

# California Fish and Wildlife **JOURNAL**

## SPECIAL ISSUE

The California Endangered Species Act: Successes and Challenges



*Journal for the Conservation and Management of  
California's Species and Ecosystems*

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# California Fish and Wildlife

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SPECIAL ENDANGERED SPECIES ISSUE

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# California Fish and Wildlife Journal

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This issue would not have been possible without the amazing team of guest editors from various programs throughout CDFW who volunteered their time and expertise for this issue.

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**MADELEINE WIELAND** has a bachelor's degree in Wildlife, Fish, and Conservation Biology from the University of California, Davis where she also worked as a research assistant on the UC Davis Wood Duck Project. Madeleine joined CDFW in 2011 as a scientific aid specializing in Scientific Collecting Permitting and Memoranda of Understanding for research on CESA listed species. Currently, Madeleine is an Environmental Scientist in the Environmental Review and Permitting program where she assists in the statewide coordination of CESA Incidental Take Permits, Safe Harbor Agreements, Voluntary Local Programs, and Habitat Restoration or Enhancement Act approvals. Madeleine is also a team lead for California's Cutting the Green Tape initiative which aims at increasing the pace and scale of ecological restoration and stewardship.

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in the Lake and Streambed Alteration (LSA) Program, specializing in cannabis cultivation permitting. Currently, Margaret helps to develop and coordinate adherence and consistent implementation of LSA Program policies relating to cannabis cultivation. She also focuses on outreach and education with cannabis farmers and is the CDFW lead contributing to the development of “Wildlife Conscious” certification for cannabis farmers.

**DANIEL APPLEBEE** is currently the Conservation and Recovery Unit Supervisor in the Wildlife Diversity Program in CDFW’s Wildlife Branch. He has worked for over 25 years as a professional wildlife biologist in California for industrial forestland owners, the Central Region’s Habitat Conservation and Timberland Conservation Programs, and the Wildlife Branch. Dan’s field experience has included designing and conducting occupancy surveys for California spotted owls (*Strix occidentalis occidentalis*); studying the habitat relationships of spotted owls in managed conifer forests; spotted owl and Northern goshawk (*Accipiter gentilis*) habitat use telemetry studies; surveying for, and studying habitat relationships of great gray owls (*Strix nebulosa*); mesocarnivore occupancy surveys; native trout (*Oncorhynchus* spp.) occupancy and composition surveys; surveys for amphibians (*Rana boylei*, *R. sierrae*, *R. draytonii*, *Anaxyrus canorus*) and blunt-nosed leopard lizards (*Gambelia sila*); spotlight transect surveys for San Joaquin kit fox (*Vulpes macrotis mutica*) and giant kangaroo rat (*Dipodomys ingens*); and mule deer (*Odocoileus hemionus*) herd composition counts. Dan received a B.S. in Wildlife Biology with a minor in Forestry from Humboldt State University and he is a Certified Wildlife Biologist.

**MAX FISH** is an environmental scientist with the Department’s Fisheries Branch. He received a B.S. in Wildlife and Fish Conservation Biology with an emphasis in Aquatic Ecology from UC Davis. He has worked for CDFW since 2007. He spent seven years working with estuarine fishes and invertebrates on the San Francisco Bay Study before moving to Sacramento to work with inland sport fishes. In his free time Max enjoys spending time outdoors with friends and family camping, fishing, and hunting.

**JENNIFER OLSON** is a Senior Environmental Scientist (Specialist) with the Department’s Coastal Habitat Conservation Planning group in the Eureka field office. She has worked for the Department since 2013 in a variety of roles, primarily focused on environmental review and permitting. She currently serves as the Caltrans Liaison for Del Norte, Humboldt, and Mendocino Counties. Prior to working for the Department, she worked as a Research Associate for the Montana Cooperative Wildlife Research Unit where she supervised field crews and data management for projects focused on life history variation in songbirds in the U.S, Venezuela, and Malaysian Borneo. Jen is originally from Minnesota and has a bachelor’s degree in Environmental Studies from the University of Minnesota-Duluth. In her free time, she enjoys birding, running, finding new places to go hiking and backpacking with her husband and her dog, and expanding her natural history knowledge about her Northern California home.

**BILLIE WILSON** is Senior Environmental Scientist (Supervisor) for North Central Region’s (Region 2) Habitat Conservation (HabCon) Program. She earned a Bachelor of Science degree in Park and Recreation Resource Management, with a concentration in Natural Resources, from CSU- Sacramento in 2004. She began her career with CDFW in the Wildlife Branch in 2002. After graduating college and working for a couple other

## Editors for this issue—continued

state agencies, she joined CDFW's Habitat Conservation Planning Branch (HCPB) where she worked for almost seven years. While in HCPB, she spent almost four years as an Environmental Scientist in the California Endangered Species Act (CESA) Program reviewing and editing hundreds of incidental take permits and consistency determinations from throughout the state. Currently, she supervises scientific and administrative staff who perform environmental review and permitting for Region 2's HabCon Programs, including Lake and Streambed Alteration Program, CESA, and CEQA, for various counties and Caltrans projects.

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**SCOTT OSBORN** is the Department's Statewide Coordinator for Small Mammal Conservation and works on CESA issues for the Mohave ground squirrel and a variety of other listed rodents, as well as the conservation of bats, insectivores, and lagomorphs. He is co-lead for CDFW's response to the threat of White-nose Syndrome in bats, is a partner in the California North American Bat Monitoring Program, and chairs the Mohave Ground Squirrel Technical Advisory Group. Scott received his B.S. degree in Biological Sciences from University of California, Irvine and M.S. and Ph.D. degrees in Ecology and Evolutionary Biology from the University of Arizona, where he studied the energetics and thermoregulatory behavior of heterothermic desert rodents. He has worked as adjunct faculty at the University of Arizona, as an environmental consultant, and joined CDFW in 1999. He is a Certified Wildlife Biologist, Past President of the Western Section of the Wildlife Society, and recipient of the Western Section's Dasmann Award for Professional of the Year in 2012.

## Introduction

RICHARD MACEDO, *Chief (Retired), Habitat Conservation Planning Branch, California Department of Fish and Wildlife*

Enacted in 1970, the California Endangered Species Act (CESA) is one of California's most recognized environmental laws and, to many, it is the most vexing of such laws. Along with the federal Endangered Species Act (passed in 1973 by President Richard Nixon), both laws were enacted to protect imperiled plant and wildlife species from extinction. CESA's notoriety generally stems from the relatively few instances where listed species have affected land use interests with resulting high-profile news stories. Spotted Owl, for example, garnered widespread news, ranging from a bellwether for lost old-growth forest habitat to a mechanism for restricting logging and other land use endeavors.

The Governor-appointed California Fish and Game Commission (Commission) is responsible for listing and delisting threatened/endangered species under CESA. The Department of Fish and Wildlife (Department) is charged with reviewing CESA petitions, preparing Status Review reports with recommendations, and providing expertise to inform the Commission's decision-making process (see flow chart on page 27 for more details). The Department is also responsible for issuing CESA-required permits and monitoring the condition of each listed species.

To date, 316 plant and animal species are protected under CESA or by preceding laws. These species range from those having very restricted geographic ranges to species inhabiting a large part of the state. For example, CESA-listed clades of foothill yellow-legged frog (*Rana boylei*) cover two-thirds of the state, while the plant species coast yellow leptosiphon (*Leptosiphon croceus*) currently occupies an area of coastal bluff roughly the size of a volleyball court.

Of California's 316 protected species, the vast majority involve plant taxa (i.e., 222 plants are currently listed under CESA or by preceding laws). These plant species do not often generate the news headlines or high-profile controversies of their animal counterparts; however, their preponderance within California's endangered species sphere dictates that plants will play a consequential role in CESA's future.

This special edition of the California Fish and Wildlife Journal follows other recently issued special editions; "Effects of Fire on California's Resources", "Impacts of Cannabis Cultivation on California's Fish and Wildlife Resources", and "Effects of Non-consumptive Recreation on Wildlife in California." While articles in these special Journal editions center on scientific research, a less predictable theme emerges, one that recognizes the importance and benefits of collaboration, finding common ground, and successfully engaging all affected interests. In truth, it has not been convention to fully embrace such elements when implementing science-based actions or regulations involving CESA. Perhaps it should if we hope to advance CESA into a more effective and valued program.

Last year marked the 150th anniversary for both the Commission and the Department. Over the past century and a half, these agencies have been tasked with implementing many new laws and responsibilities, CESA being one of the more significant of these tasks. As California's population grows, so will CESA-related challenges. For the sake of endangered

species and the future of California's natural history, it will be necessary to apply sound science and social imperatives in order to pioneer a pathway for success. Such a pathway will require partnerships comprised of diverse interests and a commitment to protect and recover endangered species while adequately responding to other interests including property rights and potential economic consequences. Property rights and economic impact concerns could conceivably be addressed by establishing a state-sponsored endowment or other funding mechanism that would serve to offset such burdens without undermining the integrity of protection and recovery measures for CESA-listed species. Without reliable and functioning partnerships along with adequate funding strategies, the future of many endangered species will remain in peril.

Recovery of CESA-listed species is an endeavor many Californians could get behind. For the conservation community, recovering imperiled species is an obvious aspiration as it aligns with important fundamentals in conservation biology. For other interests, including the regulated community, successful recovery of CESA-listed species would reduce the risk of higher project costs, prolonged construction timelines, and other burdens where affected properties support listed species. Ramping up recovery will require further commitments including funding and policy actions. Investing now in species recovery would be more convergent than today's focus which requires timely responses to listing petitions and the drafting of recommended protection measures for species that warrant CESA listing. Expanding our commitment to recovery would not only improve the condition of many imperiled species, it could also deliver what has alluded CESA's orbit thus far, common ground and a more comprehensive allegiance toward species recovery.

This issue of the *California Fish and Wildlife Journal* not only covers a wide spectrum of topics involving CESA, it also encompasses much of California's exceptional geography. Beginning with Policy and Regulations in Section 1, this issue follows with a plant section and sections covering several classes of animals: invertebrates, fish, amphibians, reptiles, birds, and mammals. Readers will also find varied reporting perspectives reflecting California's unparalleled species diversity. My gratitude to the authors of this special edition for their valuable contributions toward CESA and the imperiled species it safeguards.

## Introduction—continued

WAYNE D. SPENCER, *Chief Scientist, Conservation Biology Institute*  
JERRE ANN STALLCUP, *Conservation Biology Institute (Retired)*

California's Endangered Species Act (CESA) was passed in 1970, three years before President Nixon signed the federal ESA. For half a century, both laws have helped stem the tide of species extinctions, raise public awareness about the plight of wildlife, and underscore the need to balance species conservation with economic development. During the 21st century, advances in conservation science and innovative land-use policies have augmented species protection laws like CESA to better address our growing climate and biodiversity crises. California has shifted away from single-species protection to conserving networks of functional, sustainable, ecological communities—with all their constituent species—despite rapidly shifting baselines. This more holistic and forward-looking approach requires even more sophisticated science to deal with a non-analog future. Perhaps most important it requires even greater collaboration among all parties with a stake in healthy ecosystems.

As in so many policy arenas, California has led the nation in developing innovative strategies for conserving wildlife. As early as 1909, California passed a law protecting nongame bird nests and eggs from human exploitation. In 1957, the state began preventing “take” of certain protected animals and plants, except for scientific and educational purposes—where “take” was defined as removing, harming, or killing the species. During the 1960s the state began creating lists of Fully Protected species to identify and provide additional protection to those animals that were rare or faced possible extinction.

In 1970, California passed two landmark laws that broadened the scope of species protections: the [Species Preservation Act](#), which tasked the California Department of Fish and Game with creating an inventory of all fish and wildlife species that could be considered rare or endangered, and CESA, which defined rare and endangered species and provided some means of protecting them. In addition to prohibiting take of listed species, CESA established that protecting a species might include protecting its environment. CESA states that “All native species of fishes, amphibians, reptiles, birds, mammals, invertebrates, and plants, *and their habitats* [emphasis added], threatened with extinction... will be protected or preserved.” Those three key words: “and their habitats” formalized a fundamental principle of habitat conservation planning: We must protect species' homes to protect their lives.

CESA is more comprehensive than other state wildlife protection acts and has been amended several times. In addition to providing a mechanism for listing and protecting rare and endangered species, including plants, it also requires species recovery plans and agency consultation on state projects that may impact state-listed species. Many, if not most, counties in California have now enacted their own ordinances for protection of rare and endangered species based on CESA guidelines.

In the early 1990s conflicts between endangered species and economics ramped up, with the northern spotted owl (*Strix occidentalis caurina*) disrupting forest economies in the Pacific Northwest and the California gnatcatcher (*Polioptila californica*) stopping housing developments in southern California. The US Department of the Interior began promoting Habitat Conservation Plans (HCP) under Section 10 of the ESA to resolve conflicts for federally listed species, and the State of California passed the Natural Communities Conservation Planning (NCCP) Act (1991) to both complement and help implement CESA by encouraging landscape-scale, multi-species plans.

Often coupled, HCP/NCCP planning in California brought a new collaborative approach to species protection, in which federal and state wildlife agencies work with local jurisdictions to develop land use plans that accommodate both species conservation and economic concerns. NCCPs must be prepared at an ecologically meaningful, landscape scale, and be guided by science to conserve, manage, and monitor an interconnected and functional set of ecological reserves. The process replaces project-by-project permitting by the wildlife agencies with an “incidental take” permit issued to the local jurisdiction, which in turn can issue permits for projects consistent with their conservation plan. Thus, local jurisdictions retain their authority over local land-use decisions that may affect state or federally listed species. In return, the permitted jurisdictions implement ordinances or other local controls to help achieve the plans’ species and habitat goals.

There are now at least 19 HCP/NCCPs being planned or implemented across the state. The first of these, which received national attention during the Clinton Administration and was touted as a model for the rest of the nation, was the San Diego Multiple Species Conservation Program (MSCP). Covering large portions of the County of San Diego and 11 other jurisdictions in a global hotspot of species endemism and endangerment, the MSCP was completed in 1997 after 6 years of intensive planning and negotiation. It covers scores of both listed and unlisted species within a comprehensive reserve system that is now being implemented through a cooperative management and monitoring program.

Building on and expediting this grand experiment in conservation planning, the California Department of Fish and Wildlife recently created the Regional Conservation Investment Strategy (RCIS) program (2017). RCIS does not regulate land uses or involve species permitting. Rather, it focuses on ecosystem services—such as carbon sequestration, water conservation, and preservation of agricultural land—that may contribute to species recovery, resiliency, and adaptation to climate change.

Thus, CESA is a landmark law in a history of progressive wildlife conservation in California. What began as a safety net for the most imperiled of species has helped spur the growth of a holistic, multidisciplinary approach to understanding and conserving ecological resilience. Collaboration, partnerships, and shared responsibilities, guided by multi-disciplinary science, are key to sustaining California’s wildlife legacy. The papers assembled for this special issue on CESA policy and regulation highlight the diversity of taxa, topics, and ideas influenced by the act, including some considerations for sustaining conservation progress into the future.

