backup data drives.

1.Who collected the data; Initials of field staff are associated with each sample or set of photos collected

2.When the data was collected; The data and time of collection was recorded.

3.Where the data was collected; GPS locations are recorded for each sample and set of photos.

4.How the data was collected (description of methods and protocols); See methods section in this report.

5.The purposes for which the data was collected; Defining locations where samples and photos were taken in order to assess the ability of non-invasive sample collection and photos to monitor mountain lion populations.

6.Definitions of variables, fields, codes, and abbreviations used in the data, including units of measure; Data dictionaries are present in each excel workbook

7.The terms of any landowner access agreement(s), if applicable; Landowner access agreements generally allowed free access to sites on the properties with certain requirements for advanced notification. Due to the substantial number of different landowners, it is impractical to spell out all of those terms here, but can be provided if requested.

8.References to any related Department permits or regulatory actions; Permissions for hair sampling were in our California Dept. of Fish and Wildlife (CDFW) Scientific Collecting Permit #9875, and our UC Davis Institutional Care and Use Committee permit #22408.

9.Peer review or statistical consultation documentation; A peer expert workshop was conducted in which methodologies and strategies for accomplishing the aims of this project were discussed, and is reported herein

10.Data licensing and disclaimer language;
A. The project report to CDFW may be shared internally.
B. We request embargo from external sharing until publication of any desired peer reviewed articles relating to the project analysis has occurred,
C. Internal sharing may be done through any CDFW mechanisms. Web links will be provided for peer reviewed publications.
D. We will endeavor to publish peer reviewed articles in open access publications, but if access is

restricted a copy of the publication will be provided to CDFW upon publications. See Mountain Lion NCCP LAG Data management plan attached to this report.

All photos acquired from cubby camera sites were backed up on two hard drives, with one shipped to Dr. Manning's WSU lab, and one being retained by the UCD-WHC team. All photos acquired from other hair snare-associated cameras and those from other projects are backed up on UCD-WHC backup drives.

Task 2 - Camera Data and Hair Collection; Scat Collection and Analysis by the State: and Task 3 - Hair DNA and Camera Data Analysis:

METHODS

A probabilistic sampling design was developed by Dr.'s Manning and Vickers to inform the spatial arrangement of sampling stations that would enable inference of results across the entire range of the Santa Ana Mountains mountain lion population (Figure 2). For this, we first created a grid of 66 5x5-km cells across the study area, including a portion of Camp Pendleton Marine Corps Base, with the goal of placing a single sampling site (cubby) within each cell. This spatial resolution accounted for mountain lion space use patterns to ensure an average of four cubbies per average female mountain lion territory size (193 km^2 - 75 square miles; Vickers et al. 2017) to aid in maximizing detection probabilities. We further partitioned each 5x5-km cell into 25 1x1-km cells, used spatial information from prior collared mountain lions across the study area to quantify the frequency of mountain lion occurrence in each grid cell, and stratified the cells within this smaller-resolution grid into low, moderate, and high occurrence levels. Within each 5x5-km cell, we selected the five 1x1-km cells containing the highest estimates of occurrence and selected one of these (based on proximity to human dwellings, proximity to human trails, availability of woody debris for cubby construction, and access) as a sampling site to construct a cubby for placing a hair snare and 2 game cameras at each site (Figure 3).

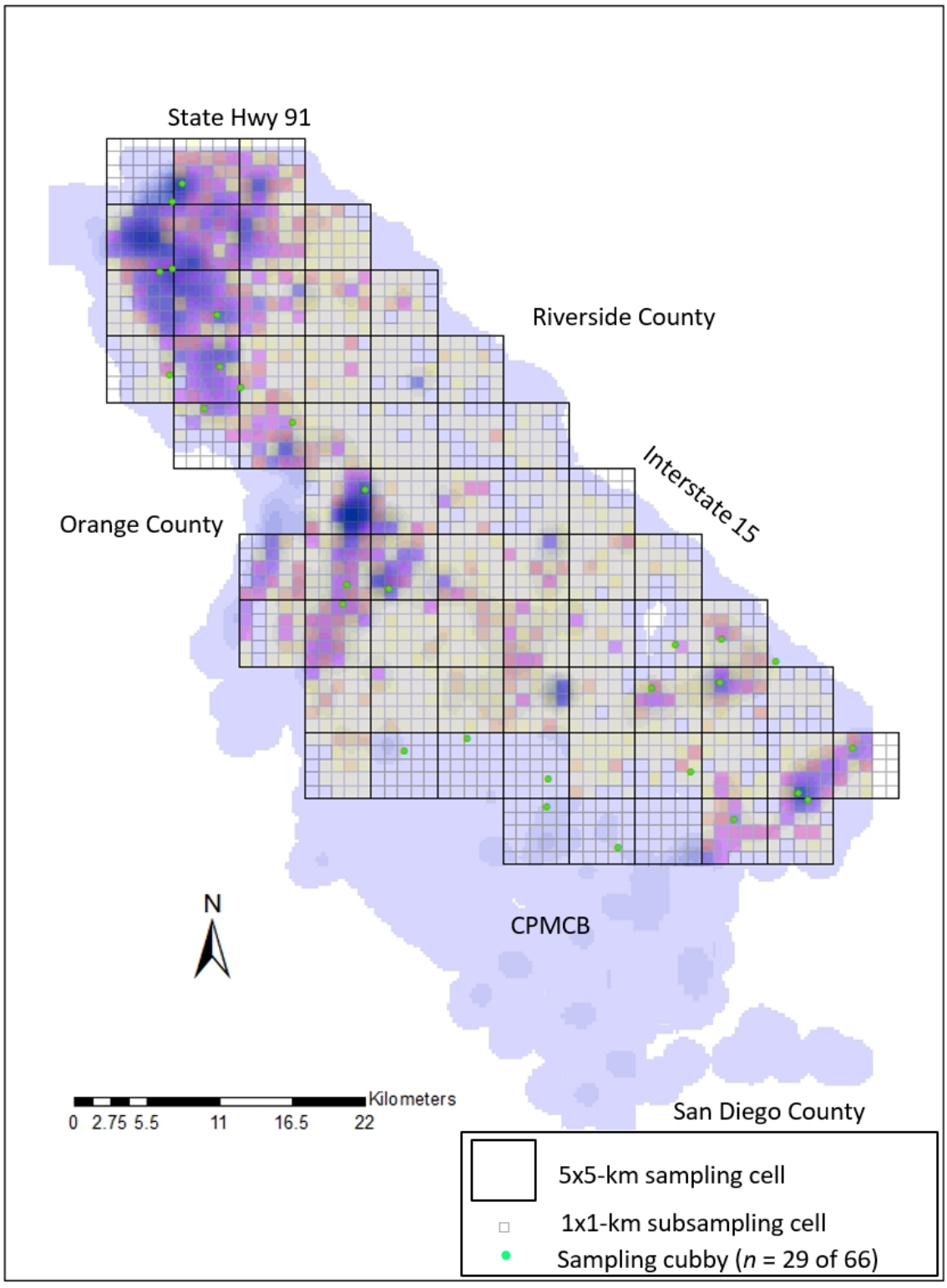


Figure 2. Sampling grids and sampling sites (cubbies) erected as of January 28, 2021. Colors denote the density of previous mountain lion GPS data points from collars.

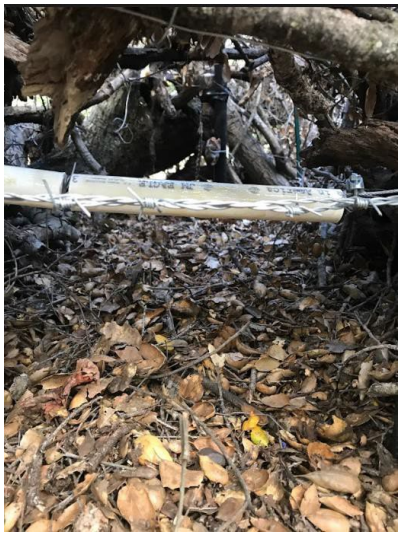
	a. Station 38-23 under construction.
	b. Camera view of Station 12-4. *Note the position of the Tanglefoot rollers and barbed wire in the vertical midline of the entrance. Although this is undesirable, it did not appear to impact sample collection, as no mountain lions were detected on camera at this site.*
	c. Photo looking inside of station 2-24 from the entrance, baited with a piece of mule deer hide and muscle, predator call, Tanglefoot (Marysville, OH) roller, a hanging feather, and barbed wire at entrance.

Figure 3 (From WSU team report): Cubby constructed of downed wood, posts, and wire to hold structure up. Sticky rollers and barbed wire hair catch are stretched across the front.

We completed development of standardized protocols and datasheets for field data collection and developed standardized dimensions and layouts of cubbies, and Dr. Vickers hired and supervised five field assistants and a sixth field crew member was provided to the project by collaborator Dave Garcelon and the Institute for Wildlife Studies. In-person, zoom, and field trainings took place to train the field crew on cubby placement and construction; hair snare maintenance and service; hair collection and storage; and labeling, organization, and filing of digital photographic records. Additionally training was held on proper opportunistic collection and storage of swabs when photos indicated the possibility of saliva being left on structures associated with the hair snare stuctures, mountain lion scat when encountered, and tissue collection from deceased animals and storage. These efforts were designed to standardize the sampling effort, reduce heterogeneous detection probabilities among mountain lions and stations, and improve mountain lion sampling and population estimation during the data analysis phase.

Other field supplies included t-posts to create a framework of 4 posts driven in the ground in a rectangle upon which to build each cubby with smooth galvanized wire between posts and downed wood, other construction equipment (hammers, wire cutters, etc), sample collection and storage supplies, and one Wasatch Wildlife Product® FurFinderR® (Magna, Utah, USA) predator call for each cubby. Calls operated only at night, emitted a 5 second recording every 30 seconds at an average of 103.5 decibels at a frequency of 2.5-3.8 kHz. Calls that were rotated at every cubby servicing visit were deer and rabbit in distress and mountain lion whistle. Cubbies were constructed similarly to those detailed by Yeager (2016) with a single entry point at the front. Rollers of PVC pipe were strung on smooth wire between steel rebar rods driven into the ground at heights initially of 14 inches above the ground but varying some during the course of the study in response to observations. These rollers were then coated with Tree Tanglefoot® (Marysville, OH) and a strand of barbed wire was also strung across the entrance at the same height.