## Species Listed Under the California Endangered Species Act

### PLANTS

Species	CESA Listing
San Mateo thornmint ( <i>Acanthomintha duttonii</i> )	Endangered
San Diego thornmint ( <i>Acanthomintha ilicifolia</i> )	Endangered
San Clemente Island bird's-foot trefoil ( <i>Acmispon argophyllus</i> var. <i>adsurgens</i> )	Endangered
Santa Cruz Island bird's-foot trefoil ( <i>Acmispon argophyllus</i> var. <i>niveus</i> )	Endangered
San Clemente Island lotus ( <i>Acmispon dendroideus</i> var. <i>traskiae</i> )	Endangered
Munz's onion ( <i>Allium munzii</i> )	Threatened
Yosemite onion ( <i>Allium yosemitense</i> )	Rare
large-flowered fiddleneck ( <i>Amsinckia grandiflora</i> )	Endangered
McDonald's rockcress ( <i>Arabis mcdonaldiana</i> )	Endangered
Baker's manzanita ( <i>Arctostaphylos bakeri</i> ssp. <i>bakeri</i> )	Rare
The Cedars manzanita ( <i>Arctostaphylos bakeri</i> ssp. <i>sublaevis</i> )	Rare
Vine Hill manzanita ( <i>Arctostaphylos densiflora</i> )	Endangered
Hearst's manzanita ( <i>Arctostaphylos hookeri</i> ssp. <i>hearstiorum</i> )	Endangered
San Bruno Mountain manzanita ( <i>Arctostaphylos imbricata</i> )	Endangered
Presidio manzanita ( <i>Arctostaphylos montana</i> ssp. <i>ravenii</i> )	Endangered
Pacific manzanita ( <i>Arctostaphylos pacifica</i> )	Endangered
pallid manzanita ( <i>Arctostaphylos pallida</i> )	Endangered
marsh sandwort ( <i>Arenaria paludicola</i> )	Endangered
Humboldt milkvetch ( <i>Astragalus agnicidus</i> )	Endangered
Clara Hunt's milkvetch ( <i>Astragalus claranus</i> )	Threatened
Long Valley milkvetch ( <i>Astragalus johannis-howellii</i> )	Rare
Sodaville milkvetch ( <i>Astragalus lentiginosus</i> var. <i>sesquimetralis</i> )	Endangered
Peirson's milkvetch ( <i>Astragalus magdalenae</i> var. <i>peirsonii</i> )	Endangered
Mono milkvetch ( <i>Astragalus monoensis</i> )	Rare
Ventura Marsh milkvetch ( <i>Astragalus pycnostachyus</i> var. <i>lanosissimus</i> )	Endangered
coastal dunes milkvetch ( <i>Astragalus tener</i> var. <i>titi</i> )	Endangered
Trask's milkvetch ( <i>Astragalus traskiae</i> )	Rare
Bakersfield smallscale ( <i>Atriplex tularensis</i> )	Endangered
Encinitas baccharis ( <i>Baccharis vanessae</i> )	Endangered
bensoniella ( <i>Bensoniella oregona</i> )	Rare
Nevin's barberry ( <i>Berberis nevinii</i> )	Endangered
island barberry <i>Berberis pinnata</i> ssp. <i>insularis</i> )	Endangered
Sonoma sunshine <i>Blennosperma bakeri</i> )	Endangered
Point Reyes blennosperma ( <i>Blennosperma nanum</i> var. <i>robustum</i> )	Rare

**PLANTS continued**

<b>Species</b>	<b>CESA Listing</b>
dwarf goldenstar ( <i>Bloomeria humilis</i> )	Rare
thread-leaved brodiaea ( <i>Brodiaea filifolia</i> )	Endangered
Kaweah brodiaea ( <i>Brodiaea insignis</i> )	Endangered
Chinese Camp brodiaea ( <i>Brodiaea pallida</i> )	Endangered
Indian Valley brodiaea ( <i>Brodiaea rosea</i> )	Endangered
leafy reed grass ( <i>Calamagrostis foliosa</i> )	Rare
Dunn's mariposa lily ( <i>Calochortus dunnii</i> )	Rare
Siskiyou mariposa lily ( <i>Calochortus persistens</i> )	Rare
Tiburon mariposa lily ( <i>Calochortus tiburonensis</i> )	Threatened
Stebbins' morning-glory ( <i>Calystegia stebbinsii</i> )	Endangered
white sedge ( <i>Carex albida</i> )	Endangered
Tompkins' sedge ( <i>Carex tompkinsii</i> )	Rare
tree-anemone ( <i>Carpenteria californica</i> )	Threatened
Tiburon paintbrush ( <i>Castilleja affinis</i> var. <i>neglecta</i> )	Threatened
succulent owl's-clover ( <i>Castilleja campestris</i> var. <i>succulenta</i> )	Endangered
Mt. Gleason paintbrush ( <i>Castilleja gleasoni</i> )	Rare
San Clemente Island paintbrush ( <i>Castilleja grisea</i> )	Endangered
Pitkin Marsh paintbrush ( <i>Castilleja uliginosa</i> )	Endangered
California jewelflower ( <i>Caulanthus californicus</i> )	Endangered
Hearsts' ceanothus ( <i>Ceanothus hearstiorum</i> )	Rare
maritime ceanothus ( <i>Ceanothus maritimus</i> )	Rare
Mason's ceanothus ( <i>Ceanothus masonii</i> )	Rare
Vail Lake ceanothus ( <i>Ceanothus ophiochilus</i> )	Endangered
Pine Hill ceanothus ( <i>Ceanothus roderickii</i> )	Rare
Catalina Island mountain-mahogany ( <i>Cercocarpus traskiae</i> )	Endangered
Camatta Canyon amole ( <i>Chlorogalum purpureum</i> var. <i>reductum</i> )	Rare
salt marsh bird's-beak ( <i>Chloropyron maritimum</i> ssp. <i>maritimum</i> )	Endangered
soft bird's-beak ( <i>Chloropyron molle</i> ssp. <i>molle</i> )	Rare
palmate-bracted bird's-beak ( <i>Chloropyron palmatum</i> )	Endangered
Howell's spineflower ( <i>Chorizanthe howellii</i> )	Threatened
Orcutt's spineflower ( <i>Chorizanthe orcuttiana</i> )	Endangered
San Fernando Valley spineflower ( <i>Chorizanthe parryi</i> var. <i>fernandina</i> )	Endangered
Sonoma spineflower ( <i>Chorizanthe valida</i> )	Endangered
Ashland thistle ( <i>Cirsium ciliolatum</i> )	Endangered
fountain thistle ( <i>Cirsium fontinale</i> var. <i>fontinale</i> )	Endangered
Chorro Creek bog thistle ( <i>Cirsium fontinale</i> var. <i>obispoense</i> )	Endangered
surf thistle ( <i>Cirsium rhotophilum</i> )	Threatened
La Graciosa thistle ( <i>Cirsium scariosum</i> var. <i>loncholepis</i> )	Threatened

## PLANTS continued

Species	CESA Listing
Presidio clarkia ( <i>Clarkia franciscana</i> )	Endangered
Vine Hill clarkia ( <i>Clarkia imbricata</i> )	Endangered
Merced clarkia ( <i>Clarkia lingulata</i> )	Endangered
Pismo clarkia ( <i>Clarkia speciosa</i> ssp. <i>immaculata</i> )	Rare
Springville clarkia ( <i>Clarkia springvillensis</i> )	Endangered
Mt. Diablo bird's-beak ( <i>Cordylanthus nidularius</i> )	Rare
seaside bird's-beak ( <i>Cordylanthus rigidus</i> ssp. <i>littoralis</i> )	Endangered
Pennell's bird's-beak ( <i>Cordylanthus tenuis</i> ssp. <i>capillaris</i> )	Rare
Wiggins' croton ( <i>Croton wigginsii</i> )	Rare
bristlecone cryptantha ( <i>Cryptantha roosiorum</i> )	Rare
July gold ( <i>Dedeckera eurekaensis</i> )	Rare
Red Rock tarplant ( <i>Deinandra arida</i> )	Rare
Livermore tarplant ( <i>Deinandra bacigalupii</i> )	Endangered
Otay tarplant ( <i>Deinandra conjugens</i> )	Endangered
Gaviota tarplant ( <i>Deinandra increscens</i> ssp. <i>villosa</i> )	Endangered
Santa Susana tarplant ( <i>Deinandra minthornii</i> )	Rare
Mojave tarplant ( <i>Deinandra mohavensis</i> )	Endangered
Baker's larkspur ( <i>Delphinium bakeri</i> )	Endangered
Cuyamaca larkspur ( <i>Delphinium hesperium</i> ssp. <i>cuyamacae</i> )	Rare
golden larkspur ( <i>Delphinium luteum</i> )	Rare
San Clemente Island larkspur ( <i>Delphinium variegatum</i> ssp. <i>kinkiense</i> )	Endangered
Mount Laguna aster ( <i>Dieteria asteroides</i> var. <i>lagunensis</i> )	Rare
beach spectaclepod ( <i>Dithyrea maritima</i> )	Threatened
slender-horned spineflower ( <i>Dodecahema leptoceras</i> )	Endangered
Cuyamaca Lake downingia ( <i>Downingia concolor</i> var. <i>brevior</i> )	Endangered
short-leaved dudleya ( <i>Dudleya brevifolia</i> )	Endangered
marcescent dudleya ( <i>Dudleya cymosa</i> ssp. <i>marcescens</i> )	Rare
Santa Cruz Island dudleya ( <i>Dudleya nesiotica</i> )	Rare
Laguna Beach dudleya ( <i>Dudleya stolonifera</i> )	Threatened
Santa Barbara Island dudleya ( <i>Dudleya traskiae</i> )	Endangered
Santa Ana River woollystar ( <i>Eriastrum densifolium</i> ssp. <i>sanctorum</i> )	Endangered
Tracy's eriastrum ( <i>Eriastrum tracyi</i> )	Rare
Indian Knob mountainbalm ( <i>Eriodictyon altissimum</i> )	Endangered
Lompoc yerba santa ( <i>Eriodictyon capitatum</i> )	Rare
Trinity buckwheat ( <i>Eriogonum alpinum</i> )	Endangered
Ione buckwheat ( <i>Eriogonum apricum</i> var. <i>apricum</i> )	Endangered
Irish Hill buckwheat ( <i>Eriogonum apricum</i> var. <i>prostratum</i> )	Endangered
Butterworth's buckwheat ( <i>Eriogonum butterworthianum</i> )	Rare

**PLANTS continued**

<b>Species</b>	<b>CESA Listing</b>
conejo buckwheat ( <i>Eriogonum crocatum</i> )	Rare
Santa Barbara Island buckwheat ( <i>Eriogonum giganteum</i> var. <i>compactum</i> )	Rare
San Nicolas Island buckwheat ( <i>Eriogonum grande</i> var. <i>timorum</i> )	Endangered
Kellogg's buckwheat ( <i>Eriogonum kelloggii</i> )	Endangered
Thorne's buckwheat ( <i>Eriogonum thornei</i> )	Endangered
Twisselmann's buckwheat ( <i>Eriogonum twisselmannii</i> )	Rare
Congdon's woolly sunflower ( <i>Eriophyllum congdonii</i> )	Rare
San Mateo woolly sunflower ( <i>Eriophyllum latilobum</i> )	Endangered
San Diego button-celery ( <i>Eryngium aristulatum</i> var. <i>parishii</i> )	Endangered
Loch Lomond button-celery ( <i>Eryngium constancei</i> )	Endangered
Delta button-celery ( <i>Eryngium racemosum</i> )	Endangered
Contra Costa wallflower ( <i>Erysimum capitatum</i> var. <i>angustatum</i> )	Endangered
Menzies' wallflower ( <i>Erysimum menziesii</i> )	Endangered
Santa Cruz wallflower ( <i>Erysimum teretifolium</i> )	Endangered
Pine Hill flannelbush ( <i>Fremontodendron decumbens</i> )	Rare
Mexican flannelbush ( <i>Fremontodendron mexicanum</i> )	Rare
Roderick's fritillary ( <i>Fritillaria roderickii</i> )	Endangered
striped adobe-lily ( <i>Fritillaria striata</i> )	Threatened
Borrego bedstraw ( <i>Galium angustifolium</i> ssp. <i>borregoense</i> )	Rare
box bedstraw ( <i>Galium buxifolium</i> )	Rare
El Dorado bedstraw ( <i>Galium californicum</i> ssp. <i>sierrae</i> )	Rare
San Clemente Island bedstraw ( <i>Galium catalinense</i> ssp. <i>acrispum</i> )	Endangered
sand gilia ( <i>Gilia tenuiflora</i> ssp. <i>arenaria</i> )	Threatened
Boggs Lake hedge-hyssop ( <i>Gratiola heterosepala</i> )	Endangered
Orcutt's hazardia ( <i>Hazardia orcuttii</i> )	Threatened
Algodones Dunes sunflower ( <i>Helianthus niveus</i> ssp. <i>tephrodes</i> )	Endangered
Santa Cruz cypress ( <i>Hesperocyparis abramsiana</i> var. <i>abramsiana</i> )	Endangered
Butano Ridge cypress ( <i>Hesperocyparis abramsiana</i> var. <i>butanoensis</i> )	Endangered
Marin western flax ( <i>Hesperolinon congestum</i> )	Threatened
Lake County western flax ( <i>Hesperolinon didymocarpum</i> )	Endangered
rock lady ( <i>Holmgrenanthe petrophila</i> )	Rare
Santa Cruz tarplant ( <i>Holocarpha macradenia</i> )	Endangered
Tahquitz ivesia ( <i>Ivesia callida</i> )	Rare
Burke's goldfields ( <i>Lasthenia burkei</i> )	Endangered
beach layia ( <i>Layia carnosa</i> )	Endangered
coast yellow leptosiphon ( <i>Leptosiphon croceus</i> )	Endangered
San Francisco lessingia ( <i>Lessingia germanorum</i> )	Endangered
Congdon's lewisia ( <i>Lewisia congdonii</i> )	Rare

**PLANTS continued**

<b>Species</b>	<b>CESA Listing</b>
Mason's lilaeopsis ( <i>Lilaeopsis masonii</i> )	Rare
western lily ( <i>Lilium occidentale</i> )	Endangered
Pitkin Marsh lily ( <i>Lilium pardalinum</i> ssp. <i>pitkinense</i> )	Endangered
Parish's meadowfoam ( <i>Limnanthes alba</i> ssp. <i>parishii</i> )	Endangered
Baker's meadowfoam ( <i>Limnanthes bakeri</i> )	Rare
Point Reyes meadowfoam ( <i>Limnanthes douglasii</i> ssp. <i>sulphurea</i> )	Endangered
Butte County meadowfoam ( <i>Limnanthes floccosa</i> ssp. <i>californica</i> )	Endangered
Sebastopol meadowfoam ( <i>Limnanthes vinculans</i> )	Endangered
San Clemente Island woodland star ( <i>Lithophragma maximum</i> )	Endangered
Mariposa lupine ( <i>Lupinus citrinus</i> var. <i>deflexus</i> )	Threatened
Lassics lupine ( <i>Lupinus constancei</i> )	Endangered
Milo Baker's lupine ( <i>Lupinus milo-bakeri</i> )	Threatened
Nipomo Mesa lupine ( <i>Lupinus nipomensis</i> )	Endangered
Father Crowley's lupine ( <i>Lupinus padre-crowleyi</i> )	Rare
Tidestrom's lupine ( <i>Lupinus tidestromii</i> )	Endangered
San Clemente Island bush-mallow ( <i>Malacothamnus clementinus</i> )	Endangered
Santa Cruz Island bush-mallow ( <i>Malacothamnus fasciculatus</i> var. <i>nesioticus</i> )	Endangered
willow monardella ( <i>Monardella viminea</i> )	Endangered
Gambel's water cress ( <i>Nasturtium gambelii</i> )	Threatened
few-flowered navarretia ( <i>Navarretia leucocephala</i> ssp. <i>pauciflora</i> )	Threatened
many-flowered navarretia ( <i>Navarretia leucocephala</i> ssp. <i>plieantha</i> )	Endangered
Twisselmann's nemacladus ( <i>Nemacladus twisselmannii</i> )	Rare
Colusa grass ( <i>Neostaffia colusana</i> )	Endangered
Amargosa niterwort ( <i>Nitrophila mohavensis</i> )	Endangered
Dehesa nolina ( <i>Nolina interrata</i> )	Endangered
Eureka Dunes evening-primrose ( <i>Oenothera californica</i> ssp. <i>eurekensis</i> )	Rare
Antioch Dunes evening-primrose ( <i>Oenothera deltoides</i> ssp. <i>howellii</i> )	Endangered
Bakersfield cactus ( <i>Opuntia basilaris</i> var. <i>treleasei</i> )	Endangered
California Orcutt grass ( <i>Orcuttia californica</i> )	Endangered
San Joaquin Valley Orcutt grass ( <i>Orcuttia inaequalis</i> )	Endangered
hairy Orcutt grass ( <i>Orcuttia pilosa</i> )	Endangered
slender Orcutt grass ( <i>Orcuttia tenuis</i> )	Endangered
Sacramento Orcutt grass ( <i>Orcuttia viscida</i> )	Endangered
Baja California birdbush ( <i>Ornithostaphylos oppositifolia</i> )	Endangered
Gander's ragwort ( <i>Packera ganderi</i> )	Rare
Layne's ragwort ( <i>Packera layneae</i> )	Rare
Geysers panicum ( <i>Panicum acuminatum</i> var. <i>thermale</i> )	Endangered
Dudley's lousewort ( <i>Pedicularis dudleyi</i> )	Rare