A 5th post was placed in the back of the cubby with a piece of PVC pipe slipped down over it, and bait (deer meat and hide), a predator call, and later in the study, a scent attractant (Russ Carmen's Canine Call), were applied to the pipe. A turkey feather was hung on monofilament line from the top of the cubby to provide a visual lure. The PVC pipe allowed all of the inducements except the feather to be lifted out of the cubby for refreshing bait and scent lure and changing batteries in the calls.

We placed 2 trail cameras (Browning Patriot Model) at each cubby site. Because one purpose of the cameras was to acquire close-up photos to allow possible identification of individuals from photos, one camera was placed 8 feet in front of the cubby facing the entrance and set to acquire photos in 5-shot bursts when triggered, followed after a 1 second interval by additional 5-shot bursts as long as the camera was being triggered (Figure 4). A second camera was placed approximately 30 feet in front of the cubby also facing the entrance for a panoramic view and set to take one photo at 30 second intervals when triggered. Though initial plans were to place a camera inside the cubbies to capture facial photos on puma entrance, experimentation revealed that distances were too short to acquire well-focused

photos.

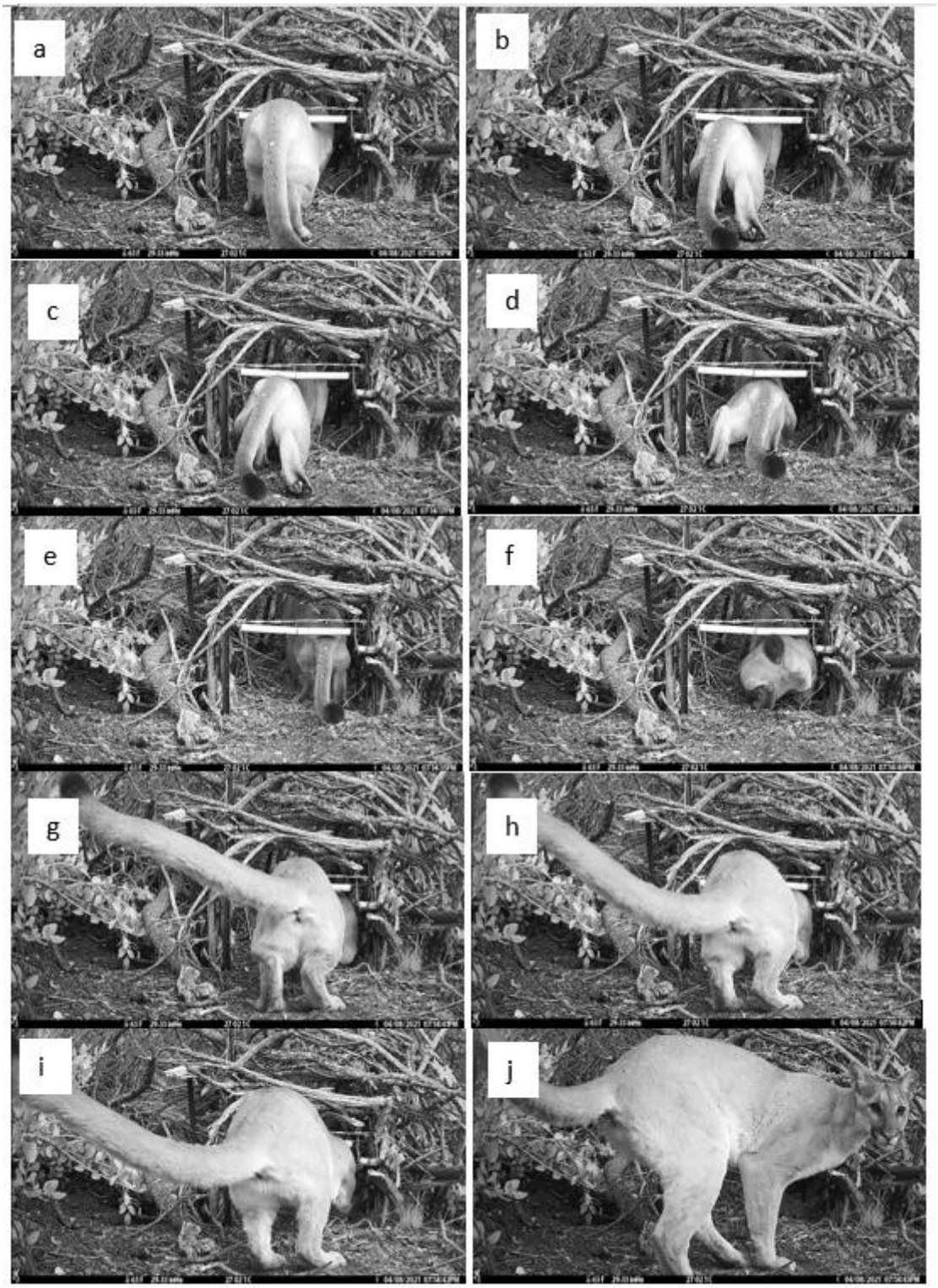


Figure 4 (From WSU team report). Sequence of photos taken by closer cubby camera set to take 5 shot bursts with 1 second interval between bursts as long as being triggered

Permissions were obtained from 13 land managers to utilize selected sampling sites across the Range. Permissions for hair sampling and capture/salvage of mountain lions were in our California Dept. of Fish and Wildlife Scientific Collecting Permit #9875, and our UC Davis Institutional Care and Use Committee permit #22408.

By the end of March 2021, 59 of the 66 potential sampling sites were fully operational with constructed cubbies and installed monitoring equipment (Figure 5). Four of the 66 sampling sites burned in the late Fall 2020 fires in Orange County, and we were only able to replace one of those in an unburned section of one of the four 5x5 km units that were affected. The habitat in three of the units was completely burned, and those were excluded from the study. In addition, sites in four units could not be accessed due to the need to cross private land to reach the sites, and being unable to get permissions in a timely fashion. Thus, the sample size was adjusted to 59 sites. At the 59 sites, field crews recorded environmental and vegetation characteristics within circular plots centered on each sampling cubby site, including cubby dimensions and placement within the landscape to use as quantitative covariates of detection probability in our population estimation models.

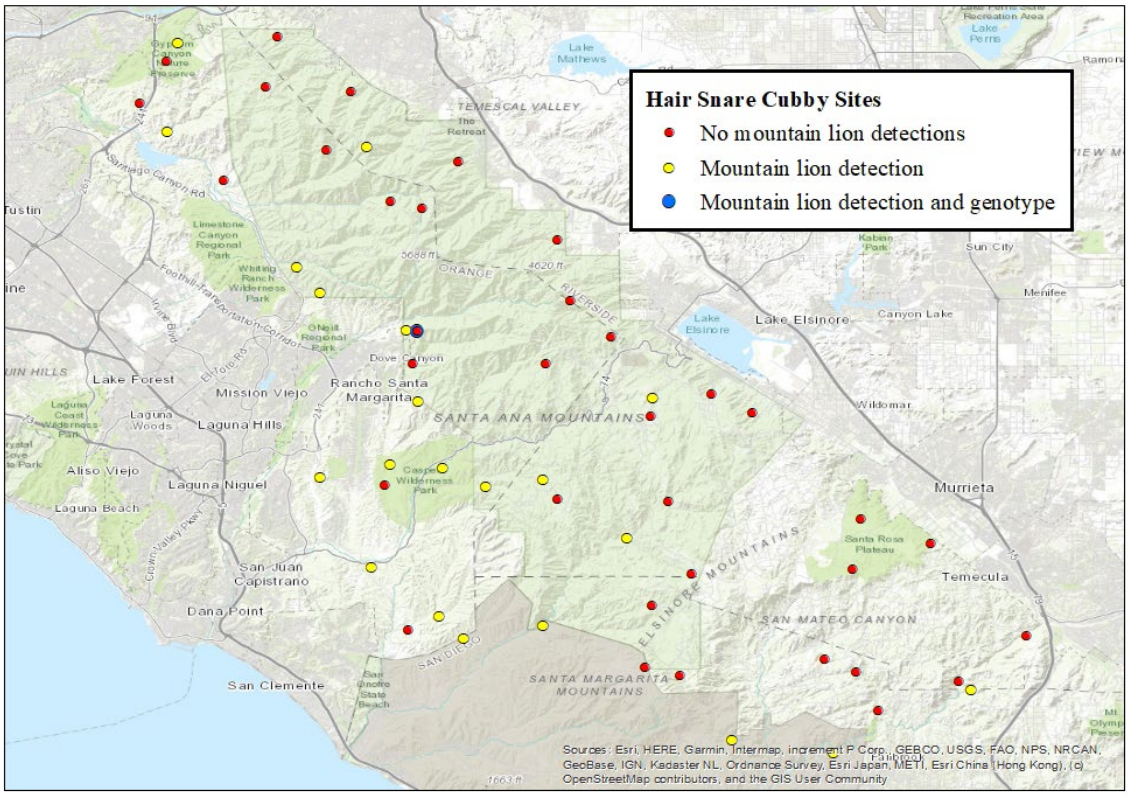


Figure 5. Cubby hair snare sites with mountain lion detections, and detections where hair captured that yielded genotypes.

All 59 sites were activated in early April (Figure 5). Sites were serviced (collection of hair samples and sticky rollers (any time lions had visited even with no hair being visually evident), as well as collection when the Tree Tanglefoot had dried or there was excess insect presence, and photograph samples, maintenance/repairs to cubby structures) weekly for the first eight weeks of the study (the original study period).

Hair samples were placed in paper coin envelopes and stored in plastic bags along with a small amount of dessicant beads. Rollers with hair suspected as present were wrapped in wax paper and also stored in plastic bags with dessicant beads in coin envelopes also in the bags.

From the WSU team report: "This differed from Yaeger where these hairs were removed from rollers in the field and preserved the same way as from the barbs. Rayon swabs (MW113; Medical Wire and Equipment) were used for environmental swabbing where lions were seen in camera trap photos interacting with cameras, wire, etc. and were preserved in lysis buffer (50 mM Tris pH 8.0, 50 mM EDTA, 50 mM sucrose, 100 mM NaCl, 1% SDS; Goldberg et al. 2003)."

Swab samples taken from environmental surfaces at alternative hair snare sites or mountain lion kills incidentally discovered later in the study were taken with Rayon swabs moistened with sterile saline, then allowed to dry and stored in protective sleeves, or were immediately processed in some cases.

Servicing was reduced to every other week for three visits due to budgetary constraints limiting the number of biologists that were in the field each week, but with additional funding from the Institute for Wildlife Studies, the monitoring period was extended to a total of 14-15 weeks for most sites. Two sets of sites had one three-week interval for reasons of scheduling and access restrictions imposed by the Marine Corps on Camp Pendleton. Two sites were active for shorter periods due to servicing having to be curtailed for safety reasons as a result of an illegal marijuana grow detection in the area of those sites. Final servicing of all sites was completed in the third week of July 2021 and cubbies were deactivated (bait, scent, and calls were removed from inside the cubbies and sticky rollers and barbed wire removed from the entrances). The cubby sites had been active for a total of 5,521 days including the site that was only active for 3 weeks (average of 93 days per site).

Suspected mountain lion hair, sticky rollers with possible hair, and swabs collected at cubby sites were forwarded to the Spatial Ecology and Conservation Genetics Lab at WSU for DNA extraction. DNA extracted there was then sent to the CDFW Genetics Research Lab in Sacramento, CA for possible individual identification.

After initial lower than expected cubby hair snare success rates, additional funding from the Institute for Wildlife Studies and other funders allowed us to modify the subset of the cubby sites where mountain lions had been recorded as visiting (n=22: 37% of the original sites) in order to test whether other hair capture designs might be more effective (Figure 6). Initially, posts that were

inside the cubbies were moved to the outside at the same site, and duct tape coated with Tree Tanglefoot placed on the posts, along with the same predator calls and scent lure, but no bait or visual lure (Figure 7). At some of the 22 sites, the scent posts with predator calls were moved to locations nearby the original cubby but still within the same subunit where more mountain lion activity (tracks, scat, marking scrapes) had been detected than at the cubby sites themselves. These 22 sites were augmented by creation of 8 additional sites using scent posts and calls in areas where mountain lion activity was known from tracking and separate camera studies for a total of 30 test sites (Figure 6).

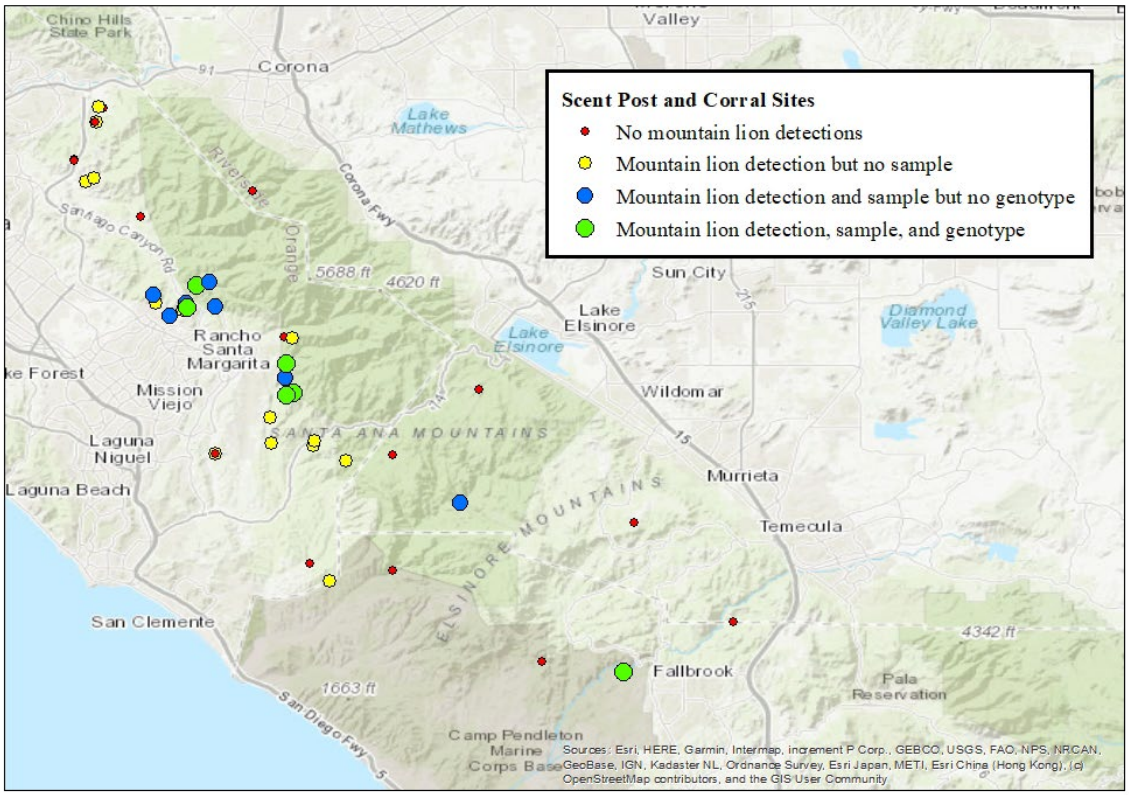


Figure 6. Alternative hair snare sites with mountain lion detections and sample collections with and without genotypes obtained.

Figure 7. Scent post with call and tape. Mountain lion rubbing against post and leaving hair.

Of those 30 sites, those that had not had lion visits after the initial evaluation period (n=52 days average) were decommissioned, and some additional sites were added at locations with likely lion passage based on tracks, sign, marking scrapes, and evidence from separate camera studies (Figure 6).

All remaining sites (n=23) were then modified with “corrals” of smooth wire covered with duct tape coated with Tree Tanglefoot surrounding the scent post (Figure 8). Other modifications were made to the posts to test hair collection potential: either no predator call and just scent, a motion activated call with scent, or a predator call plus motion activated call with scent.

Figure 8. Mountain lion stepping over wire covered with duct tape and Tree Tanglefoot and leaving hair.

Suspected mountain lion hair, and sticky rollers with possible hair, were sent to the San Diego Zoo genetics laboratory for DNA extraction. DNA extracted there was also sent to the CDFW Genetics Research Lab for possible individual identification utilizing the same SNP panel.

Swabs collected at alternative hair snare sites (scent post and corral sites) and incidentally discovered mountain lion kills were sent either to the CDFW Genetics Research Lab or to the San Diego Zoo Genetics Laboratory for DNA isolation.

In addition, scat samples that were opportunistically acquired by biologists during the project field work, tissues from mountain lion mortalities, and blood or tissue from live captures that occurred during the study period were forwarded to the CDFW Genetics Research Lab where DNA was isolated.

All DNA isolated from any source was then analyzed utilizing a Single Nucleotide Polymorphism (SNP) panel developed there for that purpose (Buchalski et al. 2022).

Both the UCD-WHC and WSU teams reviewed all photos from all cameras at the cubby sites for mountain lion presence and recorded occasions of visits and mountain lion numbers. The UCD-WHC team also reviewed all photos from the cameras at alternative hair snare sites and other cameras deployed in the Santa Ana Range by the UCD-WHC team for other projects during the study period. Mountain lion photo occasions and numbers were recorded, as well as demographic information (male

or female, kitten or subadult/adult), and individual ID's if available due to specific markings or collars. Photos of known animals were cropped to the face and grouped according to view (frontal, side, etc) and forwarded to Dr. Jeff Tracey of USGS for training of facial recognition software that he is developing as an adjunct to this project (Task 5).

The WSU lab also counted the total numbers of photos taken of both mountain lions and other animals photographed. From the WSU team report: "Camera trap images were sorted by the WSU Quantitative Wildlife Ecology and Conservation lab crew using visual examination and metadata following the method developed by Sanderson and Harris (2013), combined with characterizing the period of independence between visitation events using time between the last photograph in a series before the first photograph in the next series."

RESULTS Task 2, 3

Mountain lion visits based on photos:

Cubby sites

During the time that the primary cubby sites were active, preliminary review of photos by the UCD-WHC team found that mountain lions were detected on 44 separate occasions (one visit per 125 site activity days), at 22 of the 59 sampling sites (37% of sites). One site was abandoned after three weeks, so 58 sites were active for the majority of the study period). Each site visited had an average of 2 mountain lion visits per site (range n=1-5). At those visits, 50 or 51 mountain lions were detected due to some visits involving family groups (on one visit it is unclear whether one or two animals were present).

Due to 5 photos being taken by the camera closest to the cubby in a burst with each triggering event, any given animal triggering a camera would get photographed multiple times (Figure 4). This is why total photograph numbers reported below by the WSU lab are quite high, but are not reflective of that many individual animals having been at any given site.

Those multiple photos of each animal have allowed us to better identify certain mountain lions with distinctive markings (n=4; Figure 10) at multiple cubby sites (11 times), as well as other camera sites (28 times), and characterize the demographic group of the animal in many instances (Table 1; Figure 9).

Figure 9. Photo of a mountain lion (M294 – nicknamed "Scar") who was detected at multiple cubbies as well as other locations, and by other local photographers with trail cameras, during the study. He was collared during the course of the study but unfortunately later killed by the owner of domestic animals that he was near.

Table 1 – Number of visits of mountain lions to different classes of sites and numbers of mountain lions observed per UCD-WHC evaluations of photos.

Sites where cameras placed	Number of Detections	Number of Mountain Lions	Adult or subadult M	Confirmed adult M	Adult or subadult F	Confirmed adult F	Adult or subadult unk gender	Confirmed Subadult M	Confirmed Subadult F	Subadult unk gender	Kitten M	Kitten F	Kitten Unk gender
Cubbies and other hair snares	149	199	45	5	32	35	31	3	0	0	14	12	22
Daybeds/scrapes	5	9	0	0	0	2	3	0	0	0	2	2	0
Whiting Ranch Project	384	472	46	0	47	80	190	40	0	0	33	33	3
Capture sites	8	12	0	0	0	4	0	4	0	0	1	0	3
Other project camera sites	53	67	12	0	17	6	19	3	0	0	0	0	10
Deterrent testing sites	9	6	0	0	0	1	0	5	0	0	0	0	0
Totals	608	765	103	5	96	128	243	55	0	0	50	47	38

When the photos from the cubby sites were reviewed by the WSU team, mountain lion visits were classified as independent visits when they were more than 12 hours apart (see next paragraph), this resulted in the WSU lab review classifying the number of visits to the cubbies as 40 vs the 44

counted as independent visits by the UCD-WHC team. The review by the UCD-WHC team took into account the gender, size, and other characteristics of the animal if visits were closer together than 12 hours. It is the team's experience that males sometimes follow females at sites within a short period, or recently dispersed young may follow their mother by a few hours, thus represent independent visits even though within a 12 hour window. The 4 visits characterized by the UCD-WHC team as independent visits that were not classified as such by the WSU team were either more than 4 hours apart, or obviously different animals due to markings or gender.