**PLANTS continued**

<b>Species</b>	<b>CESA Listing</b>
white-rayed pentachaeta ( <i>Pentachaeta bellidiflora</i> )	Endangered
Lyon's pentachaeta ( <i>Pentachaeta lyonii</i> )	Endangered
Yreka phlox ( <i>Phlox hirsuta</i> )	Endangered
San Francisco popcornflower ( <i>Plagiobothrys diffusus</i> )	Endangered
Calistoga popcornflower ( <i>Plagiobothrys strictus</i> )	Threatened
North Coast semaphore grass ( <i>Pleuropogon hooverianus</i> )	Threatened
Napa blue grass ( <i>Poa napensis</i> )	Endangered
San Diego mesa mint ( <i>Pogogyne abramsii</i> )	Endangered
Santa Lucia mint ( <i>Pogogyne clareana</i> )	Endangered
Otay Mesa mint ( <i>Pogogyne nudiuscula</i> )	Endangered
Scotts Valley polygonum ( <i>Polygonum hickmanii</i> )	Endangered
Hickman's cinquefoil ( <i>Potentilla hickmanii</i> )	Endangered
Hartweg's golden sunburst ( <i>Pseudobahia bahiifolia</i> )	Endangered
San Joaquin adobe sunburst ( <i>Pseudobahia peirsonii</i> )	Endangered
Tahoe yellow cress ( <i>Rorippa subumbellata</i> )	Endangered
small-leaved rose ( <i>Rosa minutifolia</i> )	Endangered
adobe sanicle ( <i>Sanicula maritima</i> )	Rare
rock sanicle ( <i>Sanicula saxatilis</i> )	Rare
Lake County stonecrop ( <i>Sedella leiocarpa</i> )	Endangered
Owens Valley checkerbloom ( <i>Sidalcea covillei</i> )	Endangered
Cuesta Pass checkerbloom ( <i>Sidalcea hickmanii</i> ssp. <i>anomala</i> )	Rare
Parish's checkerbloom ( <i>Sidalcea hickmanii</i> ssp. <i>parishii</i> )	Rare
Kenwood Marsh checkerbloom ( <i>Sidalcea oregana</i> ssp. <i>valida</i> )	Endangered
bird-foot checkerbloom ( <i>Sidalcea pedata</i> )	Endangered
Scadden Flat checkerbloom ( <i>Sidalcea stipularis</i> )	Endangered
Red Mountain catchfly ( <i>Silene campanulata</i> ssp. <i>campanulata</i> )	Endangered
Tiburon jewel-flower ( <i>Streptanthus glandulosus</i> ssp. <i>niger</i> )	Endangered
Eureka Valley dune grass ( <i>Swallenia alexandrae</i> )	Rare
slender-petaled thelypodium ( <i>Thelypodium stenopetalum</i> )	Endangered
Santa Ynez false lupine ( <i>Thermopsis macrophylla</i> )	Rare
Pacific Grove clover ( <i>Trifolium polyodon</i> )	Rare
Monterey clover ( <i>Trifolium trichocalyx</i> )	Endangered
Greene's tuctoria ( <i>Tuctoria greenei</i> )	Rare
Crampton's tuctoria or Solano grass ( <i>Tuctoria mucronata</i> )	Endangered
Red Hills vervain ( <i>Verbena californica</i> )	Threatened
big-leaved crownbeard ( <i>Verbesina dissita</i> )	Threatened
western Joshua tree ( <i>Yucca brevifolia</i> )	Candidate

Note: those highlighted in the table above are included in this issue

**INVERTEBRATES**

<b>Species</b>	<b>CESA Listing</b>
Trinity bristle snail ( <i>Monadenia infumata setosa</i> )	Threatened
Shasta crayfish ( <i>Pacifastacus fortis</i> )	Endangered
California freshwater shrimp ( <i>Syncaris pacifica</i> )	Endangered
Crotch bumble bee ( <i>Bombus crotchii</i> )	Candidate*
Franklin's bumble bee ( <i>Bombus franklini</i> )	Candidate*
western bumble bee ( <i>Bombus occidentalis</i> )	Candidate*
Suckley's cuckoo bumble bee ( <i>Bombus suckleyi</i> )	Candidate*

Note: those highlighted in the table above are included in this issue

\*While bumblebees are not currently candidates, they were during development of the special issue. The situation is currently under litigation.

**FISH**

<b>Species</b>	<b>CESA Listing</b>
Bonytail ( <i>Gila elegans</i> )	Endangered
Clear Lake hitch ( <i>Lavinia exilicauda chi</i> )	Threatened
Colorado pikeminnow ( <i>Ptychocheilus lucius</i> )	Endangered
Mohave tui chub ( <i>Siphateles bicolor mohavensis</i> )	Endangered
Owens tui chub ( <i>Siphateles bicolor snyderi</i> )	Endangered
Modoc sucker ( <i>Catostomus microps</i> )	Endangered
Shortnose sucker ( <i>Chasmistes brevirostris</i> )	Endangered
Lost River sucker ( <i>Deltistes luxatus</i> )	Endangered
Razorback sucker ( <i>Xyrauchen texanus</i> )	Endangered
Delta smelt ( <i>Hypomesus transpacificus</i> )	Endangered
Longfin smelt ( <i>Spirinchus thaleichthys</i> )	Threatened
Coho salmon [south of Punta Gorda (Humboldt County), California] ( <i>Oncorhynchus kisutch</i> )	Endangered
Coho salmon [from Punta Gorda (Humboldt County), California to the northern border of California] ( <i>Oncorhynchus kisutch</i> )	Threatened
Steelhead [summer-run] ( <i>Oncorhynchus mykiss irideus</i> )	Candidate
Chinook salmon [winter run] ( <i>Oncorhynchus tshawytscha</i> )	Endangered
Chinook salmon [Upper Klamath-Trinity River Spring ESU] ( <i>Oncorhynchus tshawytscha</i> )	Candidate
Chinook salmon [spring-run of the Sacramento River drainage] ( <i>Oncorhynchus tshawytscha</i> )	Threatened
Bull trout ( <i>Salvelinus confluentus</i> )	Endangered
Desert pupfish ( <i>Cyprinodon macularius</i> )	Endangered
Owens pupfish ( <i>Cyprinodon radiosus</i> )	Endangered

**FISH continued**

Cottonball Marsh pupfish ( <i>Cyprinodon salinus milleri</i> )	Threatened
Unarmored threespine stickleback ( <i>Gasterosteus aculeatus williamsoni</i> )	Endangered
Rough sculpin ( <i>Cottus asperimus</i> )	Threatened

Note: those highlighted in the table above are included in this issue

ESU = Evolutionarily significant unit

DPS = Distinct population segment

**AMPHIBIANS**

Species	CESA Listing
California tiger salamander ( <i>Ambystoma californiense</i> )	Threatened
Santa Cruz long-toed salamander ( <i>Ambystoma macrodactylum croceum</i> )	Endangered
Desert slender salamander ( <i>Batrachoseps major aridus</i> ) (= <i>Batrachoseps aridus</i> )	Endangered
Kern Canyon slender salamander ( <i>Batrachoseps simatus</i> )	Threatened
Tehachapi slender salamander ( <i>Batrachoseps stebbinsi</i> )	Threatened
Shasta salamander ( <i>Hydromantes shastae</i> )	Threatened
Limestone salamander ( <i>Hydromantes brunus</i> )	Threatened
Scott Bar salamander ( <i>Plethodon asupak</i> = <i>P. stromi</i> var. <i>asupak</i> )	Threatened
Siskiyou Mountains salamander ( <i>Plethodon stormi</i> )	Threatened
Black toad ( <i>Anaxyrus</i> (= <i>Bufo</i> ) <i>exsul</i> )	Threatened
Foothill yellow-legged frog [SW/So Coast, West/Central Coast, East/So Sierra clades] ( <i>Rana boylei</i> )	Endangered
Foothill yellow-legged frog [NE/No Sierra and Feather River clades] ( <i>Rana boylei</i> )	Threatened
Cascades frog ( <i>Rana cascadae</i> )	Candidate
Southern mountain yellow-legged frog ( <i>Rana muscosa</i> )	Endangered
Sierra Nevada yellow-legged frog ( <i>Rana sierrae</i> )	Threatened

Note: those highlighted in the table above are included in this issue

**REPTILES**

Species	CESA Listing
Agassiz's desert tortoise ( <i>Gopherus agassizii</i> )	Threatened
Barefoot (=Barefoot banded) gecko ( <i>Coleonyx switaki</i> )	Threatened
Blunt-nosed leopard lizard ( <i>Gambelia sila</i> )	Endangered
Coachella Valley fringe-toed lizard ( <i>Uma inornata</i> )	Endangered

**REPTILES continued**

Species	CESA Listing
Southern rubber boa ( <i>Charina umbratica</i> = <i>C. bottae umbratica</i> )	Threatened
Alameda whipsnake ( <i>Masticophis lateralis euryxanthus</i> )	Threatened
Giant garter snake ( <i>Thamnophis gigas</i> = <i>T. couchi gigas</i> )	Threatened
San Francisco garter snake ( <i>Thamnophis sirtalis tetrataenia</i> )	Endangered
Pacific leatherback sea turtle ( <i>Dermochelys coriacea</i> )	Candidate

Note: those highlighted in the table above are included in this issue

**BIRDS**

California condor ( <i>Gymnogyps californianus</i> )	Endangered
Swainson's hawk ( <i>Buteo swainsoni</i> )	Threatened
Bald eagle ( <i>Haliaeetus leucocephalus</i> )	Endangered
Elf owl ( <i>Micrathene whitneyi</i> )	Endangered
Great gray owl ( <i>Strix nebulosa</i> )	Threatened
Northern spotted owl ( <i>Strix occidentalis caurina</i> )	Threatened
California black rail ( <i>Laterallus jamaicensis coturniculus</i> )	Threatened
Light-footed Ridgway's (= clapper) rail ( <i>Rallus obsoletus levipes</i> )	Endangered
California Ridgway's (= clapper) rail ( <i>Rallus obsoletus obsoletus</i> )	Endangered
Yuma Ridgway's (= clapper) rail ( <i>Rallus obsoletus yumanensis</i> )	Threatened
Greater sandhill crane ( <i>Antigone [=Grus] canadensis tabida</i> )	Threatened
California least tern ( <i>Sternula antillarum browni</i> )	Endangered
Marbled murrelet ( <i>Brachyramphus marmoratus</i> )	Endangered
Scripps's murrelet (=Xantus's murrelet) ( <i>Synthliboramphus scrippsi</i> )	Threatened
Guadalupe murrelet (=Xantus's murrelet) ( <i>Synthliboramphus hypoleucus</i> )	Threatened
Western yellow-billed cuckoo ( <i>Coccyzus americanus occidentalis</i> )	Endangered
Gilded (=Gilded northern) flicker ( <i>Colaptes chrysoides</i> = <i>C. auratus chrysoides</i> )	Endangered
Gila woodpecker ( <i>Melanerpes uropygialis</i> )	Endangered
Willow flycatcher ( <i>Empidonax traillii</i> )	Endangered
Arizona Bell's vireo ( <i>Vireo bellii arizonae</i> )	Endangered
Least Bell's vireo ( <i>Vireo bellii pusillus</i> )	Endangered
Bank swallow ( <i>Riparia riparia</i> )	Threatened
Inyo California towhee ( <i>Melozone crissalis eremophilus</i> )	Endangered
Belding's savannah sparrow ( <i>Passerculus sandwichensis beldingi</i> )	Endangered
Tricolored blackbird ( <i>Agelaius tricolor</i> )	Threatened

Note: those highlighted in the table above are included in this issue

**SMALL MAMMALS**

<b>Species</b>	<b>CESA Listing</b>
Riparian brush rabbit ( <i>Sylvilagus bachmani riparius</i> )	Endangered
San Joaquin (= Nelson's) antelope squirrel ( <i>Ammospermophilus nelsoni</i> )	Threatened
Mohave ground squirrel ( <i>Xerospermophilus [= Spermophilus] mohavensis</i> )	Threatened
Morro Bay kangaroo rat ( <i>Dipodomys heermanni morroensis</i> )	Endangered
Giant kangaroo rat ( <i>Dipodomys ingens</i> )	Endangered
Fresno kangaroo rat ( <i>Dipodomys nitratoides exilis</i> )	Endangered
Tipton kangaroo rat ( <i>Dipodomys nitratoides nitratoides</i> )	Endangered
Stephens' kangaroo rat ( <i>Dipodomys stephensi</i> )	Threatened
San Bernardino kangaroo rat ( <i>Dipodomys merriami parvus</i> )	Candidate
Amargosa vole ( <i>Microtus californicus scirpensis</i> )	Endangered
Salt-marsh harvest mouse ( <i>Reithrodontomys raviventris</i> )	Endangered

Note: those highlighted in the table above are included in this issue

**MAMMALS – CARNIOVORES & UNGULATES**

<b>Species</b>	<b>CESA Listing</b>
Gray wolf ( <i>Canis lupus</i> )	Endangered
Island fox ( <i>Urocyon littoralis</i> )	Threatened
San Joaquin kit fox ( <i>Vulpes macrotis mutica</i> )	Threatened
Sierra Nevada red fox ( <i>Vulpes vulpes necator</i> )	Threatened
Mountain lion [Southern California ESU] ( <i>Puma concolor</i> )	Candidate
Wolverine ( <i>Gulo gulo</i> )	Threatened
Humboldt (= coastal) marten ( <i>Martes caurina humboldtensis</i> )	Endangered
Pacific fisher [Southern Sierra Nevada ESU] ( <i>Pekania [=Martes] pennanti</i> )	Threatened
Guadalupe fur seal ( <i>Arctocephalus townsendi</i> )	Threatened
Peninsular desert bighorn sheep [Peninsular CA DPS] ( <i>Ovis canadensis nesloni</i> [=cremnobates])	Threatened
Sierra Nevada (= California) bighorn sheep ( <i>Ovis canadensis sierrae</i> = <i>O. c. californiana</i> )	Endangered

Note: those highlighted in the table above are included in this issue

ESU = Evolutionarily significant unit

DPS = Distinct population segment

# CESA Policy & Regulations



# CESA Policy and Regulations

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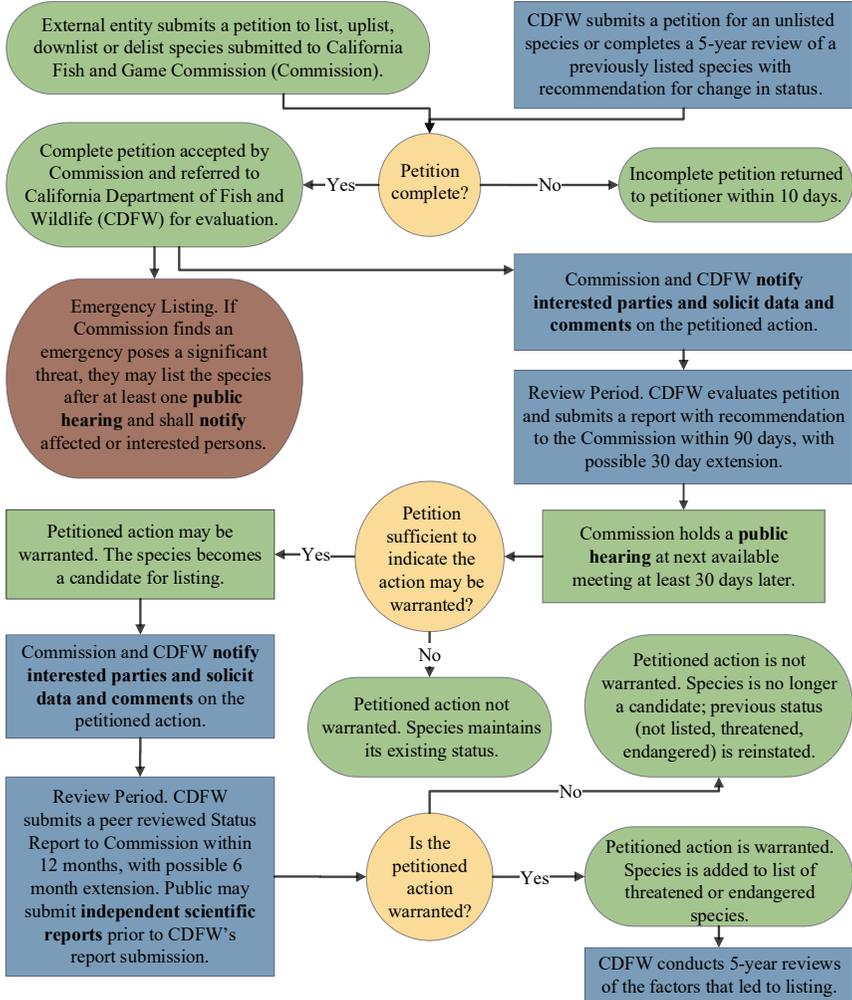
1. Native Plant Program staff monitor a population of a listed plant species at CDFW's Hallelujah Junction Wildlife Area. Photo Credit: Raffica La Rosa, CDFW
2. Native Plant Program staff census a population of a listed vernal pool plant species in Tulare County. Photo Credit: Cherilyn Burton, CDFW
3. Frog species determination survey (*Rana boylei*), Spanish Creek, Plumas County. Photo Credit: Sandra Jacks, CDFW
4. USGS biologist Cory Overton prepares a newly radio-tagged Ridgway's rail (*Rallus obsoletus*) for release at Arrowhead Marsh, in Alameda County. Photo Credit: Marcia Grefrsud, CDFW
5. CDFW staff leads a field trip for the First Lego League Robotics Team to learn about California tiger salamanders in Livermore, CA for their Animal Allies Challenge; the team later went on to win the Inspiration Award for their work. Photo Credit: Dung Le, Parent
6. Frog (*Rana* spp.) species surveys—netting along Spanish Creek, Plumas County. Photo Credit: Sandra Jacks, CDFW



# Process for Listing Species Under the California Endangered Species Act



This is a simplified flowchart designed to give a broad overview of the process. For more detailed information, see Fish and Game Code sections 2070–2079, California Code of Regulations, Title 14, section 670.1, and Government Code section 11344–11348.