From the WSU team report on the cubby portion of the study: "A total of 178,017 photos were recorded across the 58 stations during the study period, with 140 photos of mountain lions (Table 2). To estimate the number of independent photograph events of mountain lions (e.g., condense multiple photographs of the same individuals during a single visit to a single photograph event), we counted the number of hours between independent events by evaluating the asymptote of the relationship between time interval between events and number of events detected (Figure 10). Based on the stabilization of this relationship at approximately 12 hrs, we chose this as the time period between photographic events where they became temporally independent on average. Visitation events were highest in the first two weeks, with some variation over time (Figure 11), revealing a pattern of waning interest or possible trap shyness. Visitation within the 24-hr daily cycle was highest between 4pm and 4am (Figure 12)."

Table 2. Number of photos by species recorded at 58 game camera stations associated with hair snare stations for detecting mountain lions in the Santa Ana Mountains, California.

Species	Total # of photos
bobcat	715
mountain lion	140
coyote	1057
deer	681
domestic	514
fox	4261
ghost/blur	156161
human	2797
interaction	21
opossum	850
other	10676
unidentifiable	144
Total	178017

Note: 'other' includes rodents, birds, insects, vegetation movement, etc.

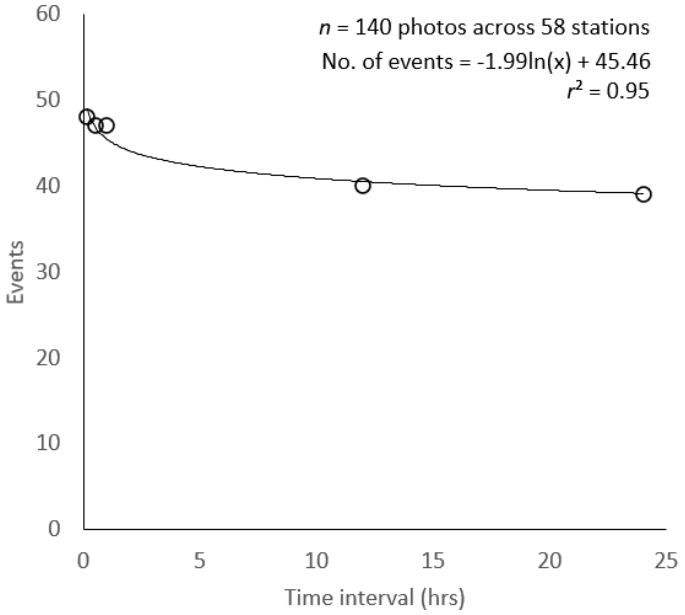


Figure 10. Relationship between number of lion visitation events detected by camera trapping and time interval used to determine independence of those events. Time intervals (10 min, 30 min, 1 hr, 12 hrs, and 24 hrs were selected by the authors for this post-hoc assessment.

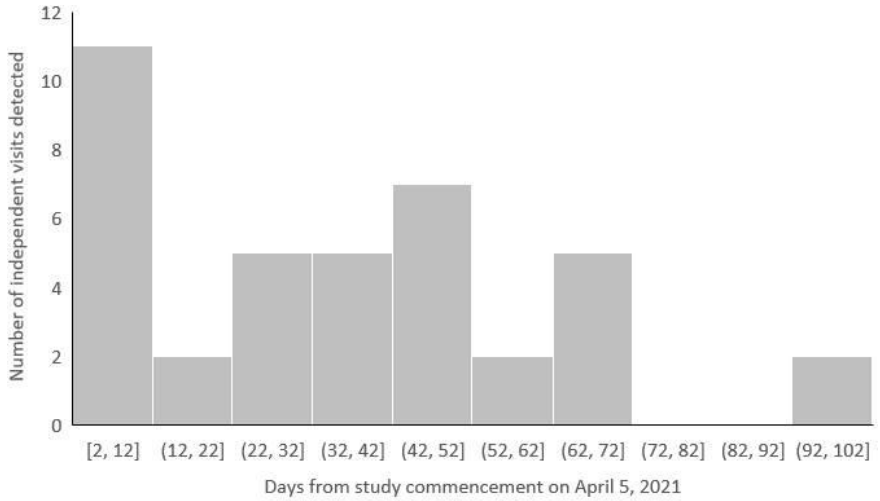


Figure11. Histogram of the number of independent lion visits detected at cubby sites over entire study period. Independence of detection events based on 12-hr interval.

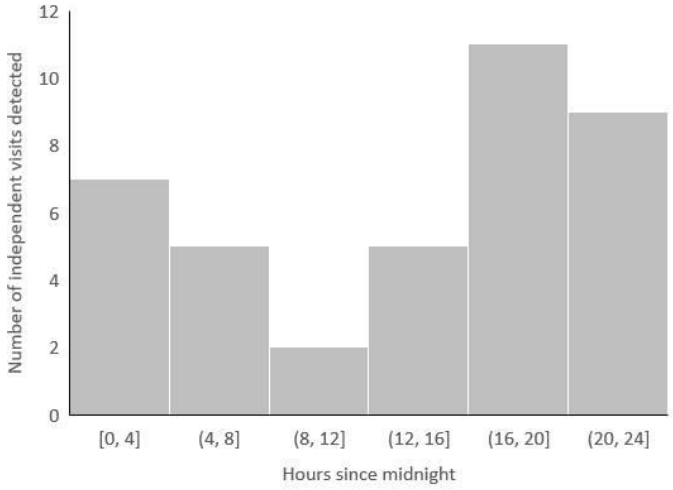


Figure 12. Histogram of detection times of lion visitation events to cubbies over entire study period. Independence of detection events based on 12-hr interval."

Alternate hair snare designs:

Scent post sites

Thirty scent post sites were active for 1551 days (average 52 days per site). UCD-WHC review indicated that mountain lions were detected on 40 occasions (one visit per 39 site activity days), with 48 individual animals detected due to family groups being present on several occasions (Table 1; Figures 6,7).

Wire and sticky tape corral sites

The 23 sites that were modified to hair snare corrals were active for 2145 days (an average of 90 days per site) with detections of lion visits on 80 occasions (one visit per 27 days of site activity) and 113 mountain lions detected Table 1; Figures 6,8). Most sites were deactivated by December 2021. Three sites were kept active until 2/8/2022.

Overall totals for all hair snare designs were 149 visits with 199 individual mountain lions in the photos (Table 1). At all hair snares combined, the demographic group (male, female, dependent kitten, adult/subadult) could be ascertained from photos 83% of the time. The demographic breakdown was 151 adults/subadults and 48 dependent kittens since family groups were present on a significant number of occasions. For adults/subadults, females outnumbered males 67 to 53, with the gender of 31 adults/subadults undetermined, and an approximate ratio of adults/subadults to kittens of 3:1.

In addition, mountain lion photos were taken at multiple other sites in the Santa Ana Range for other projects during this study period. Other cameras deployed for other studies during the project period recorded mountain lions on an additional 459 occasions, with 566 animals photographed. Photos were taken of 479 adults/subadults and 87 kittens on these occasions, with the age class of 3 animals undetermined. Of the adults/subadults, 157 were female, 110 male, and 212 undetermined (Table 1).

In all, mountain lions were detected on 608 occasions with photos of 765 individuals being taken (Table 1, Figure 13). This wide array of cameras on the landscape has allowed us to accumulate a larger picture of the demographic breakdown of a significant percentage of the population that was photographed. Thus overall during the study, the ratio of adults/subadults to kittens was approximately 4:1, and the ratios of females to males (when sex could be determined) was approximately 3:2. Approximately 20% of the total cameras were present in a relatively small area of the range so sampling is biased by territorial animals that were repeatedly photographed on those cameras, nevertheless we think that the information from these cameras adds to our understanding of the population.

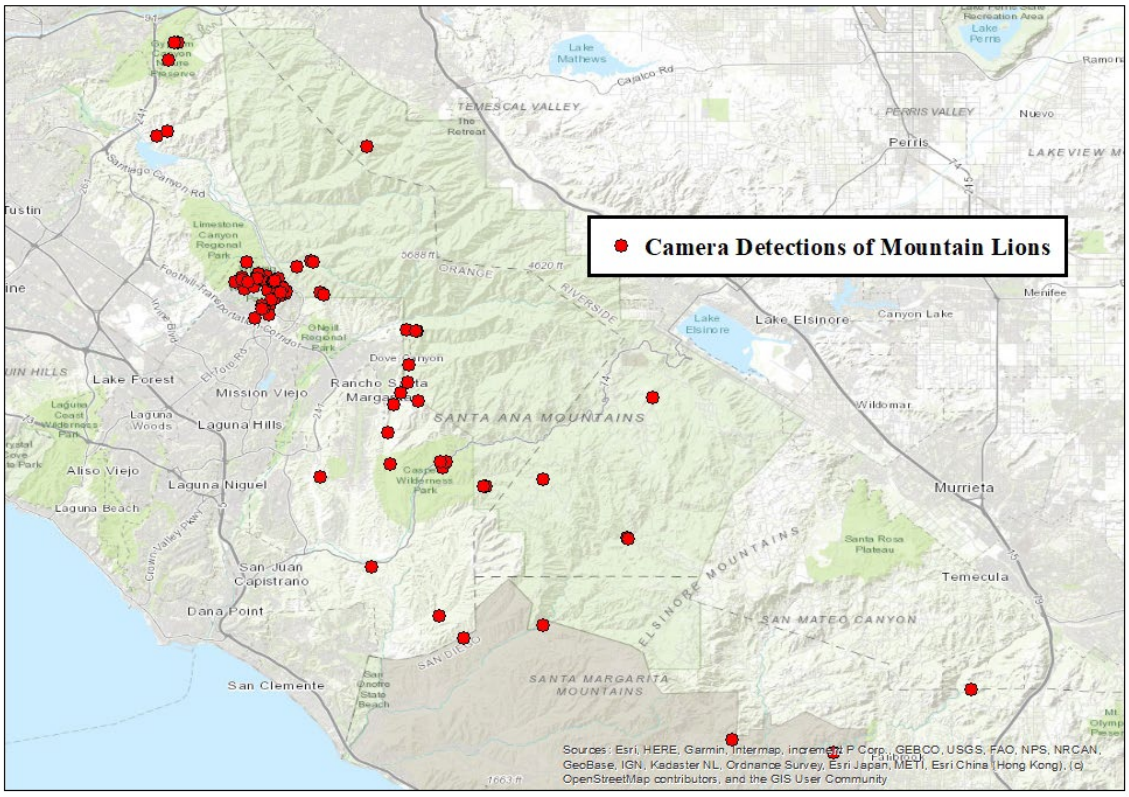


Figure 13. Locations where mountain lions were detected by cameras during the study period.