ESSAY

## **An overview of the listing process under the California Endangered Species Act**

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The California Endangered Species Act (CESA) is one of the most important legal tools available to the Fish and Game Commission and Department of Fish and Wildlife to protect the State's wildlife resources. The listing process, together with the prohibitions in section 2080 of the Fish and Game Code, are the law's regulatory teeth. At the same time, because any interested person can petition to list a species, the listing process invites citizen participation in the regulatory scheme. Listing decisions can be the difference between persistence and extinction of a species. They can also cause severe economic disruption and, for this reason, should in our view be made with due deliberation and based on the best available scientific information. Here we describe the complex roadmap that petitions must navigate and that is intended to assure that only native species that need protection get it.

**Key words:** CESA, endangered species, listing

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At the heart of the California Endangered Species Act (CESA) is a process whereby species are listed as endangered or threatened and such species are afforded broad protections. This process—which both provides for the listing of species, as well as prohibits conduct that could harm the species—is intended to halt the decline of species at risk of extinction and, ultimately, contribute to the conservation of such species so that they may be removed from the list of endangered and threatened species. Below we provide an overview of this process, including a description of the procedures whereby the Fish and Game Commission (Commission) considers whether to list a species, and a discussion of the role of the Department of Fish and Wildlife (Department) in the listing process.

Section 2070 of the Fish and Game Code provides that “[t]he commission shall establish a list of endangered species and a list of threatened species.” CESA defines an endangered species as “a native species or subspecies of a bird, mammal, fish, amphibian, reptile, or plant which is in serious danger of becoming extinct throughout all, or a significant portion, of its range due to one or more causes, including loss of habitat, change in habitat, overexploitation, predation, competition, or disease” (Fish & G. Code § 2062). It defines a

threatened species as “a native species or subspecies of a bird, mammal, fish, amphibian, reptile, or plant that, although not presently threatened with extinction, is likely to become an endangered species in the foreseeable future in the absence of the special protection and management efforts required by this chapter” (Fish & G. Code § 2067).

Section 2071 requires the Commission to adopt guidelines so “an interested person may petition the commission to add a species to, or to remove a species from either the list of endangered or the list of threatened species.” Those guidelines are set out in Section 670.1, Title 14, of the California Code of Regulations. In the alternative, section 2072.7 of the Fish and Game Code provides that the Department may “recommend to the commission that it add a species to, or remove a species from, either the list of endangered species or the list of threatened species.”

### THE SCOPE OF CESA

CESA extends to “native species or subspecies of a bird, mammal, fish, amphibian, reptile, or plant” (Fish & G. Code §§ 2062 (definition of endangered species), 2067 (definition of threatened species), 2068 (definition of candidate species)). By its plain language, it does not extend to other forms of life though the argument has been made that the broad definition of “fish” in the Fish and Game Code expands the protections CESA provides beyond the plain meaning of fish to include all invertebrates. In 1998, Assembly member Keith Olberg requested an opinion of the Attorney General regarding the eligibility of insects for listing under CESA (Cal. Op. Atty. Gen. 98-105). In response, the Attorney General opined: “Insects are ineligible for listing as a threatened or endangered species under the California Endangered Species Act” (*Id.*). He further explained: “These definitions limit the application of CESA to birds, mammals, fish, amphibians, reptiles, and plants. Insects do not fall within any of these categories” (*Id.*).

The Attorney General’s opinion reaffirmed an earlier decision by the Office of Administrative Law that insects are not subject to listing under CESA. In 1980, certain parties sought to list the Smith’s blue butterfly (*Euphilotes enoptes smithi*), and the Lange’s Metalmark butterfly (*Apodemia mormo langei*) under CESA. The Commission voted to list both butterflies, and the decision went to the Office of Administrative Law. As authority for its position that the butterflies could be listed, the Commission reasoned that (1) the definitions of endangered and threatened species expressly include fish, (2) section 45 of the Fish and Game Code expressly defines fish to include invertebrates, (3) insects are invertebrates, (4) insects are therefore fish, and, (5) insects can be listed. The Office of Administrative Law determined that the Commission acted outside its authority in listing the two butterflies, concluding that, despite the Commission’s arguments, insects are not fish (Price 1981). Thereafter, the Commission rescinded its prior action.

While not binding, the U.S. Fish and Wildlife Service’s long-held position – articulated in *Federal Register* notices regarding various insect species and citing communications with the California Department of Fish and Wildlife for support – is consistent with that taken by the California Attorney General’s Office and the State’s Office of Administrative Law. At least as far back as 1997, a *Federal Register* notice regarding the Callippe Silverspot butterfly (*Speyeria callippe callippe*) and Behren’s Silverspot butterfly (*Speyeria zerene behrensii*) stated “The California Endangered Species Act (CESA) does not provide protection to insects (sections 2062, 2067 and 2068, Fish and Game Code)” (USFWS 1997). More recently, the U.S. Fish and Wildlife Service affirmed its prior position, for example, stating in

2011, in a notice regarding the Casey's June beetle (*Dinacoma caseyi*), that "The California Endangered Species Act (CESA) provides protections for many species of plants, animals, and some invertebrate species. However, insect species, such as Casey's June beetle, are not afforded protection under CESA" (USFWS 2011).

In 2018, the Xerces Society and others filed a petition to list four subspecies of bumble bee as endangered under CESA. In response, the Department issued a 90-day evaluation recommending the Commission accept the petition, and the Commission voted to accept the petition, thereby determining that listing the species may be warranted. Soon thereafter, a number of organizations representing the farming community filed a petition in Superior Court challenging the Commission's action on the grounds that listing the subspecies of bumble bees is precluded as a matter of law. The court decided in favor of petitioners, holding "the absence of authority to list insects under CESA, either as fish or otherwise, is clear" (*Almond Alliance et al. v. Cal. Fish & Game Com.* (13 Nov 2020, Super. Ct. Sacramento County No. 34-2019-80003216)). The Commission appealed the trial court's decision, and that appeal is pending.

## THE DEFINITION OF SPECIES

Neither CESA nor the Fish and Game Code generally define the term "species." But the definitions of endangered and threatened species (Fish & G. Code §§ 2062, 2067) both expressly encompass "native species or subspecies." The species concept has a rich history in the biological sciences. Species are commonly defined as groups of individuals that are morphologically or genetically distinct from other groups of individuals or as groups of individuals that can breed among themselves and that do not breed with other groups (Primack 2006). Over time, within the scientific community and beyond, there have been strident disagreements regarding the application of these definitions, in part because of their legal and policy implications.

The subspecies concept is both biological and legal. As a result, it has been the subject of greater controversy than the species concept. For example, after the Commission listed one population of Coho Salmon (*Oncorhynchus kisutch*) on the northern California coast as a threatened species, and another population of Coho Salmon on the central coast as an endangered species, the California Forestry Association challenged the listing decisions. The trial court upheld the Commission's decisions, and the Court of Appeal affirmed.

The Court of Appeal began by noting that the proper interpretation of the term "species or subspecies" in the statute is a question of law that the courts review de novo (*California Forestry Assn. v. California Fish & Game Commission* (2007) 156 Cal. App. 4th 1535, 1544). It went on to explain that the Commission and Department determined to list the two populations of Coho Salmon to maintain the diversity of the species in order to contribute to its preservation (*Id.* at 1546-1547). Ultimately, the Court of Appeal deferred to the Commission's decision to list two evolutionarily significant units of Coho Salmon, noting this is consistent with the liberal construction it accords laws such as CESA (*Id.* at 1548-49). The decision signals the willingness of the judiciary to defer to the Commission and Department to determine when a listing of a population of a species (but not the entirety of the species) is appropriate.

Some commentators have objected to the Court of Appeal decision. Schiff and Thompson (2010) argue that the court erred by reading the statute too expansively, so as to extend it to include evolutionarily significant units (ESU). They point out that whereas

the federal Endangered Species Act includes species, subspecies, and distinct population segments (DPS), CESA only includes species and subspecies. They also note that the two Acts are similar in many respects, and argue that the decision of the California legislature not to include the term “distinct population segments” in the definitions of endangered and threatened species is strong evidence that CESA does not extend to DPSs or ESUs. But for the time being, the liberal construction of the statute adopted by the Court of Appeal stands. The Court of Appeal, in a recent case concerning the Coho Salmon confirmed that, similar to how a population can only be listed if it qualifies as a species, subspecies, or ESU, a population can only be delisted if it (1) can be defined as a separate species, subspecies, or ESU, and (2) if the determination can be made that the separate species, subspecies, or ESU is not endangered (*Central Coast Forest Assn. v. Fish and Game Commission* (2017) 18 Cal.App.5th 1191, 1239-1240).

The Commission has, in several instances since the Coho Salmon decision was upheld, listed subspecies. For example, the Commission voted in 2016 to list the Southern Sierra Nevada Evolutionarily Significant Unit of the fisher (*Pekania pennanti*) as a threatened species (Fish and Game Commission 2016). Similarly, the Commission voted in 2020 to list the Southwest/South Coast, West/Central Coast, and East/Southern Sierra clades of the foothill yellow-legged frog (*Rana boylei*) as endangered and the Northeast/Northern Sierra and Feather River clades of the species as threatened (Fish and Game Commission 2020a). Also, in 2020, the Commission voted to make the Southern California/Central Coast ESU of mountain lions (*Puma concolor*) a candidate species (Fish and Game Commission 2020b). The decision was controversial and could lead to litigation that addresses the definition of species (Sahagun 2020).

In a case decided in early 2019, a trial court addressed the other prong of the “native species or subspecies” requirement in CESA, namely whether a species is native to California (*Cal. Cattlemen’s Assn. v. Cal. Fish & Game Commission* (Jan. 28, 2019, Super. Ct. San Diego County, No. 37-2017-00003866-CU-MC-CTL)). At issue in the case was the Commission’s decision to list the gray wolf (*Canis lupus*).

According to the administrative record, gray wolves historically inhabited most of the United States, including much of California, until they were extirpated from California almost 100 years ago. In December 2011, a lone gray wolf known as OR-7 dispersed from northeastern Oregon’s gray wolf population and was observed crossing the Oregon-California border in both directions, multiple times. Shortly thereafter, in 2012, the Center for Biological Diversity and others submitted a petition to list the gray wolf to the Commission. In its 12-month review of the petition, the Department noted that the presence of OR-7 in California in late 2011 was the first wolf sighting in California since extirpation in the 1920s. It went on to explain that there has been no breeding population of gray wolves in California for many decades and that, as a consequence, the Department considered information on the status of the species from other states when undertaking its review. Ultimately, the Department recommended against listing.

The Commission sided with petitioners and against the Department, opting to list the gray wolf as endangered. Agriculture and ranching groups challenged the Commission’s listing decision on the grounds that the gray wolf did not meet the criteria for listing set forth in section 2062 (Fish & G. Code § 2062). Specifically, the groups argued that the listing was improperly based on the presence of a non-native subspecies of gray wolf, that the intermittent presence of a single wolf did not warrant a finding that the gray wolf’s

range included California, and that the gray wolf was not at risk of extinction throughout its entire range, which extended beyond California. The court disagreed, holding that section 2062 of the Fish and Game Code permits the listing of native species in addition to native subspecies, and that furthermore, the Commission's scientific finding that OR-7 possessed some genetic markers of a native California subspecies (*Canis lupus nubilus*) was entitled to deference. The court also held that the Commission could reasonably find that listing was necessary to protect the gray wolf from extinction in California based on the intermittent presence of OR-7 and other wolves, along with the possibility that a breeding population might be established in California in the foreseeable future.

While this decision is notable, particularly because the gray wolf is an iconic species in the history of the western United States, its implications for future listing decisions are limited, as the fact that the gray wolf was native to and present in California in the relatively recent past was not in dispute.

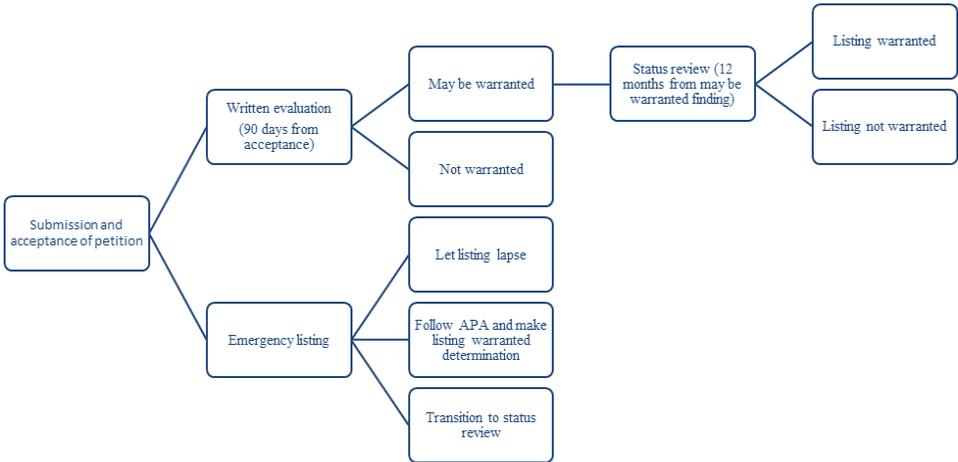
## THE PETITION

The listing process commences with the submission of a petition to the Commission. The significant milestones in the process are illustrated in Figure 1. Petitions must be submitted on an authorized petition form (Cal. Code Regs., tit. 14, § 670.1(a)). To be accepted, a petition must include sufficient scientific information that the petitioned action may be warranted (Fish & G. Code § 2072.3). The Fish and Game Code provides that it must include information regarding “the population trend, range, distribution, abundance, and life history of a species, the factors affecting the ability of the population to survive and reproduce, the degree and immediacy of the threat, the impact of existing management efforts, suggestions for future management, and the availability and sources of information,” and “the kind of habitat necessary for species survival, a detailed distribution map, and any other factors that the petitioner deems relevant” (*Id.*).

In response to a petition to list the western Joshua tree (*Yucca brevifolia brevifolia*), the Commission recently found that the petition provides sufficient information to indicate that the petitioned action may be warranted, thereby making the species a candidate for listing. The Commission's decision is notable because the petition did not include information on “population trend” and “abundance,” which arguably must be included in a petition under section 2072.3. The petition stated that “a reliable estimate of Joshua tree population size is not available,” and that “no range-wide population trends have been documented” (Center for Biological Diversity 2019). Further, the Department's 90-day evaluation affirmed “[t]he Petition does not present an estimate of western Joshua tree population size, nor does it provide evidence of a range-wide population trend” (Department of Fish and Wildlife 2020). Nonetheless, the Commission concurred with the Center for Biological Diversity and Department that climate change, combined with other factors including habitat loss, provide grounds to designate the species a candidate for listing. The decision is being challenged in Superior Court and the outcome of the lawsuit may provide greater clarity regarding the extent to which petitioners are required to provide information on each of the factors identified in section 2072.3.

Commission staff must review a petition for completeness. An incomplete petition shall be returned to the petitioner by Commission staff within 10 days of receipt (Cal. Code Regs., tit. 14, § 670.1(b)). If the petition is deemed complete, the Commission is required to

refer it to the Department (Fish & G. Code § 2073). Commission staff must submit notice of complete petitions to the Office of Administrative Law for publication in the California Regulatory Notice Register, at the time the petition is transmitted to the Department for evaluation (Fish & G. Code § 2073.3; Cal. Code Regs., tit. 14, § 670.1(c)). Such notice must



**Figure 1.** Significant milestones in the CESA listing process.

include the date and location of the Commission meeting at which the petition is formally scheduled for receipt. Commission staff has treated this review as largely perfunctory.

## THE DEPARTMENT'S WRITTEN EVALUATION

Within 90 days of receipt of the petition, the Department is required to submit a written evaluation of the petition to the Commission (Fish & G. Code § 2073.5(a)). The Department's Wildlife Branch and Habitat Conservation Planning Branch staff coordinate evaluation of petitions. The evaluation of whether or not the petition is complete is to be based on the petition and other relevant information the Department possesses or receives (*Id.*). It must address each of the following petition components.