Samples collected:

Cubby sites

The UCD-WHC biologists were instructed to send any sticky rollers that had been present at a mountain lion visit, had visible hair, or that had become covered with debris, along with any hair collected from barbs, or swabs of environmental surfaces that might have had saliva deposited, to the WSU Goldberg lab.

From the WSU team report: "The WSU lab received 209 samples, including rollers, hair, and swabs. Comparing these with camera trap data and field notes to determine the presence and entry of lions associated with each sample, we selected 43 roller samples and 9 swabs to be extracted. The rest of the samples were rollers collected when they became full of debris and were not associated with the presence or detection of lions. Roller samples from the same day and cubby were combined and rollers and envelopes with no hairs were excluded, resulting in 21 hair samples."

Scent post sites

Suspected mountain lion hair was collected via rollers or barbs on 8 of 40 mountain lion visits (20 %)

Wire and sticky tape corral sites

Suspected mountain lion hair was collected via rollers or barbs on 26 of 80 mountain lion visits (32%)

Other sites

Additionally, while other camera studies were going on or sites serviced, seven hair samples were collected from apparent mountain lion day beds, scrapes, or kill sites at locations where cameras identified six individual lions. In total, 73 suspected mountain lion hair samples from all sources were collected during the study period.

As noted above, a percentage of visits to sites were by family groups. In some instances it was possible to determine which animal left hair; in others, hair may have been left by more than one animal, making DNA identification of each individual separately difficult or not possible.

In addition, in some interactions with the sites, mountain lions "mouthed" or interacted physically with the call or a camera but did not leave hair, and swabs for possible DNA isolation (n=25+) were

taken at those locations, as well as at some mountain lion kill sites. Some of those swabs had DNA isolated at WSU, some at CDFW, and some at the San Diego Zoo Genetics Laboratory.

Samples of blood and/or tissue for DNA were also collected from mountain lions discovered deceased (n=7) or captured (n=11) in the SAM during the study period, as well as animals that were known deceased or that were captured in the ePR.

Genetic analysis and ID assignment

METHODS

Cubby samples

From the WSU team report: "The number of days between a lion detection on camera and collection of swab or hair sample from the field ranged from 1 to 55 days with a median of 7 days. Hair samples ranged from 1 to 10 follicles (maximum used in the extraction) with a median of 3 hairs. Samples from barbs were not combined with roller samples from the same visit. In total there were 30 extractions. Samples were extracted using the DNeasy tissue kit (Qiagen, Inc.), with the addition of a Qiashredder step after digestion for the swabs and final elution in 100 µl. To determine whether the samples had useable DNA and were from lions, we first used 0.14 µM each of CB534 and Tcytbthr primers (Engstrom et al. 2004), the latter extended to 5'CTTCATTCTTTGGTTTACAAGACC3', in a reaction with 1X Qiagen Multiplex PCR Master Mix, 0.5X Q solution (Qiagen, Inc.), and 3 µl extracted DNA in a 21 µl reaction. The thermal profile was 95°C for 15 minutes, followed by 30 cycles of 95°C for 30 sec, 54-47°C touchdown for 90 sec, and 72°C for 60 sec, ending with 60°C for 30 minutes. This targets a 678 bp fragment. After low success (only 4.21.21 41-1-A amplified), we then used FelidID-F and PCon-R (Davidson et al. 2014), which amplifies a 130 bp fragment, in a PCR with 1X Qiagen Multiplex Mix, 0.5X Q Solution (Qiagen), and 0.10 µM of each primer in 21 µl total volume. The thermal profile consisted of initial denaturation step of 95°C for 15 minutes followed by 35 cycles of 94°C for 30 seconds, 46°C for 1.5 minutes, and 72°C for 1 minute followed by 60°C for 30 minutes. Results were evaluated in 2% agarose gels.

DNA testing of Tanglefoot samples:

We tested our extraction techniques on hairs pulled from lion skin and stored in Tanglefoot to mimic roller samples prior to extracting field samples to ensure that inhibitors would be removed by our extraction protocol. We created 6 replicates of 10 hairs with follicles in Tanglefoot, then extracted them after 1 day. We added a Qiashredder step after digestion for 3 of the samples and amplified the extracts with the longer mtDNA marker. All samples successfully amplified. We then stored three additional 10-follicle samples in Tanglefoot at room temperature for two months, extracted and then amplified them using the shorter fragment. All these samples also amplified successfully."

Scent post and Wire and sticky tape corral site samples

Hair and swab samples taken by the UCD team at scent post and corral hair snare sites, as well as other sites where opportunistic samples occurred, were sent to the San Diego Zoo Genetics Lab where they used extraction techniques that are in use there for hair samples from many different species. After extraction, DNA samples were sent to the CDFW Genetics Research Lab for confirmation as to species and attempted assignment to individual ID's.

Scat that was opportunistically collected by field crews, and tissue/blood that were collected during the study period from deceased or captured mountain lions, had DNA extracted at the CDFW Genetics Research Lab as per standard protocols in use there.

Some samples of blood and tissue from the ePR were included in the sample mix, as well as hair, swabs, and tissue/blood collected at the San Diego Zoo Wild Animal Park from a mountain lion captured there and deer that had been predated.

All DNA was then analyzed and individual ID's assigned when possible as per protocols described in Buchalski et al. (2022).

RESULTS

From the WSU team report: "All negative controls tested negative. Eight samples amplified for the shorter mtDNA marker and were sent to the CDFW lab for further analysis (Table 3). We used the date/time-stamped photographs from cameras at each station to estimate the number of days a sample was in the field before collection. Of the hair samples analyzed, samples that failed to amplify for lion identification at the species level mostly had 4 or fewer follicles or had been in the field for 4 weeks or more before collection. Because this was just a lion identification test, we cannot distinguish if these samples were not from lions or were not of high enough quantity and quality for amplification. The difference in amplification from the barb and roller samples collected on the same visit for the longer mtDNA lion identification test indicates that some degradation of samples was occurring in the hair on the rollers, either while in the field or after collection.

Of the 8 samples that amplified for lion mtDNA, 2 were successfully genotyped to individual at nuclear DNA markers (4.21.21 41-1 A and B). These were the only samples that had 10 follicles, and the sample was collected only 3 days after the lion visit. This visit had an adult female and two subadults seen on the station camera. Hair from the barbs was identified to be from a male and hair from the rollers from a female. It also may be noteworthy that we recovered mtDNA from swabs collected from a 3-6-inch diameter piece of woody debris that a lion appeared (in date/time-stamped photographs) to sniff and/or rub its face on apparently 2 weeks prior. This 2-week period we calculated under the assumption that we received all of the photographic data; however, the timestamp reported in the field data summary

received from UC Davis did not match those in the photographs, indicating that either the data summary was inaccurate or photos may have been missing."

Table 3. Samples that amplified for mountain lion mtDNA. *indicates some uncertainty as to when the lion visited.

Lab ID	type	follicles	days in field
5.23.21 19-25	hair from rollers	8	2
5.22.21 21-3	hair from rollers	5	3
4.21.21 41-1-A	hair from barbs	10	3
4.21.21 41-1-B	hair from rollers	10	3
5.4.21 56-1	hair from rollers	4	22
5.23.21 19-25	cubby swab		2
6.13.21 28-3-A	branch swab		14*
6.13.21 28-3-B	branch swab		14*

Overall, success rates for genotyping to individual ID's varied substantially between sample types (Table 4; Figure 14). Tissue/blood is not listed because it is essentially 100%, with rare exception. All samples of that type that were acquired during this study and forwarded to the CDFW Genetics Research Laboratory were successfully genotyped there. One tissue sample sent to the WSU lab was too deteriorated to isolate DNA.

The greatest success (60%) was with swabs from the San Diego Zoo that were taken from wound areas on predated animals – probably because of the saliva of the mountain lion being quite fresh on the skin around the wounds. Swabs taken at cubbies from the environment were not very successful (8%). Deriving individual ID's from scat was moderately successful (44%) and hair from each project team was similar (20 and 28%).

Table 4. CDFW Genetics Research Laboratory success rates genotyping to a specific ID by sample type.

Genotyping Success Rates			
Extracted By/Sample Type	genotyped	total rec'd	%
UCD Hair	15	54	0.28
UCD Scat*	21	48	0.44
SDZ Swab	12	20	0.60
UCD Swab	2	25	0.08
WSU Hair	2	5	0.20
total	52	155	0.34

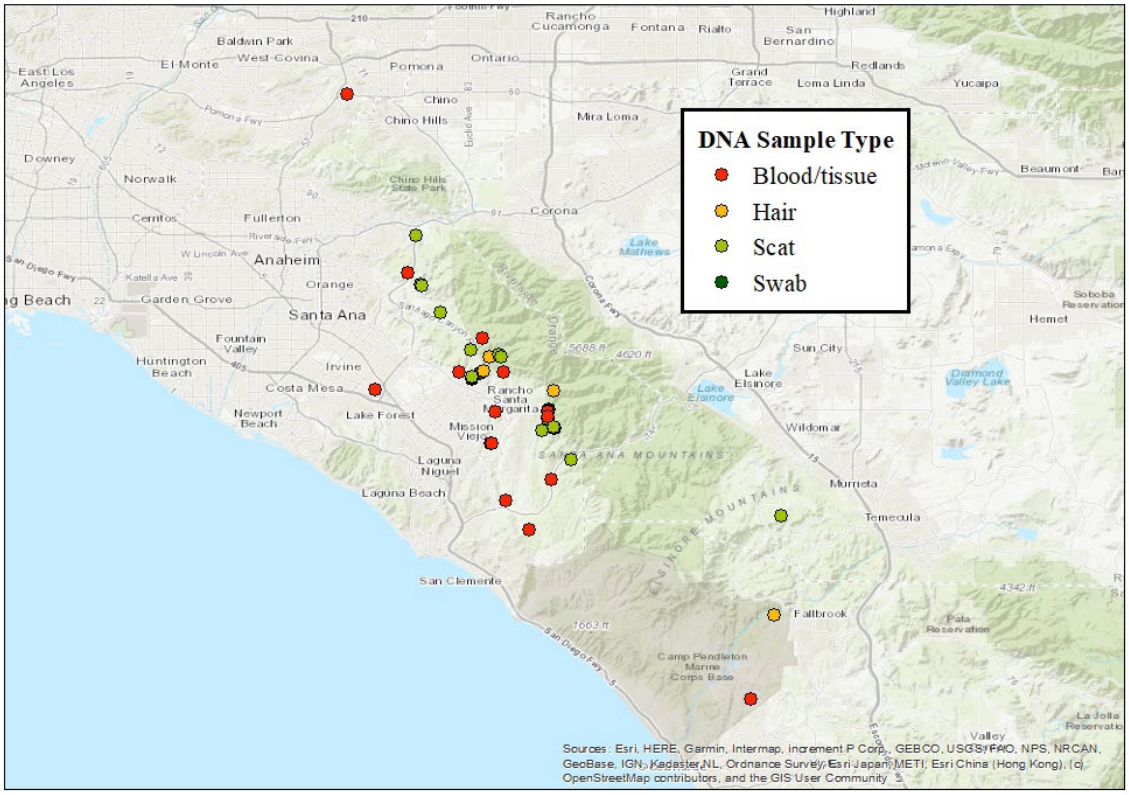


Figure 14. Locations where different sample types yielded a discrete genotype ID.

Each DNA collection method detected the following numbers of animals in the SAM (Table 5; Figure 14):
Blood and tissue from mortalities and captures – n=18*
Scat opportunistically collected – n=14
Hair from snares and opportunistic collection – n=9
Swabs from hair snare or kill- n=2
*Tissue from one additional deceased animal was recovered but the WSU lab was unable to provide DNA from that animal due to deterioration of the tissue, thus it is not certain whether this animal was sampled by other means.

Combining analyses of the different sample types allowed identification of 29 unique individuals with 17 individuals being detected by only one method, and 12 being detected by 2-4 methods (Table

5, Figure 15). Table 5 below shows the detail as well as the demographic group that each animal was in if known. ID's containing an "F" denotes females and "M" denotes males, and "U" denotes individuals where sex could not be determined in the analysis.

The age as determined by direct exams or cameras of the 29 animals identified by genetics in the Santa Anas (including 5 animals detected only after mortality; Table 5) were:
Confirmed Adults (over 30 mo, or female with kittens) – (n=7; 5F, 2M)
Adults or Subadults (Fully grown animals not with mother but exact age unknown) – (n=5; 2F, 3M)
Confirmed Subadults (18-30 mo.) – (n=5; 1F, 4M)
Kittens (0-18 mo) – (n=6; 4F, 2M)
Age unknown (scat detection only) – (n=6; 2F, 2M, 2 could not be determined)

Table 5 – Sources of DNA by which 29 individual mountain lions were identified.

ID	Number of times DNA detected and source				Number of times distinctly marked pumas detected by photos at hair snare sites	GPS Collared	Samples taken at	Known Adult/known subadult/Adult or subadult/ dependent kitten when sampled
	Blood/tissue	Scat	Hair	Swab				
F126	1	2				Pre study	Capture/opportunistic	Known Adult
F270	1						Mort	Known Adult
F291	1	1				During study	Capture/Kill site	Dependent Kitten
F292	1						Capture	Dependent Kitten
F302	1						Mort	Dependent Kitten
F306	1						Mort	Known Subadult
F312	1		2		12	Post study	Capture/Hair snares	Known Adult
F315	1	1				Post study	Capture/Opportunistic	Known Adult
F320	1	1	1			Post study	Capture/Opportunistic/hair snare	Known Adult
F322	1						Mort	Dependent Kitten
F361			1				Hair snare	Adult or subadult
F363			1				Hair snare	Adult or subadult
F365		1					Opportunistic	Unk
F368		1					Opportunistic	Unk
M259	1						Mort	Known Adult
M294	1	3			6	During study	Capture/Opportunistic	Known Adult
M299	1					During study	Capture	Kitten age but independent
M305	1						Mort	Adult or subadult
M313	1	4	2		5	Post study	Capture/Opportunistic/hair snare	Known Subadult
M316	1		2			Post study	Capture/hair site	Known Subadult
M317	1	1				Post study	Capture/Opportunistic	Known Subadult
M318	1	1	1	1			Mort/Opportunistic/Kill site	Known kitten then subadult
M321	1					Post study	Capture	Known Subadult
M362			2		12		Hair snare	Adult or subadult
M364			2	1			Hair snares/swab at hair snare	Adult or subadult
M369		1					Camera site	Unk
M370		1					Opportunistic	Unk
X366		1					Opportunistic	Unk
X367		1					Opportunistic	Unk

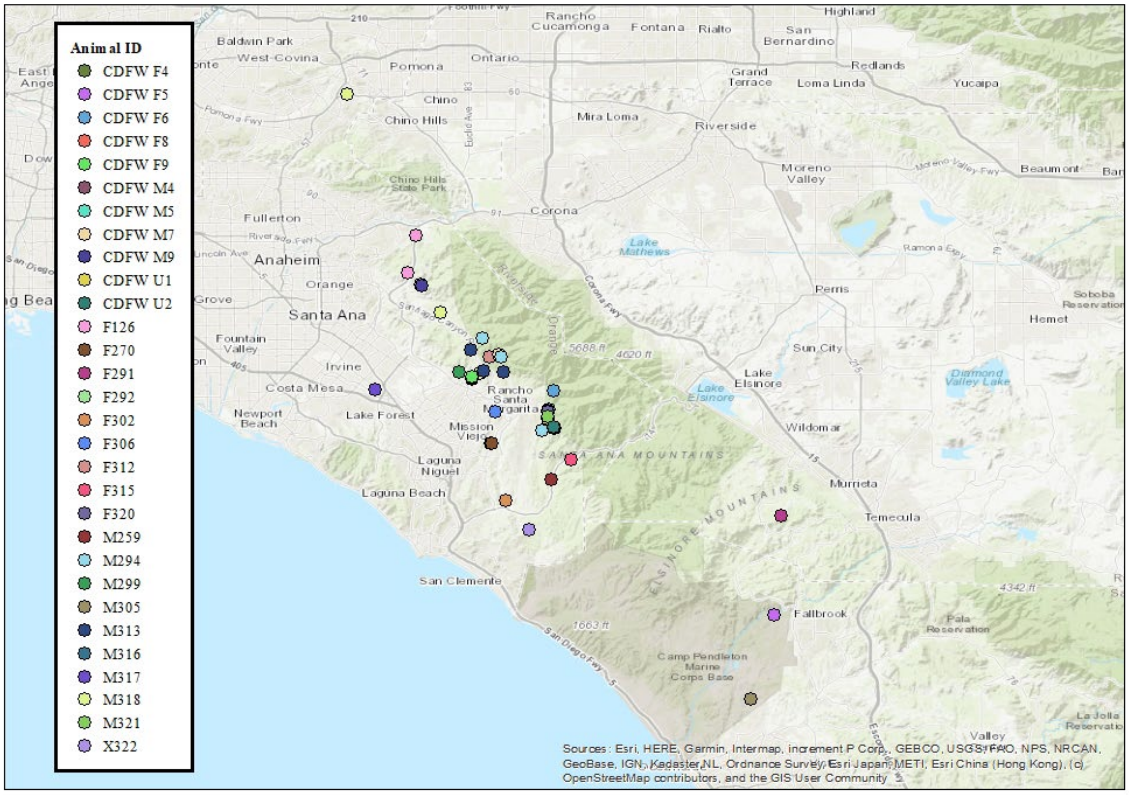


Figure 15 – Locations where samples were taken that yielded discrete genotype ID's.

Task 4 – Development of a population estimate

METHODS

Though using spatial mark-recapture analytic techniques with the results of DNA analysis of hair from the hair snare cubbies, and photos, was the intent at the beginning of the project, the low numbers of hair samples collected that resulted in discrete ID's precluded that technique being utilized. This small sample size and low recapture rate precluded conducting spatial-mark recapture modeling using genotypes from hair to develop a population estimate for mountain lions in the Santa Ana Mountains. However, our collaborator Dr. Justin Dellinger employed the approach developed by Cooley et al. (2009), and used by others (Beausoleil et al. 2013, Dellinger et al. 2018, Elbroch et al. 2020, Beausoleil et al. 2021, Kertson and Keren 2022), to estimate mountain lion density and abundance based on multiple data types including GPS radio-collars (Figure 16) and other occurrence data (e.g., photos of known animals and genetic samples from uniquely identified individuals).