- (A) population trend;
- (B) range;
- (C) distribution;
- (D) abundance;
- (E) life history;
- (F) kind of habitat necessary for survival;
- (G) factors affecting the ability to survive and reproduce;
- (H) degree and immediacy of threat;
- (I) impact of existing management efforts;
- (J) suggestions for future management;
- (K) availability and sources of information; and
- (L) a detailed distribution map.

(Cal Code Regs., tit. 14, § 670.1(d)(1)). In addition, it must be accompanied by a recommendation that the petition be rejected or accepted and considered (Fish & G. Code

§ 2073.5(a)). Upon a request by the Director of the Department, the Commission may grant the Department an extension of up to 30 days to complete the evaluation (*Id.* § 2073.5(b)).

### **CANDIDACY**

After the Department releases its written evaluation, the Commission must schedule both the petition and written evaluation for consideration at the Commission’s next available meeting, but not sooner than 30 days after receipt of the petition and written evaluation (Fish & G. Code § 2074). At the meeting, the Commission is obliged to accept written materials and oral testimony (*Id.* § 2074.2(a)). After the conclusion of testimony, the Commission may either close or leave open the hearing (*Id.* § 2074.2(a), (d)). If the Commission closes the hearing, it may either continue the meeting on the petition or make a finding (*Id.* § 2074.2(d), (e)).

The Commission may make one of two findings with respect to a petition to list: (1) it may find that the petition does not provide sufficient information to indicate that the petitioned action may be warranted and reject the petition or (2) it may find that the petition provides sufficient information to indicate that the petitioned action may be warranted and publish a notice of finding that the petition is accepted for consideration (Fish & G. Code § 2074.2(e)). In making the decision whether a listing may be warranted, the Commission must weigh the evidence for and against candidate listing and decide essentially a question of fact in the process (*Natural Resources Defense Council v. Fish & Game Com.* (1994) 28 Cal. App. 4th 1104, 1116). As the Third District Court of Appeal explained, “the section 2074.2 phrase ‘petition provides sufficient information to indicate that the petitioned action may be warranted’ means that amount of information, when considered in light of the Department’s written report and the comments received, that would lead a reasonable person to conclude there is a substantial possibility the requested listing could occur” (*Id.* at 1125; *see also Center for Biological Diversity v. Fish and Game Commission* (2008) 166 Cal. App. 4th 597, 609-10). In both cases referenced above, the Court of Appeal overturned the determination of the Commission that the petition to list was not warranted. In contrast, there are no reported cases in which a party has successfully challenged a Commission determination that a petition to list is warranted based on the information included in the petition. That said, the Commission has on a number of occasions determined that a petition to list is not warranted.

If the Commission finds that the petition does not provide sufficient information to indicate that the petitioned action may be warranted, it must publish a notice of its finding that the petition is rejected in the California Regulatory Notice Register (Cal. Code Regs., tit. 14, § 670.1(e)(1)). If the Commission accepts a petition that recommends listing the species as endangered or threatened, the Commission shall include in its notice of finding that the petitioned species is a candidate species (Fish & G. Code § 2074.2(e)(2)). The Commission must publish the notice in the California Regulatory Notice Register (Cal. Code Regs., tit. 14, § 670.1(e)(2)).

### **STATUS REVIEW**

If a petition is accepted, the Commission is required to make reasonable attempts to notify affected and interested parties and to solicit data and comments on the petitioned action from as many persons as is practicable (Fish & G. Code § 2074.4). In addition, the

Department is required to commence a status review of the species that is the focus of the petition (*Id.* § 2074.6). Within 12 months of the date of publication of a notice of acceptance of a petition for consideration, the Department is required to produce a report (or status review) that indicates whether the petitioned action is warranted, includes a preliminary identification of the habitat that may be essential to the continued existence of the species, and recommends management activities and other recommendations for recovery of the species (*Ibid.*). The Department's Wildlife Branch or Habitat Conservation Planning Branch staff coordinate preparation of the status review.

The status review must be peer reviewed and based upon the best scientific information available (*Id.*). Interested parties may recommend reviewers to the Department, but the process for selecting reviewers is in the Department's sole discretion. The Department does not compensate persons who conduct peer reviews. As a practical matter, it can be difficult to attract experts in the field to the peer review process because it is time consuming and does not involve any remuneration. Moreover, individuals that are willing to participate in the peer review process may have an interest in the outcome of the petitioned action, which makes finding neutral peer review participants challenging.

Where the Department fails to include proper peer review in its status report, it cannot serve as the basis for the Commission's decision on a petition for listing (*Center for Biological Diversity v. Cal. Fish & Game Com.*, No. CGC-10-505205 (San Francisco Sup. Ct. 23 July 2012)). The Superior Court's decision underscores the importance of peer review in the CESA listing process and the weight that CESA places on relying on credible science (or the best available scientific information) in making listing decisions.

Prior to releasing the final status review, the Department must evaluate and respond in writing to the independent peer review and amend the draft status review report as appropriate (*Id.*). Commission regulations define peer review as "the analysis of a scientific report by persons of the scientific/academic community commonly acknowledged to be experts on the subject under consideration, possessing the knowledge and expertise to critique the scientific validity of the report" (Cal. Code Regs., tit. 14, § 670.1(f)(2)). Both the draft and final status reviews are part of the administrative record and included in the materials provided to the Commission before it makes a final listing decision.

Commission regulations also provide that interested parties who wish to submit a detailed written scientific report to the Commission must do so not later than the time the Department submits its report and that reports received thereafter may not be considered (Cal. Code Regs., tit. 14, § 670.1(h)(1)). They further provide that such parties may seek independent and competent peer review of the report prior to submission and include all comments from the reviewers with the report to verify that peer review has been solicited (*Id.* § 670.1(h)(2)). In practice, interested parties routinely submit comments on the Department status review but rarely prepare an alternative status review of the species.

## THE FINAL LISTING DECISION

The Commission is required to schedule the petition for final consideration at its next meeting after receiving the Department's final status review (Fish & G. Code § 2075; Cal. Code Regs., tit. 14, § 670.1 (g)(1)). At the meeting, the Commission is obliged to accept written materials and oral testimony (Fish & G. Code § 2075.5(a)). After the conclusion of testimony, the Commission may either close or leave open the hearing (*Id.* § 2075.5(a), (d)). After the hearing is closed, the administrative record for the Commission's decision

is closed (*Id.* § 2075.5(b)). The administrative record may only be reopened if there is a change in state or federal law or regulation that has a direct and significant impact on the Commission's determination as to whether the petitioned action is warranted or if the Commission determines that it requires further information to evaluate whether the petitioned action is warranted (*Id.* § 2075.5(c)).

The Commission may make one of two findings with respect to a petition to list: (1) it may find that the petitioned action is not warranted, in which case the finding shall be entered in the Commission's records and the petitioned species shall be removed from the list of candidate species, (2) that the petitioned action is not warranted but listing the species at a different level than petitioned is warranted (i.e., petition to list as endangered is not warranted but listing as threatened is), or (3) it may find that the petitioned action is warranted, in which case the Commission shall publish a notice of that finding and a notice of proposed rulemaking pursuant to the Administrative Procedure Act, to add the species to the list of endangered species or the list of threatened species (Fish & G. Code § 2075.5(e)). The typical practice of the Commission is to vote on the adoption of findings at a subsequent meeting in order to allow staff to prepare written findings consistent with the action taken. Where the Commission is changing the status of a species, it must submit the change in status to the Office of Administrative Law for filing with the Secretary of State and publication in the California Code of Regulations (*Id.* § 2075.5(e)(2)).

One consequence of the fact that the Commission votes on whether the action is warranted at one meeting then votes on adoption of findings at a subsequent meeting is that the Commission has an opportunity to re-visit its prior decision at the subsequent meeting. By and large, this is irrelevant because the same Commissioners who vote on whether the action is warranted vote at the next meeting on the adoption of findings. But if Commissioners are absent from a meeting or if a Commissioner resigns his or her post or is replaced, then it is possible such a change in the voting members will lead to a circumstance where the vote on adoption of findings becomes a vote on reconsideration. There is no evidence this is what the Legislature intended. But absent statutory or regulatory clarification that the findings adopted must support the prior determination, there is the possibility of reconsideration.

A petition to delist may be granted on the basis of a determination that the factors that were the basis for listing no longer threaten the continued existence of the species or because the decision to list was in error in light of the available evidence (*Central Coast Forest Assn. v. Fish and Game Commission* (2017) 2 Cal.5th 594, 604-605; *Central Coast Forest Assn. v. Fish and Game Commission* (2018) 18 Cal.App.5th 1191, 1204-1205.). In the case referenced above, the California Supreme Court found that a petition that presents new evidence to the Commission regarding the status of an already-listed species must still be reviewed by the Commission in the same way it would review a petition for listing (*Central Coast Forest Assn. v. Fish and Game Commission, supra*, 2 Cal.5th at p. 606). The California Supreme Court found that a delisting petition that provided new evidence that a population of Coho Salmon should not have been listed as endangered originally was the proper method of challenging the original listing decision (*Id.*). On remand, the Court of Appeal found that the Commission appropriately determined that the petitioned action of delisting the populations of Coho Salmon south of Punta Gorda (Humboldt County) was not warranted (*Central Coast Forest Assn. v. Fish and Game Commission, supra*, 18 Cal. App.5th at pp. 1224-1235). This was, in part, because there was neither evidence that those populations of Coho Salmon consisted entirely of hatchery stocks nor evidence that the

populations south of Punta Gorda were non-native. Thus, the Coho Salmon populations could not be considered ESUs, as is required to either list or delist only a population of a species under CESA.

## EMERGENCY LISTING

The Fish and Game Code authorizes the Commission to adopt a regulation adding a species to the list of endangered species or to the list of threatened species as an emergency regulation if the Commission finds that there is an emergency posing a significant threat to the continued existence of the species (Fish & G. Code § 2076.5). The Commission is required to notify affected or interested persons of the adoption of such an emergency regulation (*Id.*). To date, the only species that the Commission has listed on an emergency basis is the tricolored blackbird (*Agelaius tricolor*). The Commission listed the tricolored blackbird on an emergency basis in December 2014. The Commission subsequently let the emergency listing lapse, by vote, in June 2015.

The Administrative Procedure Act (Cal. Gov't Code §§ 11340–11365) governs emergency regulations promulgated by state agencies, including emergency listing decisions. Subsection 11346.1(b)(1) states “if a state agency makes a finding that the adoption of a regulation or order of repeal is necessary to address an emergency, the regulation or order of repeal may be adopted as an emergency regulation or order of repeal” (Cal. Gov't Code § 11346.1(b)(1)). Further, Government Code section 11346.1 states as follows: “No regulation, amendment, or order of repeal initially adopted as an emergency regulatory action shall remain in effect more than 180 days unless the adopting agency has complied with Sections 11346.2 to 11347.3, inclusive, either before adopting an emergency regulation or within the 180-day period.” (*Id.*).

In light of the requirements of the Administrative Procedure Act, the Commission may let an emergency listing rule lapse after 180 days, comply with the requirements set forth in the Administrative Procedure Act then adopt the listing rule on a permanent basis, or elect to follow the traditional listing procedure by requesting a written evaluation from the Department and determining whether the petition provides sufficient information to indicate that the petitioned action may be warranted.

## PROTECTIONS AFFORDED LISTED AND CANDIDATE SPECIES

Once a species is listed under CESA, it is afforded broad protections that are intended to serve the dual purpose of halting the decline of the species, while also contributing to the conservation of the species so that it may ultimately be removed from the list of endangered and threatened species. Specifically, pursuant to Fish and Game Code section 2080, “[n]o person or public agency shall import into this state, export out of this state, or take, possess, purchase, or sell within this state, any species, or any part or product thereof, that the commission determines to be an endangered species or a threatened species, or attempt any of those acts” (Fish & G. Code § 2080; Cal. Code Regs., tit. 14, § 783.1). The term “take” is defined to mean “[h]unt, pursue, catch, capture, or kill, or attempt to hunt, pursue, catch, capture, or kill” (Fish & G. Code § 86). These acts are prohibited with respect to listed species and candidate species, as candidate species receive the same legal protections afforded to endangered or threatened species (Fish & G. Code § 2085). Penalties for violating the take prohibition range from \$25,000 to \$50,000 for each violation, one-year imprisonment, or both fine and imprisonment (Fish & G. Code § 12008.1).

The Department may authorize the take of a listed species if certain conditions are met. The following is a non-exhaustive list of take authorizations available under CESA: (1) Through permits or memoranda of understanding, the Department may authorize individuals, public agencies, universities, zoological gardens, and scientific or educational institutions, to import, export, take, or possess any endangered species, threatened species, or candidate species for scientific, educational, or management purposes (Fish & G. Code § 2081(a)); (2) Through incidental take permits, the Department may authorize take that is expected to occur incidental to otherwise lawful activities (*Id.*, § 2081(b)); (3) Through safe harbor agreements, the Department may offer, in exchange for actions that contribute to the recovery of listed species, formal assurances to private landowners that their activities will not be deemed to violate the take prohibition (*Id.*, §§ 2089.2–2089.26); (4) Through a consistency determination, the Department may authorize take of a listed species, provided the species is also listed under the federal Endangered Species Act, take authorization has been obtained pursuant to that Act, and the authorization is consistent with CESA (*Id.*, § 2080.1); (5) Through the Natural Community Conservation Planning program, the Department may authorize take pursuant to comprehensive conservation and management plans (*Id.*, §§ 2800–2835); and (6) Through voluntary local programs, the Department may authorize farmers and ranchers to take listed species incidental to routine and ongoing agricultural activities (*Id.*, §§ 2086–2089).

The Commission has separate authority under section 2084 of the Fish and Game Code to authorize the taking of any candidate species based on the best available scientific information (Fish & G. Code § 2084). To utilize this authority, the Commission must adopt a regulation either simultaneous with its decision to make a species a candidate for listing or at a subsequent meeting. This is commonly referred to as a 2084 regulation. Historically, the Commission made greater use of this authority under section 2084 than it has in the past 10 years. This decline in the use of its authority is a consequence of a challenge to a 2084 regulation for the black-backed woodpecker (*Picoides arcticus*) that led to an out-of-court settlement and stipulated dismissal of the lawsuit. (This history is recounted in subsequent litigation between the parties with respect to attorney’s fees, *Center for Biological Diversity v. Fish & Game Commission* (28 Jan 2014, A137889) [nonpub. opn.] Since then, the Commission has adopted 2084 regulations for two candidate species: tricolored blackbird and western Joshua tree. In both cases, petitioners and the Department did not oppose the regulations. This could indicate an unwillingness on the part of the Commission to adopt 2084 regulations that are opposed by petitioners, the Department, or both.

## CONCLUSION

CESA, much like its federal counterpart, provides a comprehensive scheme for petitioning, reviewing, listing, and authorizing take of threatened and endangered species. However, unlike its federal counterpart, CESA contemplates a petition process that plays out before two agencies – the Department and the Commission – and relies on a definition of species and subspecies that is arguably narrower than that employed by the federal Endangered Species Act. The latter of these two distinctions may not hold over time as a consequence of the position taken by the Commission and the judiciary in recent years. But when the Commission takes steps that arguably push the limits of its authority – as some argue it has done in recent actions regarding bumble bees and mountain lions – interests groups may be expected to turn to the other branches of government to make their case for or against

listing. In the coming years, it seems likely that the courts and perhaps even the legislature will play as meaningful a role in the evolution of CESA as the Commission and Department.

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## FULL RESEARCH ARTICLE

# The Explicit Integration of Species Conceptual Models and Species Distribution Models as a Best Practice for Systematic Conservation Planning in California

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Natural Community Conservation Plans (NCCPs) represent the most powerful tool in statute for regional and systematic conservation planning for species at risk in California. This study examines the use of species conceptual models (SCMs) and species distribution models (SDMs) in such planning. Eighteen Natural Community Conservation Plans (NCCPs) were analyzed to determine if or how explicit connections were made between both types of models for a covered species and key components of its conservation strategy. Results indicate plans were strong in the use of SDMs, however, each deferred preparing or using SCMs to later management and monitoring phases. A more effective best planning practice for developing a conservation strategy is to explicitly integrate SCMs and SDMs during plan preparation.