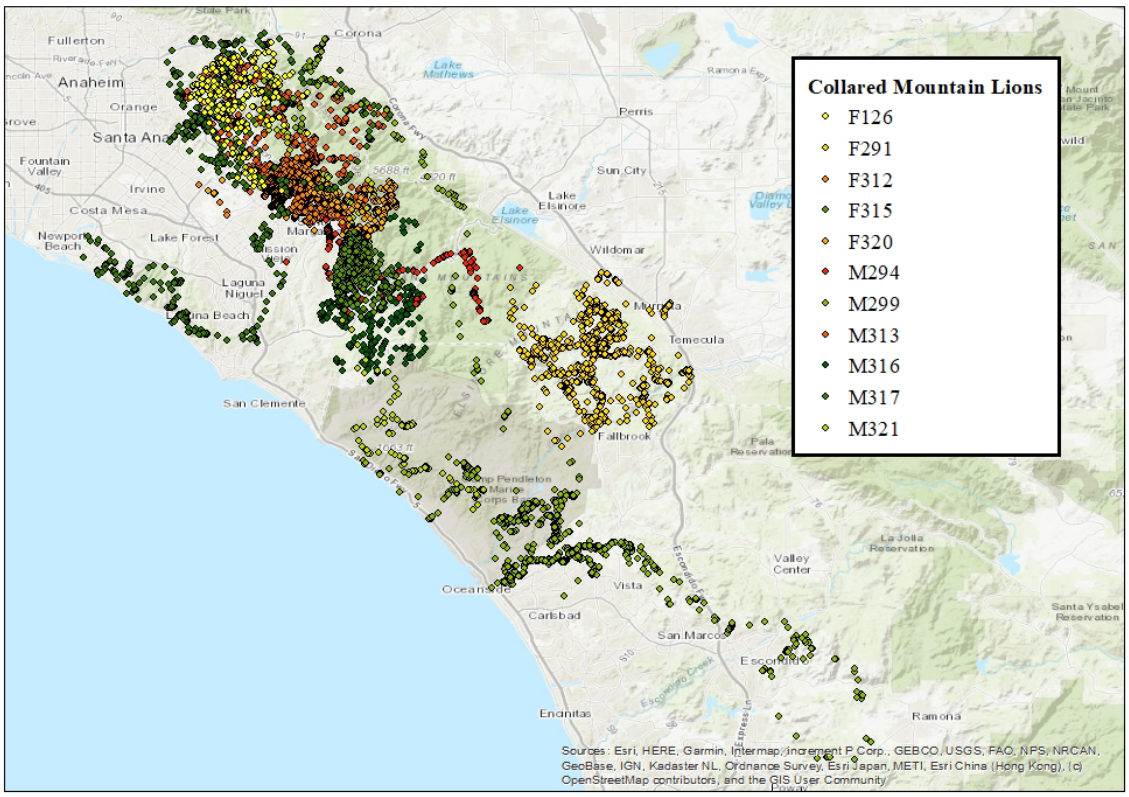


Figure 16. GPS collar data from mountain lions collared during the study period

We estimated mean annual densities of mountain lions (number of mountain lions/100 km^2) in the Santa Ana Mountains as the number of animals multiplied by the mean proportion of male and female locations that fell inside a mean annual 95% composite kernel home range of collared females (Cooley et al. 2009). For uncollared mountain lions that were detected via genetic analysis of hair and/or scat, or that were uniquely identifiable via camera photos (Kelly et al. 2008), we used the mean proportion of marked animals that fell within the 95% composite kernel home range of collared females. We back-calculated the life span of each marked and unmarked mountain lion to the beginning of the study, its birth date (females), or immigration date (males; Stoner et al. 2006, Robinson et al. 2008).

RESULTS – Task 4

Of the 73 suspected mountain lion hair samples collected during the hair snare effort in the Santa Ana Mountains, DNA from 49 was ultimately screened at the Buchalski lab. Seven failed to amplify as mountain lion, 28 were mountain lion but did not amplify to derive a specific genotype, and 14 samples did amplify to a specific genotype and were assigned an ID. Nine individuals (5 males and 4 females)

were represented in the 14 samples, which equates to a recapture rate of 1.56. Five of these individuals were also detected via other means during the study.

When considering all sample types (e.g., blood, scat, hair, and swab), we detected 29 individual mountain lions in the Santa Ana Mountains (Table 5). Of these 29 individuals, 12 were detected using multiple sample types (Table 5). Nineteen individuals were detected via opportunistic sampling of captured and deceased mountain lions while 14 individuals were detected via opportunistic scat collection (Table 5). Combining the mortality and capture related samples with scat samples would have resulted in 25 individuals detected of the 29 that were detected by all methods during this study. Thus, it appears possible that opportunistic sampling of deceased and captured animals (e.g., during 'no-harm-no-foul' conflict settings or collaring for other reasons) as well as scat collection could be a viable method for monitoring mountain lion demographics in the Santa Ana Mountains.

The primary thing that differentiates the opportunistic sampling of dead or captured animals, and camera data, from non-invasive sampling via scat is the additional demographic detail that can be gained (Table 1). For example, you can generally determine the age class, and maybe reproductive status, of an animal when examining the carcass or body, and in many cases from camera photos. Whereas with non-invasive samples such as scat, one can only tell the sex, not the age or reproductive status, of the animal detected. The additional demographic information from the other methods can provide valuable information and provide insight when a population is limited genetically and demographically (Benson et al. 2019).

Using the approach detailed above in the Methods, we derived a density estimate of 1.6 mountain lions per 100 km^2. Using information on suitable habitat in the Santa Ana Mountains from Dellinger et al. (2020) and Benson et al. (2019) (which excludes the Chino Hills), we then derived an abundance estimate of 24.5 - 32 mountain lions throughout the area dependent on the inclusion or exclusion of the Chino Hills. Though likely occasionally used by mountain lions as we have shown in this study, it is unlikely that the density or amount of use in the Chino Hills is more than a fraction of the use in the remainder of the SAM. Thus we would expect the overall abundance to be at the lower end of this range. This estimate does not differentiate between demographic classes, thus is not intended to suggest this number of territorial adults are likely present, and is more appropriately regarded as a minimum population estimate.

An alternative method of population estimation utilizing extrapolation of findings from a statewide scat DNA-based study (Dellinger et al. in prep) and habitat mapping suggests a somewhat higher density estimate of 2.12 (1.9-2.3) animals per 100 sq. km. This estimate is based on the expectation of minimal detection of scat from animals less than 10-12 months of age, and extrapolation from scat sampling done in 12 different regions of the state. One of those regions however did include the eastern Peninsular Range adjacent to the Santa Anas. This density estimate results in an estimated population of 32.4 (95% CI: 29-35.2) total animals above 10-12 months of age.

2. Benson et al. (2019) estimated the capacity of the range as5-7 adult males and 11-14adult females based on territory sizes and habitat availability (exclusive of the Chino Hills, which were presumed to be unoccupied or minimally occupied). Thus, both estimates, along with our minimal population of 17 adults and subadults, is not inconsistent with the Benson capacity estimate.

Benson et al. (2019) estimated the capacity of the Range as 5-7 adult males and 11-14 females (exclusive of the Chino Hills) based on territory sizes,habitat availability, and Beier and Barrett (1993)'s estimate of a population density of 1.05 animals per 100 sq. km. (which included the Chino Hills which are currently presumed to be unoccupied or minimally occupied). This density estimate is, somewhat less than both our current density estimatesNevertheless, both our estimates are relatively consistent with these previous estimates since those estimates were inclusive of adults only and assumed uniform density across the range.

Of the 29 known animals detected during this study period, only around one quarter (n=7) were confirmed adults, with 6 confirmed subadults, 5 adults/subadults but exact age not known, 6 animals of unknown age (scat only), and 5 confirmed kittens. If the 5 adults/subadults and 6 animals of unknown age were split between adults and subadults as with the confirmed animals, then the number of adults in the minimum population count could be in the range of 12 or more. Given that most of our sampling success was on the western side of the range where density is highest, these findings are not inconsistent with the density and abundance estimates or capacity estimates outlined above

These findings suggest that there is value in taking every advantage of opportunities to collect DNA from mountain lions, whether systematic or opportunistic, including taking swabs from animals that mountain lions are found to be feeding on.

Task 5 – Photo software development

RESULTS – Task 5

Analysis of photos has yielded information on sex and differentiated between dependent kittens and adults/subadults for approximately half of the animals photographed. Four animals that have been photographed multiple times have distinctive markings, and 3 of the 4 have also been captured and sampled (Table 5, Figure 9).

During the course of the study our team, including dedicated volunteers, has cropped over 4,000 photos of known animals to highlight the head and facial features. These photos came from our study in southern California, and those of our collaborative project in Modoc and Lassen Counties with the Institute for Wildlife Studies, Dr. Quinton Martins with the Living with Lions Project in Sonoma-Napa Counties, and Dr. Mark Elbroch of Panthera who contributed photos from 3 different

projects. These cropped photos have been forwarded to Dr. Jeff Tracey of USGS for training of software for potential automated detection of individuals. Additional cropping of photos is continuing as the UCD-WHC team continues to have significant numbers of cameras in the field on several projects.

Additionally, the UC Davis field team has been experimenting with field methods for acquisition of better-quality photos for this purpose using the cameras, predator calls, modified calls that are motion triggered, and scent lure utilized in the hair snare sites. These have yielded many high-quality images that will then be matched with individual lion IDs once DNA samples from those sites and times are genotyped.

Task 6 – DNA analysis of tissue and blood, and pedigree updating

METHODS

This task is being completed by Dr. Holly Ernest at the University of Wyoming, PI Vickers, Dr. Buchalski at CDFW, and Dr. Kyle Gustafson at Arkansas State University to update the pedigree created by the same researchers for the SAM and ePR populations and published previously (Gustafson et al. 2017).

Updating of the population genetic structure, minimum population size, and other genetic parameters will also result from this collaboration. Pedigree work will be completed along with comparisons between the SNP and microsatellite techniques, and a separate publication developed for peer review, in summer 2022.

RESULTS – Task 6

High quality DNA was isolated by Dr. Ernest's lab from 34 of 35 samples of tissue and blood collected prior to 2020. Microsatellite characterization of the 34 samples has been completed for comparison to previous samples and pedigree updating. Dr. Buchalski has also genotyped these same samples using the SNP array that he has developed for individual identification, including the majority of earlier samples that were incorporated into the original published pedigree from the region (Gustafson et al. 2017), and the statewide SNP-based analysis recently published by Dr. Gustafson (Gustafson et al. 2022). DNA from this study period from hair, scat, tissue/blood, and swabs was also genotyped by Dr. Buchalski's SNP array as noted earlier in this report – yielding 26-30 additional individuals that will be included in the analysis if possible.