**Key words:** endangered species, habitat conservation planning, Natural Community Conservation Planning (NCCP), species conceptual models, species distribution models

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This is a study of the explicit and effective integration of species models into the planning processes of Natural Community Conservation Plans (NCCPs). As discussed herein, NCCPs are California's most powerful tool in statute for species conservation on a regional scale, with a higher standard for conservation than federal Habitat Conservation Plans (HCPs). In statute, NCCPs (California Fish & G. Code § 2800 et seq.) are an alternative to the project-by-project incidental take permitting process (Fish & G. Code § 2081) under the California Endangered Species Act (CESA). Such systematic and regional conservation planning is a critical ongoing need for the state and identifying "best practices" for all aspects of creating these plans is also an ongoing need. California has more than 2,000 plant species and more than 400 animal species that are considered to be at risk – meaning they are already state or federally listed as threatened or endangered or are at risk for becoming so (CDFW 2020a). The state's population is near 40 million and is expected to reach 45 million by the year 2035 (CDOF 2019).

Species models serve to gather the collective scientific knowledge of a species (Franklin 2009). Species account models (SAMs) are verbal accounts, yet they provide

conceptual information such life history, habitat use, geographic range, distribution, threats, and population trends and so may be considered a form of modeling (Andelman et al. 2001). Management-oriented species conceptual models (SCMs), in graph form, clarify assumptions regarding a species' relationship to ecosystem components, stressors, and its response to potential management actions (Atkinson et al. 2004; Hopkins 2004). They also identify remaining uncertainties, key to hypothesis testing in an adaptive management and monitoring context. SCMs provide a bridge between the goals and objectives of a conservation plan and the conservation measures or management actions assumed necessary for achieving them and thus preparing them at the time a conservation plan is being written would be a best practice.

Explicitness in the development and use of species distribution models (SDMs) in conservation planning, particularly reserve design, would also be a best practice. The design of a reserve network is inherently spatial. SDMs provide spatial data on both known occurrences of a species as well as environmental variables thought to predict its occurrence. They also provide the rule base linking species occurrences to environmental variables (Franklin 2009). Explicitly disclosing rules and assumptions for mapping the predicted distribution of a species allows a user to both replicate a model and evaluate uncertainty in the prediction.

SAMs, SCMs and SDMs are communication tools, for stakeholders in the present and future. Over the lifetime of a plan, often 50 years or more, they can serve as a marker for future planners on the knowledge and assumptions guiding scientists and planners during the time at which a plan was approved.

Regional conservation planning is a tool for resolving potential conflicts between economic development (e.g., urbanization, agriculture) and threatened and endangered (listed) species, especially in biologically rich areas of the state that face high levels of growth and development (Atkinson et al. 2004). State and federal wildlife agencies in California have two primary statutes to accomplish species conservation—state Natural Community Conservation Plans (NCCPs) under California Fish and Game Code Section 2800 et seq. and federal Habitat Conservation Plans (HCPs) under the Endangered Species Act Section 10(a)(1)(B). These plans are intended to establish large reserve networks of permanently protected lands and long-term programs designed to conserve, mitigate for, and manage species legally “covered” by a plan while they allow compatible and appropriate development (Presley 2011). In California, an HCP can be implemented without an NCCP. However, all NCCPs are joint state and federal NCCP/HCPs, although hereafter they will be referred to as NCCPs. NCCPs in California may also be thought of as systematic conservation plans, the term “systematic conservation planning” having come from the seminal and highly cited work of the same name published in *Nature* by Christopher Margules and Robert Pressey (2000).

NCCPs may, in fact, be among the best examples of government-sponsored systematic conservation planning. Both NCCPs and HCPs provide conservation benefits beyond that of traditional approaches to endangered species conservation, which allow limited “incidental take” of species in exchange for habitat mitigation actions or offsets, often on a project-by-project basis (McKenney and Kiesecker 2010:174). This practice results in uncoordinated or piecemeal mitigation, far less effective than a coordinated, regional approach (Underwood 2010). NCCPs and HCPs provide coordinated mitigation and conservation actions that can result in larger blocks of higher quality and more connected habitats (Noss et al. 1997). Underwood (2010) demonstrated the effectiveness of this approach by comparing two large areas of San Diego County and finding that the portion with a multispecies NCCP/HCP had implemented 5–10 times more area for conservation of rare species than the portion practicing project-by-project or piecemeal mitigation.

Beyond this, NCCPs are subject to an even higher standard for conservation than HCPs. To approve an HCP under the federal Endangered Species Act (16 USC §§ 1531–1544), the federal government must find that the taking of a species by a proposed project will not “appreciably reduce the likelihood of the survival and recovery of the species in the wild” (Section 10(a)(1)(B)(iv)). By legislative intent, this finding is treated as equivalent to the language in Section 7(a)(2) – that a proposed project “... is not likely to jeopardize the continued existence of any endangered species or threatened species or result in the destruction or adverse modification of habitat” for the species. (See USFWS 2007 for example of equivalence language.) Effectively the standard is one of “no jeopardy” (Pollak 2001).

To approve an NCCP under California’s Endangered Species Act (Fish & G. Code §§ 2050–2089), the state government must find that “the development of reserve systems and conservation measures in the plan area provides, as needed for the conservation of species: ...the establishment of ...one or more reserves or other measures that provide equivalent conservation of Covered Species within the Plan Area and linkages between them and adjacent habitat areas outside the Plan Area” (Section 2820(a)(4)(B)). “Conservation” means “to use, and the use of, methods and procedures within the plan area that are necessary to bring any covered species to the point at which the measures provided pursuant to Chapter 1.5 (commencing with Section 2050) [The California Endangered Species Act] are not necessary” (Section 2805(d)). Requiring that a species be brought to the point of no longer requiring protection under the California Endangered Species Act is effectively a standard of recovery (Hopkins 2004; Presley 2011).

Thus, NCCPs are mandated to provide both recovery and habitat connectivity beyond plan boundaries for covered species, a powerful combination for the conservation of a species across its entire geographic range. Greco (2020) examined the mix of conservation standards across the geographic range of the federally and state-listed threatened giant garter snake (*Thamnophis gigas*) in California, finding only 14% of the range to be subject to an NCCP recovery standard and concluding that the variation in standards could have significant implications for recovery.

In this study, we examined the integration of species conceptual models and spatial models in the NCCP planning process (see Table 1 for a typology of models used in NCCPs). Often such models are included as appendices in NCCPs, but it is not always clear if and how they are used in the planning process. Specifically, we looked at how management-oriented species conceptual models (SCMs) and species distribution models (SDMs) are currently used in NCCPs – to guide biological goals and objectives, adaptive management and monitoring, and reserve design (see Fig. 1). Calls in the literature for explicitness in how models translate into conservation strategies and reserve designs come from Atkinson et al. (2004), Franklin et al. (2011), Guisan et al. (2013), and Tulloch et al. (2016). Specifically regarding SDMs, researchers have shown how the vast majority of research focuses on methods rather than implementation in the context of systematic conservation planning, implying that research on the explicit connections between SDMs and reserve design strategies is rare (Mair et al. 2018, Guisan et al. 2013). We assessed past and current practice regarding model integration (i.e., SAM, SCM, SDM) for systematic conservation planning throughout California.

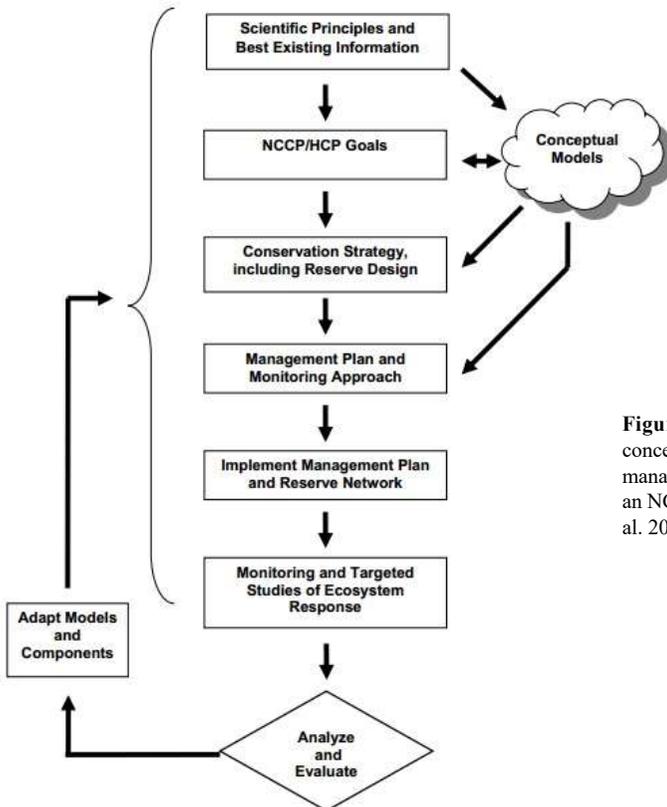
We examined four central research questions, presented here with some key background information related to each question. First, what is the level of modelling done in the planning phase of an NCCP and do NCCPs prepare SCMs and SDMs during this phase? All NCCPs are in one of two phases: planning or implementation. In the planning

**Table 1.** Typology of models used in NCCPs. According to Andelman et al. (2001), verbal accounts, mathematical formulae, and graphical diagrams are all structural variations of conceptual models. The conceptual model of interest here is a “management-oriented” species conceptual model (SCM). The spatial model of interest is a species distribution model (SDM).

Model Used in NCCP	Model Type and Structure	Alternate Terminology for Model Structure	Model Inputs in NCCPs
Species Account Model (SAM)	Conceptual model: verbal (text-based) account	Species account Ecological account Species profile	Legal status Species description Life history Habitat associations Geographic range Home range or territory size Distribution of occurrences in the plan area Threats Population trends Abundance estimates
Management-oriented Species Conceptual Model (SCM)	Conceptual model: graphical diagram	Influence diagram (Clemen 2001)  Conceptual diagram (Goodwin and Wright 1991)  Envirogram (Andrewartha and Birch 1984, James et al. 1997)  Causal web (Andelman et al. 2001)	Measurable aspects of response (e.g. habitat quality, patch occupancy, population size)  Anthropogenic threats Natural drivers Management actions Critical uncertainties
Species Distribution Model (SDM), using discrete or continuous variables	Spatial model: distribution map	Species distribution model (Franklin 2009)  Index of habitat suitability or potential occupancy (Barrows et al. 2005)	Mapped occurrences or population locations  Classified (expert opinion) suitable habitat – mapped as discrete polygons in vector format (if habitat features can be mapped) Or  Environmental variables that correlate with species presence – mapped as a composite of continuous variables in raster format

**Table 1** continued

Model Used in NCCP	Model Type and Structure	Alternate Terminology for Model Structure	Model Inputs in NCCPs
Population Viability Analysis (PVA), depending on available data	Conceptual model: mathematical formula	Count-based population viability analysis (Santa Clara Valley Habitat Plan 2012)  Count-based extinction analysis (Morris et al. 1999)	Known numbers of adults Population growth rates Reproductive rates
Spatial PVA (SPVA), depending on available data	Spatial model: spatially-explicit movement model with population size and demography	Individual model Occupancy map Population projection (size and demography)  (Schumaker 1998, 2010)	SDM (with habitat suitability) PVA parameters Movement parameters



**Figure 1.** A flow diagram of where conceptual models fit into the adaptive management and monitoring process for an NCCP. (Reprinted from: Atkinson et al. 2004).

phase, an Enrollment Agreement or Planning Agreement has been signed by the permittee organizations and the state and federal wildlife agencies who will approve and permit the plans. As the plan is being developed, one or more administrative drafts may be produced internally before a draft is prepared for public review (Presley 2011). A core component of each NCCP is the conservation strategy, consisting of both the biological goals, objectives and conservation measures for the plan and a strategy for reserve design (Hopkins 2004). In the implementation phase, a plan has been approved and permitted and progress on the conservation strategy, such as acquisition of reserve lands, is actively underway.

The second research question is: are there explicit connections between the SCMs and the proposed adaptive management and monitoring program for each plan and, if so, how are they made? Beginning with the NCCP Act of 2003 each plan is required to contain an adaptive management and monitoring program (Atkinson et al. 2004). “Adaptive management” as defined in the NCCP Act “means to use the results of new information gathered through the monitoring program of the plan and from other sources to adjust management strategies and practices to assist in providing for the conservation of covered species” (NCCPA 2003).

This leads to our third research question: are there explicit connections between SCMs and the biological goals and objectives of each plan and, if so, how are they made? Goals, objectives, and conservation measures differ across plans in how they are used. Generally, however, goals are broad statements of desired outcomes that set the direction for an NCCP (e.g., conservation for a covered species in perpetuity), objectives are specific and measurable statements detailing how each goal can be achieved (e.g., a specified quantity of acres of some specific habitat type for a covered species) and conservation measures describe actions (e.g., acquire land in fee title). The term “conditions for coverage” is also sometimes used in the biological goals and objectives section of an NCCP to describe specific actions that must be taken for a species to be considered conserved and thus eligible for coverage under a plan.

Our final research question is: are there explicit connections between SDMs and the reserve design of each plan and, if so, how are they made? For the purposes of this study, the reserve design is defined to include both the measurable reserve acreage commitments in the biological goals and objectives and the spatial design of the reserve system for the entire plan area. As stated previously, NCCPs must provide a connected reserve system, with linkages between reserves inside the plan area and to adjacent habitat areas outside of the plan area (NCCPA 2003).

## METHODS

The primary methodology used to explore and answer these questions was a keyword search of planning documents from 18 NCCPs in California that are either approved or in public draft form (Table 2). Nineteen NCCPs met this initial set of criteria, but one was dropped, the San Diego MSCP La Mesa Subarea Plan. The plan included only a brief statement describing its consistency to a programmatic NCCP, but otherwise contained none of the necessary elements to stand on its own as an NCCP. Of the remaining 18 plans, 15 have been approved and permitted and three are in public draft form, generally the final stage before public comments are reviewed and the plan is finalized and submitted to the state and federal wildlife agencies. Approved and permitted plans that are considered “Subarea Plans” to larger programmatic NCCPs were treated as unique plans. Programmatic NCCPs serve as “umbrella” plans. They do not receive permits and were not included as unique plans. However, they were considered as contributors of conservation analyses and modeling to

**Table 2.** NCCP/HCPs in California presented in order of permit year or public draft year. Plans with a permit year are approved and in the implementation phase. Those with a public draft year are still in the planning phase.

Plan #	Approved and Permitted NCCP/HCPs	Permit Year	NCCP Act Version	Scientific Advisor Report
1	San Diego Gas & Electric	1995	1991	n/a
2	County of Orange Central and Coastal Subregion	1996	1991	n/a
3	San Diego Multiple Species Conservation Program (MSCP) Poway Subarea Plan	1996	1991	n/a
4	Kern Water Bank	1997	1991	n/a
5	San Diego MSCP City Subarea Plan	1997	1991	n/a
6	San Diego MSCP County Subarea Plan	1998	1991	n/a
7	San Diego Multiple Habitat Conservation Program (MHCP) Carlsbad Subarea Plan	2004	2001	n/a
8	Western Riverside Multi-Species Habitat Conservation Plan (MSHCP)	2004	2001	n/a
9	San Diego MSCP Chula Vista Subarea Plan	2005	2001	n/a
10	East Contra Costa County	2007	2003	Huntsinger et al. (2003)
11	Coachella Valley Multiple Species Habitat Conservation Plan (MSHCP)	2008	2003	Noss et al. (2001)
12	San Diego County Water Authority	2011	2003	Rahn et al. (2008)
13	Santa Clara Valley Habitat Plan	2013	2003	Spencer et al. (2006a)
14	Orange County Transportation Authority	2017	2003	Rahn et al. (2011)
15	Yolo NCCP/HCPs in Public Draft Form	2019 Draft Year	2003 NCCP Act Version	Spencer et al. (2006b) Scientific Advisor Report
16	Rancho Palos Verdes	2018	2001	n/a
17	Butte Regional Conservation Plan	2019	2003	Spencer et al. (2007)
18	Placer County Conservation Plan	2019	2003	Brussard et al. (2004)

their subarea plans. All documents were publicly available as downloads from the websites of organizations serving as lead entities for the plans. A lead entity is generally a local government with land use planning authority, but it is not required to be so; regional authorities for water and transportation and private companies also undertake NCCPs (Hopkins 2004).

To address the first question regarding the level of modelling done in the planning phase of an NCCP, we searched each plan document using model names, model types, and alternate terms for model structure as keywords, entries in columns 1–3 of Table 1, respectively. The goal was to determine which of the model inputs (Column 4 entries) were present for species in a plan. The criteria for including model inputs as present are listed in Table 3. Model inputs were considered present if they were included for one or more species in a plan. They were also considered present if, in the case of subarea plans, direct reference was made to a species model in a programmatic NCCP. Of the six subarea plans, three relied wholly on species models produced for a programmatic NCCP and three both referenced models in a programmatic NCCP and included models for species in the subarea plan that were not included in the programmatic NCCP.

**Table 3.** Criteria for including model inputs as present in NCCPs

<b>Model Inputs</b>	<b>Criteria for Including Model Inputs as Present</b>
	<b>Species Account Model (SAM)</b>
Legal status	listing status as threatened or endangered under the California or federal Endangered Species Acts or rare under the California Native Plant Protection Act (FGC §§ 1900–1913) inclusion on any administrative watch lists such as California Species of Special Concern
Species description	physical description, growth habit in the case of plants, and/or any level of taxonomic information
Life history	pattern of survival, life cycle, and reproduction events for a species
Habitat associations	for terrestrial wildlife species, habitat types, natural community types, or landcover types known to be suitable for a species meeting one or more life history requirements; for fish, stream reaches or water bodies with the proper conditions, such as temperature or flow rate or connectivity, to support one or more stages of a species' life history; for plants, inclusion of plant associations, soil type, hydrology, slope, or elevation
Geographic range	description and/or map of the limits of distribution globally, in North America or in California
Home range or territory size	for terrestrial wildlife species, reference to the distance an individual travels in meeting life history requirements; for species that are also territorial, reference to the average territory size for an individual, or a range of sizes depending on habitat conditions or gender
Distribution of occurrences in the plan area	general description of population locations, often in the absence of surveys for the entire plan area
Threats	anthropogenic threats such as habitat loss or fragmentation, exotic species introduction, uncontrolled grazing, pollution, pesticide use, or noise disturbance
Population trends	globally, in North America, in California, or in the plan area (if known); located through keyword searches on “trend”, “population”, “declining”, “stable”, and “increasing”

Table 3 continued

Model Inputs	Criteria for Including Model Inputs as Present
Abundance estimates	globally, in North America, in California, or in the plan area (if known), expressed as a range of population size or as a density estimate
Measurable aspects of response	Identification of variables for measuring the response of natural communities, species or populations to management actions such as habitat quality, patch occupancy or population size, respectively
<b>Management- oriented Species Conceptual Model (SCM)</b>	
Anthropogenic threats	threats such as habitat loss or fragmentation, exotic species introduction, uncontrolled grazing, pollution, pesticide use, or noise disturbance expressed as management issues in a conceptual diagram
Natural drivers	drivers of change such as fire or hydrologic regimes directly connected to response variables in a conceptual diagram
Management actions	mitigating actions directly connected to anthropogenic threats in a conceptual diagram
Critical uncertainties	outstanding research questions for completing or updating a conceptual diagram
<b>Species Distribution Model (SDM), using discrete or continuous variables</b>	
Mapped occurrences or population locations	occurrences presented as points in vector format in a GIS, either as maps in a plan document or available digitally as Geographic Information Systems (GIS) data
Classified (expert opinion) suitable habitat – mapped as discrete polygons in vector format (if habitat features can be mapped)	for terrestrial wildlife species, habitat types, natural community types, or landcover types known to be suitable for a species meeting one or more life history requirements, sometimes incorporating a minimum habitat patch size; for fish, stream reaches or water bodies with the proper conditions, such as temperature or flow rate or connectivity, to support one or more stages of a species' life history; for plants, inclusion of plant associations, soil type, hydrology, slope, or elevation
Or	(no instances found)
Environmental variables that correlate with species presence – mapped as a composite of continuous variables in raster format	
<b>Population Viability Analysis (PVA), depending on available data</b>	
Known numbers of adults	count-based or other methods of determining the number of adults
Population growth rates	calculated rate based on a ratio between number of adults in any given year and number of adults one year later

Table 3 continued

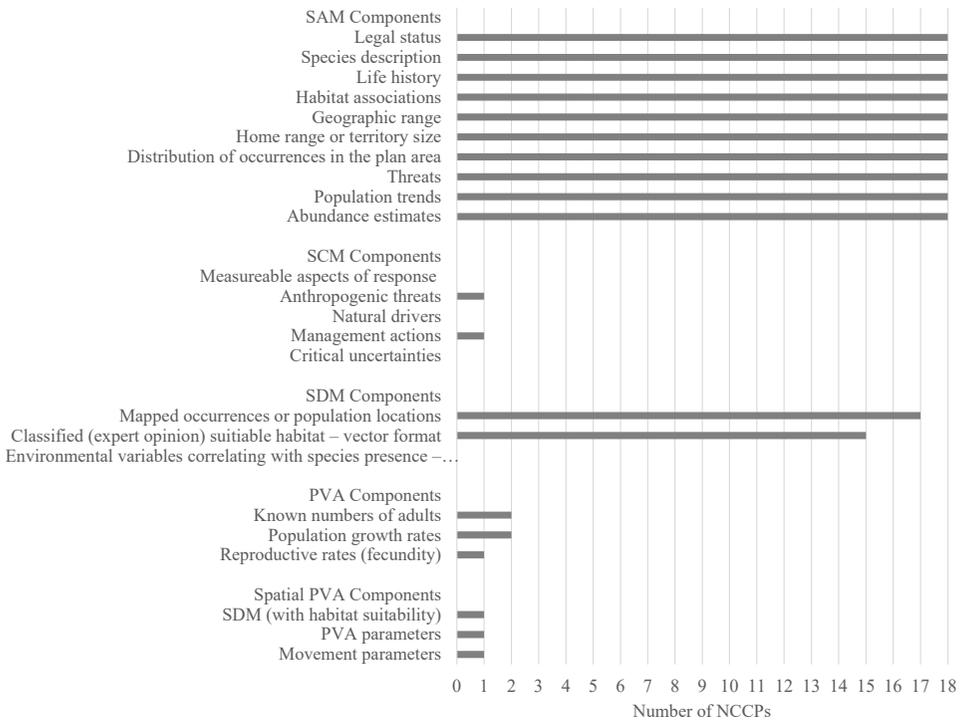
Model Inputs	Criteria for Including Model Inputs as Present
Reproductive rates	fecundity, based on survival and fertility rates
<b>Spatial PVA (SPVA), depending on available data</b>	
SDM (with habitat suitability)	one of more of the SDM components listed above
PVA parameters	one or more of the PVA components listed above
Movement parameters	one or more spatial components of a PVA that affect habitat suitability such as connectivity of habitat on a landscape or dispersal ability of a species

To find explicit connections between the SCMs and the proposed adaptive management and monitoring program, our second question, we conducted a keyword search in the adaptive management and monitoring chapter of each plan for direct reference to the name and location of the species models in the documents for that plan. Only one plan was found to contain SCMs, so to determine intent to create SCMs among the remaining plans, we also searched for “concept” and “model.” The search for explicit connections between SCMs and biological goals and objectives, our third question, was also limited because only one plan was found to contain SCMs. Therefore, to see how any level of species modeling may be influencing biological goals and objectives in the remaining plans, we looked at connections between the one SAM component that is in common with an SCM, namely, threats. “Threat” was used as a keyword in the search, as were “enhance” and “restore,” action words found among biological goals and objectives for reducing or mitigating threats.

Finally, to research explicit connections between SDMs and reserve design, we looked in two locations within each plan. First, for connections to reserve acreage commitments, we searched the biological goals and objectives section for direct reference to the name and location of the SDMs in the documents for that plan. If this yielded no results, the keywords “model,” “occurrence,” and “occupied” were used to query for the components of an SDM. Second, for connections between the SDMs and the reserve design strategy for a plan, we employed several keywords that lead to descriptions of how SDMs may be used together, all of which can be found among the collection of planning documents: “criteria,” “principle,” “concept,” “rule,” “consideration,” “design,” “assembly,” “synthesis,” and “process.”

## RESULTS

Results for the level of modeling done in the planning phase of each NCCP are presented in Fig. 2. All 18 NCCPs contained the full suite of components for text-based SAMs. Only one NCCP prepared SCMs for its covered species in the planning phase (Plan #18 of Table 2). Regarding SDMs, 17 of the 18 plans (94%) contained maps of known occurrences for each covered species. Fifteen plans or 83% also presented expert-based habitat suitability maps for each covered species as discrete polygons in a vector-based GIS, wherein each mapped polygon represents one habitat type on the ground modeled as suitable for a species and contains a single habitat suitability value. No plans presented habitat suitability maps as a composite of continuous environmental variables in a raster-based GIS, wherein each

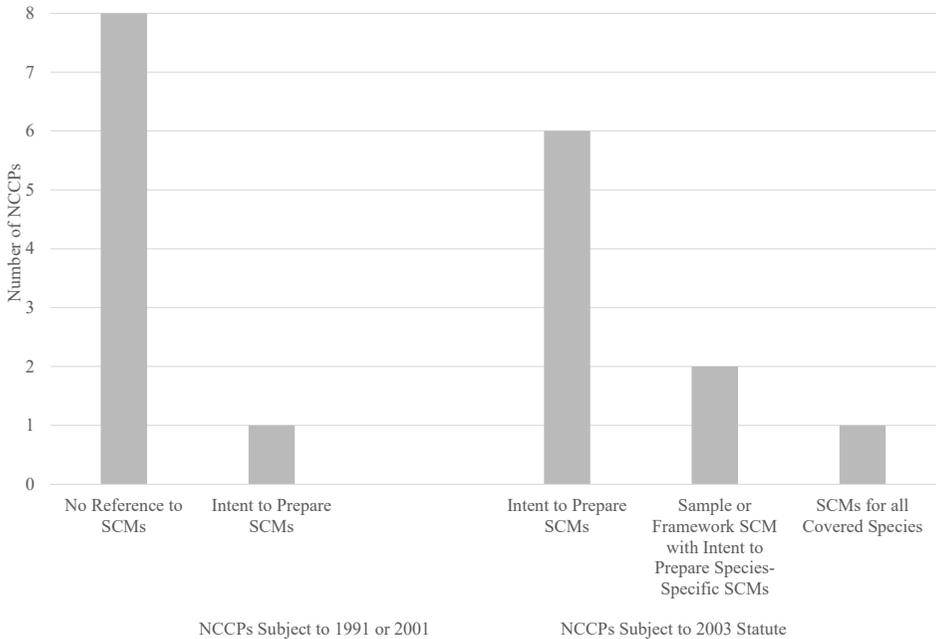


**Figure 2.** The model components and levels of species modeling found among existing NCCPs.

cell in a pixelated mapped surface has a habitat suitability value representing the summed value of layers of environmental variables for that cell on the ground, creating a “surface” of habitat suitability that is continuous. (See Chang 2019 for a full comparison of vector and raster data formats in GIS.) Just two plans (#7, #13) contained some components of Population Viability Analysis (PVA) species models and, in each case, for a single species only. One of these two plans (#7) also had components of a spatial PVA.

Results for explicit connections between the SCMs and the proposed adaptive management and monitoring program are presented in Fig. 3. A full 50% of the eighteen plans made no reference to SCMs. Only one plan (#18) prepared SCMs at the planning phase but did not declare intent to use and refine them until a future date. Two additional plans (#14, #15) prepared a single sample or framework SCM for use in preparing future species-specific SCMs. Six plans declared an intent to develop and use SCMs in their adaptive management and monitoring chapters (#8, #10, #11, #12, #13, #17). Significantly, eight of these nine collective plans referencing SCMs represent the plans in Table 2 subject to approval through the NCCP Act of 2003, which added the requirement for an adaptive management and monitoring component.

Beginning with a 2000 amendment to the NCCP Act, new NCCPs were also required to incorporate independent scientific input, although several plans already underway with signed Planning Agreements were “grandfathered” in and exempted from this requirement. A search of scientific advisory reports prepared at the start of the planning process for each NCCP required to prepare such an analysis (Table 2) revealed that seven out of

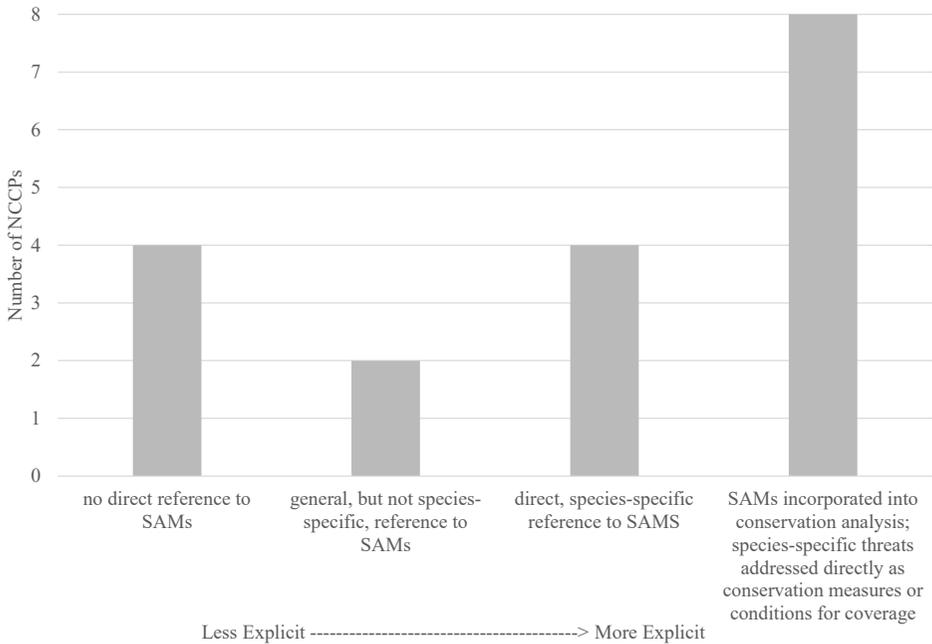


**Figure 3.** Among early NCCPs ( $n = 9$ ), only a portion reference species conceptual models (SCMs) in their adaptive management and monitoring chapters. Among NCCPs subject to the 2003 statute ( $n = 9$ ), most plans present an intent to prepare SCMs in the future.

eight recommended the use of SCMs in adaptive management and monitoring. One such report called for the development of SCMs ideally up front, that is, in the planning phase (Rahn et al. 2008). It is worth noting that Atkinson et al. (2004) was published shortly after the NCCP Act of 2003, placing SCMs as necessary inputs to the adaptive management and monitoring process (Fig. 1).

Results of the search for connections between threats described in SAMs and biological goals and objectives are shown in Fig. 4, categorized and presented in order from least to most explicit connections. Six of the eighteen plans, or one-third, either made no direct reference or a very general one to the SAMS when presenting biological goals and objectives, indicating they were used but unclear about how. Two-thirds of the plans made direct references to SAMS in species-specific biological goals and objectives. The most explicit connections were made by eight plans (#5, #6, #7, #8, #9, #12, #16, #17), who chose to present an analysis of SAM components, such as threats, side-by-side with species-specific biological goals and objectives as a justification for them. Thus, it was transparent and immediate to see how the models and model assumptions were used.

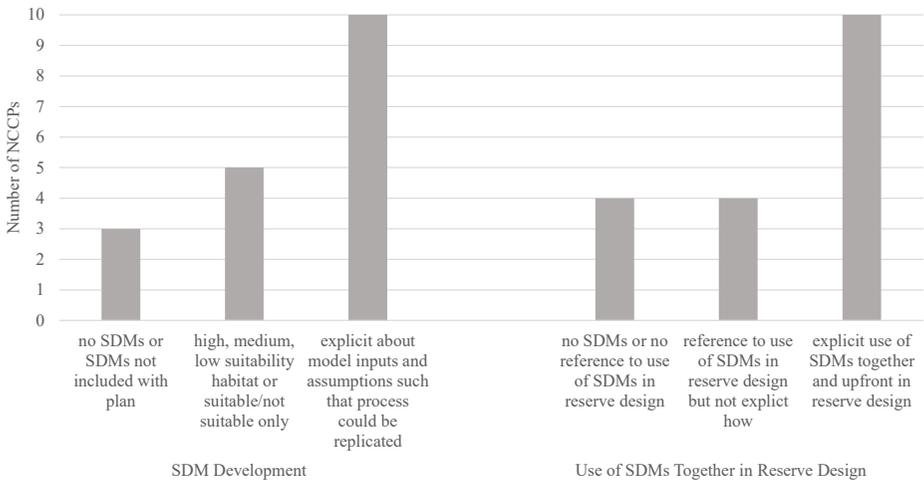
Finally, we examined explicit connections between SDMs and reserve designs. Each of the 17 plans that contained SDMs made a direct reference to their use in the conservation strategy, which includes reserve design, and the one remaining plan (#4) made reference to text-based information in the SAMs it contained. However, it was not always explicit how SDMs and SAMs were used. Since modeled suitable habitat is used along with occurrence data as the basis for reserve acreage commitments by species, the next question to examine



**Figure 4.** There is a range of explicitness among NCCPs in how SAMs connect to biological goals and objectives.

was how explicit the process was for using classified (expert opinion) suitable habitat in each SDM. The results are shown in Fig. 5. Of the 15 plans that had this SDM component, five plans (#2, #9, #11, #15, #16) had habitats ranked as “high”/ “medium”/ “low” or “suitable”/ “unsuitable” for a species, without disclosing model inputs or assumptions, such as habitat types selected or minimum habitat patch size applied. Thus, these models could not be replicated just with the information contained in the plan. Ten plans were explicit about how each suitability map was created such that the process could be replicated in a GIS.