Other genetic analyses using tissue and blood based DNA are beginning in a separate study that is also using genetic samples collected in the Santa Ana Mountains and the adjacent portions of the larger UC Davis study area. This study being led by UC Santa Cruz researchers is a whole genome analysis study

that will utilize samples from across the state to analyze DNA from 500 individual mountain lions. A special focus will be three potentially threatened populations along the coast - the Santa Ana Mountains, Santa Monica Mountains, and Santa Cruz Mountains populations. Information from this study will add significantly to our understanding of the Santa Ana Mountains population genetics.

Task 7 – Population estimation technique comparisons

RESULTS – Task 7

Using demographic (e.g., survival and reproduction), genetic (e.g., levels of inbreeding and relatedness), and spatial (e.g., GPS locations) data, and previous research (Beier and Barrett 2003), Benson et al. (2019) developed a population viability analysis for the Santa Ana Mountains that estimated between 16-21 adults in the population.

Using genetic capture-recapture data derived from scat, and GPS locations from radio-collars, Dellinger et al. (unpublished data) estimated an average of 1.5 (95% CI: 1.3-1.7) mountain lions per 100 sq.km. statewide, and extrapolation from that statewide average would result in an abundance of 23 (95% CI: 19.9-26) mountain lions in Santa Ana Mountains exclusive of the Chino Hills. This estimate is intended to include subadults and adults but not kittens.

However, as seen in the earlier results, a more refined estimate extrapolated from the statewide scat-based study (Dellinger et al. unpublished data) that takes into account habitat quality in the Santa Anas yielded an estimated density of 2.12 per 100 sq.km., and an abundance of 32.4 (95% CI: 29-35.2) animals exclusive of the Chino Hills.

The density estimate derived from the data collected herein at 1.6 is in the middle between the extrapolation from the statewide scat-based study (1.5 - 2.12 per 100 sq. km.), and the densities calculated by Beier and Barrett (1993) and Bensons et al. (2019) of 1.05 per 100 sq. km. However, it should be noted that the radio-collar data used to define the study area in our estimate from this study primarily included females that circulated on the western side of the Santa Ana Mountains where primary productivity is higher than the drier eastern side of the range. This higher primary productivity likely means mule deer abundance is higher on the western side of the range as compared to the eastern side. This greater primary productivity, and thus higher primary prey abundance, could allow mountain lion density to be higher in this portion of the range than elsewhere in the range (Stoner et al. 2018).

The naturally low variation in mountain lion density (Beausoleil et al. 2021), and the small size of the Santa Ana Mountains relative to mountain lion spatial requirements (Dellinger et al. 2020), means such variation in density on the western and eastern side of the range would be slight and difficult to detect at pertinent spatial scales.

Thus, our density estimates could be expected given that our approach attempted to account for all age classes. Overall, our efforts demonstrate that multiple data streams can allow one to derive defensible mountain lion density and abundance estimates that are comparable to other methods. Detecting sigficant change in population density and abundance over time however will require use of the same method or combination of methods repeatedly at regular time intervals – perhaps every 3 years - to be able to detect population decline in a timely enough fashion to allow intervention .

Camera based approaches with newer analytical techniques, and possibly long term radio collars, should also be considered and tested for long term population monitoring.

Tasks 8 and 9 – Conducting a workshop and reporting its results

A workshop was held via Zoom with 6 experts in population estimation and mountain lions specifically in late March, along with 1 expert in habitat management. The experts workshop came to no specific conclusions or recommendations regarding long term monitoring of mountain populations in the region, though there was general agreement that the strategies for the SA and ePR ranges may need to be different. There was also general agreement that detecting a population decline in the SA range would be difficult or impossible in early stages unless intensive monitoring via collars is ongoing. The SA range may benefit more from having marked animals (collared/otherwise sampled and marked) on the landscape to assist in mark-recapture based assessments (Beausoleil et al. 2013, 2021). The small size of the population means that detecting changes at the population level is much more difficult until they are quite significant. The ePR could potentially be effectively monitored with techniques like periodic scat surveys combined with some combination of the techniques recommended for the SAM.

Task 10 – Reporting

Dr. Vickers generated this report with the assistance of Dr. Dellinger. Dr.'s Manning and Goldberg created a separate report with one of their graduate students, T.M. Hopkins, detailing only the cubby-based hair snare project details. That report is referred to and quoted in this report as "WSU team report."

Discussion

Initial results suggest that scent posts out in the open and along travel-ways, especially if augmented with surrounding wire with sticky tape are more effective hair collection devices than the original cubby arrangements in the summer environment in southern California. However, even those arrangements were not effective enough to yield adequate numbers of captures and recaptures of individuals to allow analytical analysis to yield significant population information by itself. This is partly due to the low success rate amplifying DNA recovered from the hair that was collected, which

may be related to the environmental conditions, time between hair snare checks, laboratory techniques used (though DNA from scat and tissue amplified relatively well), or other unknown factors. It is possible that a more focused effort (shorter in time frame) with improved hair-catch devices could be more effective, but this would have to be tested.

It is evident also that any of the hair snare techniques tested are labor intensive, in that they require construction time and recurring visits to sampling sites. Efficiency and cost of using these methods is highly dependent on how long hair can be expected to remain viable in the environment under summer conditions or at other times of year. This determines the needed frequency of staff visits and the person-hours required. Because visit intervals became longer in the second and third phases of the study, this became more of an issue and also led to drying of the tape and Tree Tanglefoot, reducing its effectiveness for capturing hair. Later in the study animals also seemed to become less responsive to the predator calls, likely because some animals had likely run across them multiple times at that point.

From the WSU team report: "The results from this study and sampling design reveal that southern California mountain lions can be lured to sampling stations by using automated predator calls. Over the 101-day study, 140 photographs of lions visiting cubby stations were obtained. Based on the estimated 12-hr period of independence between detection events, this translated to an estimated 40 temporally independent spatially explicit detections. This indicates that as observed in Colorado mountain lions by Yeager (2016), the automated predator call was effective at attracting mountain lions, which is an important step towards implementing non-invasive sampling. However, despite Yeager's relatively high success at luring lions into their cubbies and collecting fur with barbed wire and Tanglefoot rolls, we unexpectedly had low success at luring lions into cubbies (based on photographs and hair samples). This greatly reduced our ability to acquire hair samples necessary for identifying individuals and the sex of lions that visited our stations.

One reason for this may be related to the dimensions of our cubbies, albeit the design was to construct cubbies with dimensions similar to those constructed by Yeager (2016), our cubbies appeared to be small relative to the size of the lions, as photographs revealed adult lions that did enter were unable to turn around while inside (Figure 4). Such small dimensions may have deterred lions from entering. Alternatively, the carrion (e.g., deer hide and meat) appeared to be removed by mesocarnivores soon after each time the meat was refreshed, leaving a cubby without the fresh meat lure for the remainder of the 2-week period. This may not only explain the observed lack of interest in entering cubbies, but also the observed decline in station visits after the first week (27% of the 40 independent detection events occurred during the first biweekly period; Figure 11). Such rapid drops in detection events are generally viewed as subsequent disinterest or shyness of sampling/trap stations, which should be accounted for in designing future population monitoring efforts.

Individual ID in this study was only obtained from samples that had 10 follicles and was successfully obtained from hairs stored in Tanglefoot. All samples that tested as lions for species ID were obtained

from samples collected within 22 days of deposition and had at least 4 follicles. Almost all of the samples that failed species ID had 4 or fewer follicles. The camera trapping data indicated that there were also many visits by lions to the cubbies where they did not leave hair on rollers or barbs. This indicates a primary limitation for this method is getting lions to interact with hair collection devices at trapping sites, and to leave enough hair behind for genotyping. Results also indicate that prompt collection of those hairs may be important for preventing degradation under these field conditions."

However, by utilizing multiple streams of data and DNA sources, our UCD-WHC team was able to generate a density estimate using alternative methodologies. This density estimate is consistent with our expectations for this population based on previous research both there and elsewhere in the state. This gives some comfort that density and abundance estimates for the Santa Anas can be achieved in more than one way or group of ways, but that determining the most cost efficient methods overall remains dependent on future testing – pending scat dog testing in the Santa Anas and possible testing of other analytical methods utilizing camera arrays.

The work here points out that detecting population change of significance in a population of this size is a challenge, and the best long term monitoring methods are not yet completely clear. Nevertheless, this work has moved us and the wildlife agencies closer to the ability to determine strategies that will balance costs with the information that can be gained with different techniques.

Acknowledgments

The field crew and collaborative efforts of volunteers for this project have been essential. Our primary field crew members have worked many long hours in the field, and they are: Armand Amico, Calvin Duncan, James Bourdon, Juan Gonzalez, Lina Vu, and Rich Codington. Laura Coley Eisenberg, manager for Rancho Mission Viejo Reserve monitored and serviced cubby sites established on the Rancho Mission Viejo Reserve. Nate Redetzke of Marine Corps Base Camp Pendleton monitored and serviced cubbies established on the Base. Biologists at the Fallbrook Naval Weapons Station assisted with access to that facility. Collaborators at The Institute for Wildlife Studies and Dr. Jessica Sanchez supplied supplemental equipment for the 2021 field season.

Volunteer Ishwinder Battoo has assisted in organizing and shipping samples and reading camera cards, along with volunteer Robert Detrano. The Cleveland National Forest, CDFW, Orange County Parks, Irvine Ranch Conservancy, Santa Margarita Ecological Reserve, Bureau of Land Management, Wildlands Conservancy, Western Riverside County Regional Conservation Authority, Audubon Starr Ranch, the Conservation Fund, The Nature Conservancy, The Center for Natural Lands Management, and Rivers and Lands Conservancy have all allowed land access to conduct project activities. The UC South Coast Research and Extenstion Station has hosted the UC Davis team and its equipment. This large and complex sampling effort would not have been possible without the cooperation of all of these entities and persons, and we are extremely grateful.

Scientific collaborators have included Dr, Mike Buchalski, Dr. Caren Goldberg, Dr. Jessica Sanchez, Dave Garcelon, Kristen Ahrens, Dr. Mrinalini Watsa, Dr. Cynthia Steiner, Dr. Matthias Tobler, Dr. Jeff Tracey, Trish Smith, and Dr. Kris Preston

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