Regarding use of the models together in the creation of a reserve design, three levels have been distinguished and are shown in Figure 5 from the least to the most explicit. Three of the 17 plans containing at least one component of an SDM did not describe a process of using the models together in the reserve design (plans #9, #13, #14). Four plans made reference to using SDMs in their reserve designs but were not explicit about how their individual SDMs may have been used together and upfront (plans #10, #15, #17, #18). Ten NCCPs described processes for using SDMs together and upfront for prioritizing lands in a reserve system (plans #1, #15, #2, #3, #5, #6, #7, #8, #12, #16). Spatial analysis methods used by the 10 plans included: (1) “hotspot” analyses, in which landscape data are divided into standard units such as square or hexagon grid cells and point-based species occurrence data is assessed for each cell to locate areas of high density or “hotspots,” (2) formal and “informal” GAP analyses (USGS 2019), in which suitable habitat and/or known occurrences for one or more targeted species on a landscape is overlain with existing conserved lands to locate “gaps” in conservation, and (3) a composite habitat evaluation model (Ogden 1995), which consists of high priority habitat for selected covered species, wildlife corridor data, and a habitat value index. The habitat value index itself represents seven input data layers



**Figure 5.** In SDM development, not all NCCPs are explicit enough about model inputs such that the process can be replicated. In the use of SDMs together and upfront in reserve design, NCCPs vary in explicitness.

weighted and combined to assess relative biological value: soils known to support sensitive plant species, adverse edge effects, habitat element features (e.g., presence of cliffs, springs, or ponds), ecotone index, habitat diversity index, rarity of natural habitats, and potential to support covered species.

## DISCUSSION

The results reveal that all plans create species models and reference them to some degree and most plans utilize SAMs in developing biological goals and objectives. Thus, there is a practice of connecting what is known about a species' life history, habitat use, geographic range, distribution, threats, and population trends to what would be appropriate objectives for that species in a strategy for conservation. Especially effective in conveying the reasoning behind a conservation strategy were those eight plans (44%) that presented an analysis of SAM components, such as threats, side-by-side with species-specific biological goals and objectives as a justification for them.

What is most concerning is the almost non-existent use of SCMs in the planning phases of NCCPs. Only one plan developed SCMs up front, and even this plan did not utilize these models in developing a framework adaptive management and monitoring program. It is strongly recommended that plans develop and utilize SCMs up front, so these models can inform key components of the plan, as indicated in Figure 1 (Atkinson et al. 2004). As stated previously, SCMs are a bridge between the goals and objectives of a conservation plan and the conservation measures or management actions assumed necessary for achieving them. They also serve to identify priorities for monitoring and critical uncertainties that still need research (Atkinson et al. 2004), information that would be beneficial, especially when scoping the long-term cost of a plan. Franklin et al. (2011), in prefacing a case study for developing a monitoring program for the San Diego Multiple Species Conservation Program (MSCP), acknowledge that conservation plan objectives can be set too broadly to identify monitor-

ing priorities during plan development and that monitoring and management often require more resources than are acknowledged or even known at the planning stage. Without proper funding at the outset, effectiveness monitoring for an NCCP can be significantly delayed.

There may be any number of reasons for the underutilization of SCMs in the planning phase of an NCCP. One is simply a lack of specificity in the requirements of the NCCP Act related to adaptive management and monitoring. In approving an NCCP, the state must find that the plan “integrates adaptive management strategies that are periodically evaluated and modified based on the information from the monitoring program and other sources” (Section 2810(a)(2)) “contains a monitoring program” (Section 2810(a)(7)) and “contains an adaptive management program” (Section 2810(b)(8)). Author MP has worked as an agency scientist reviewing NCCPs and preparing findings, and has found that these chapters are often very brief, with some planners considering that to define a management and monitoring program at the planning stage before reserve lands are acquired would be premature.

Although it would be premature to write individual management plans, SCMs with explicit assumptions are key to developing a management and monitoring framework to guide management planning across an entire reserve system. Managers of individual reserves must be able to translate the goals and objectives of a conservation plan into a work plan for management and monitoring—over time, in the context of a reserve network, and in the face of uncertainty. SCMs clarify assumptions regarding a species’ relationship to ecosystem components, stressors, and its response to potential management actions (Atkinson et al. 2004; Hopkins 2004). If SCMs were to be developed while a conservation plan was still being written, the goals of the plan might be specified in a way that makes management targets and monitoring objectives obvious (Franklin et al. 2011).

Explicit assumptions included as part of a SCM are especially important in an adaptive management context, which necessarily treats assumed causal relationships between modeled ecological variables as hypotheses (Woodward et al. 1999; Barrows et al. 2005; Franklin et al. 2011; Runge et al. 2011). Wrote one participant as feedback in a San Diego workshop designed to develop SCMs: “... the way this modeling process was done, identifying stressors and using available life history information was a good approach. I think we came up with some interim management methods/tasks that could be used immediately... to help protect occupied Hermes [a butterfly species] habitat from fire while we wait for research questions to be answered about dispersal and other unknowns.” (Lewison et al. 2012)

Plans varied in describing the creation of SDMs and their use together and upfront in reserve design. In creating SDMs, the five plans that simply ranked habitats as “high”/“medium”/“low” or “suitable”/“unsuitable” for a species, without disclosing model inputs or assumptions may indeed have engaged in an explicit modeling process among scientists without publishing details of the process in the plan, but this should be discouraged as a planning practice for NCCPs. More explicitness makes the planning process more transparent and inclusive to other stakeholders because interested parties are able to replicate the models and understand the assumptions made in using them. It is strongly recommended that metadata accompany each SDM. Ideally, it would include all input data sets, their sources, their limitations of use as described by their creators, and the assumptions modelers made in assembling them to create a SDM. Because knowledge of a species distribution is often coarse or incomplete, understanding data available and methodological choices used to create a model is key to its appropriate use (Sofaer et al. 2019).

When it comes to using SDMs together and upfront in reserve design, it is signifi-

cant that two of the three plans that did not conduct such an analysis are led by regional authorities with the ability to commit their own lands to a reserve system, and this factor is what likely drove the initial design. Among the remaining fourteen plans, one might have expected more spatial analysis among newer plans, with greater GIS capacity and access to more spatial data layers than were available to planners in the 1990s, but there does not appear to be a trend related to the year of plan approval. Researchers have acknowledged the difficulty in selecting an appropriate modeling method for multiple species, known as an ensemble modeling strategy (Lin et al. 2018). It is significant that two thirds of plans employed an ensemble strategy upfront in reserve design. Once again, it is recommended that modelers disclose any assumptions made or data limitations noted in the process of compiling multiple SDMs. For example, several NCCPs employed a process similar to that of the California Department of Fish and Wildlife (CDFW) Areas of Conservation Emphasis (ACE II) – which includes indices of biological value by hexagonal unit across the state, derived from occurrence data and range maps of multiple species. The metadata for ACE II not only discloses reasoning for the unit (hexagon) and scale (hexagon size) of analysis chosen, it also acknowledges that values are influenced by the data (or lack of data) available for any given species in any given region of the state. Included with the data are recommendations for appropriate use (CDFW 2015).

The intent of this paper has been to understand current practice regarding the integration of species models in systematic conservation planning, with an aim to improve practice overall, rather than to highlight the shortcomings of any individual plan. With a powerful and far-reaching statute to guide them and only fifteen plans approved statewide, NCCPs are in many ways still experimenting with best practices. Early publications have taken the form of case studies (Pollak 2001, for example) or guidance documents with “lessons learned.”

Universally, plans were strong in including detailed SAMS, while they varied in their ability to connect models directly to biological goals and objectives. Two-thirds of plans were explicit in SDM creation such that individual species models could be replicated in a GIS with the information provided in the plan and two-thirds of plans demonstrated ways that SDMs can be used together in reserve design. We found the greatest room for growth in the use of SCMs for adaptive management and monitoring planning, which may be facilitated by more detailed requirements in statute regarding the adaptive management and monitoring component of a plan. Professional training in the creation and use of SCMs may also help. To this end, NCCPs in the implementation phase that have developed SCMs for monitoring would be an ideal resource.

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ESSAY

## **Development of ecologically meaningful, multiple-species conservation strategies under the California and U.S. Endangered Species Acts**

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The California and U.S. Endangered Species Acts prohibit take of protected species, but allow for authorization of take incidental to otherwise lawful activities provided the take is minimized and mitigated. Incomplete and inconsistent ecological information can limit the contribution of mitigation plans for incidental take, especially those for multiple species, to species persistence. Many such plans focus on acquisition and management of coarse-resolution land-cover or land-use types. These classifications may not coincide with a species' resource requirements (its habitat) or the greatest constraints to its viability. Complementing acquisition with rigorous research on population biology, stressors, and habitat use and quality may be much more effective than preservation of putative but unproven habitat. Such adaptive conservation can be applied to species with restricted or extensive distributions. When the distribution and ecology of geographically restricted species are well-known, then connectivity analyses, sometimes complemented by spatially explicit, mechanistic population models, may inform habitat acquisition and management. When little information exists on the ecology or vital rates of a restricted species, we suggest assessment of occupancy, habitat use, or demography; tracking individuals' movements; and evaluation of habitat quality. Acquisition and management of local lands that may not serve as habitat is unlikely to contribute to conservation of extensively distributed species with range-wide declines. Instead, we suggest that conservation efforts for these species emphasize strategic acquisition of open space

(large, undeveloped areas that are more likely to serve as high-quality habitat), potentially in locations distant from the permit area. The above areas of research can inform optimization of conservation locations. Many mitigation decisions are based on assumptions drawn from limited data. Inclusion of scientific research in development and implementation of mitigation plans for incidental take can strengthen the plans' information content, improve the ecological success acquisition and management, and advance conservation of protected species.

**Key words:** adaptive management, connectivity, demography, habitat quality, mitigation, occupancy, optimization, population models, reserve management, spatial prioritization

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Conservation of species listed under the California Endangered Species Act (CESA; California Fish and Game Code sections 2050–2089.25) or U.S. Endangered Species Act (ESA; U.S. Code, Title 16, sections 1531–1544), especially on private lands, is hampered by ecological information that is limited and often is inconsistent among species and locations, and by the difficulty of reliably estimating species-specific effects of allowable land uses. These gaps in understanding make it difficult to evaluate whether conservation actions, including acquisition of potential habitat, are likely to compensate for the effects of ongoing land use and other regulated activities. Gradual, optimized establishment of conservation areas that is augmented by species-specific research may enable adaptive management that allows protected species to persist locally and regionally. Here, we focus primarily on terrestrial species on private lands, but much of our discussion is transferable to aquatic species and public lands.

### Permits for Incidental Take

The CESA and ESAs prohibit the take of species, subspecies, or, under the ESA, distinct population segments (collectively referenced herein as *species*) listed as endangered or threatened. The CESA also prohibits take of species that are candidates for listing. In general, exceptions are made, via permit, if the take is incidental to otherwise lawful activity and will not jeopardize the continued existence of the species. Under the ESA, individuals or entities that are proposing actions that do not require other federal permits, approvals, funds, or actions, and therefore are ineligible for Section 7 consultation, may apply for incidental take permits under Section 10(a). The applicant's habitat conservation plan (HCP) details how they will meet requirements for issuance of an incidental take permit. The ESA requires that an HCP specify the effects of the take and the steps that will be taken to minimize and mitigate those effects.

The CESA conditions issuance of incidental take permits on minimization and full mitigation of the impacts of the proposed taking. It is unlawful to take a species listed under the CESA, regardless of whether it is listed under the ESA, without additional state authorization. Individuals or entities may obtain an incidental take permit under Section 2081(b) of the California Fish and Game Code provided that the impacts of the proposed take are minimized and fully mitigated. If a species is listed under both the ESA and CESA, and an incidental take statement or permit is obtained under Section 7 or 10(a), respectively, of the ESA, then the California Department of Fish and Wildlife may determine that the federal authorization is sufficient to fulfill the requirements of the CESA. California and federal

law allow for some other exceptions to the take prohibitions. For example, Safe Harbor Agreements encourage voluntary conservation by landowners that will contribute to species recovery, and some scientific, educational, and management activities are eligible for permits.

Minimization and mitigation plans for incidental take under the CESA, or HCPs for such take under the ESA, may be developed for a single species or for multiple species (for simplicity, although the language of the CESA and ESA differ, we henceforth use *mitigation plans* to encompass the requirements of both acts). In California, plans may be developed for species that are listed or candidates for listing under the CESA. Federal plans may be developed for species that are listed or that may be listed over the duration of the permit. In recent years, the agencies that implement the ESA (US Fish and Wildlife Service and National Marine Fisheries Service) generally have discouraged inclusion in incidental take permits of species for which the likelihood of listing is low, data to inform conservation actions are quite limited, and occurrence in the plan area is uncertain (e.g., USFWS and NMFS 2016). Nevertheless, because private landowners generally seek assurances that future listings of species will not incur additional financial burdens or land-use restrictions, it is not uncommon for federal incidental-take mitigation plans to cover unlisted species. For example, of the 168 HCPs approved in Region 8 (California and Nevada) as of March 2020, 50 included taxa that were not listed under the ESA (ECOS 2020). In many cases, these species are designated as taxa of conservation concern by resource management agencies or taxonomic authorities.

### **Concepts of Habitat in Mitigation Plans for Incidental Take**

At both the California and federal levels, most species-specific mitigation plans focus on reducing threats to the species, acquiring areas that appear to function as habitat for the species, or increasing the quality and configuration of the species' habitat. Habitat, a species-specific construct, encompasses the space within which a species lives or can live and the abiotic and biotic elements in that space that generally are required for survival and persistence (Hall et al. 1997; Morrison and Hall 2002). The quality and configuration of a species' habitat affect its population dynamics and relations with other species (Pulliam 1988; Dunning et al. 1992; Watkinson and Sutherland 1995) and its connectivity, usually defined as the probability that genes or individuals move among patches of the species' habitat (McRae 2006; McRae et al. 2012). Although habitat is a central concept in plans to mitigate incidental take, plans use many different methods to assess habitat.

The ecology, and the breadth and depth of ecological knowledge, of species that are included in a plan for mitigation of incidental take varies. Gaps in demographic, environmental, and genetic data are common, even for listed species. The gaps can be exacerbated when a species occurs largely on private land to which access is relatively limited. In such circumstances, detailed understanding of phenomena affecting survival and reproduction across the species' range or within a given region often is limited. Even the information on occurrences that is included in many mitigation plans, particularly for species that are not listed, may not be collected or collated rigorously. The California and federal endangered species permitting agencies generally require an assessment of whether a given species has been documented or reasonably might be expected to occur in the planning area. Applicants often base these determinations on a limited number of surveys, complemented by searches of Natural Heritage Program or similar data, and the use of vegetation or other biotic or abiotic attributes as surrogates for habitat. The latter sources have limited information content

because they rarely account for observed absences, detection probability (the likelihood of observing a species given its presence; MacKenzie et al. 2003), temporal variation in presence and abundance, or the fact that presence may be transient and not reflect local recruitment.

The manner in which a species' habitat is represented in plans for mitigation of incidental take under the CESA and ESA may not fully reflect existing knowledge about habitat structure, composition, and function. Instead, descriptions and quantifications of habitat tend to rely on coarse-resolution land-cover or land-use types (e.g., grassland, pasture, shrubland, woodland, wetland). For example, the Pacific Gas & Electric Company San Joaquin Valley Operations and Maintenance HCP and Section 2081(b) permit estimated the percentage of area of 14 land-cover and land-use types within the plan boundary that might function as habitat for 48 species that were covered by the permits (Jones and Stokes 2007). Similarly, the South Sacramento HCP (which also received a Section 2081(b) permit for seven species listed under CESA) included a binary classification of whether 17 land-use and land-cover types were associated with potential presence of 29 covered species (County of Sacramento et al. 2018). Coarse representations of habitat make it difficult to estimate occupancy, abundance, survival, and reproduction, and therefore to identify the factors that most constrain survival and reproduction, the actions most likely to increase the species' survival and reproduction, and metrics of success. Several multiple-species HCPs in California incorporated the concept of umbrella species, or species that, if conserved, will confer protection to numerous co-occurring species (Gerrard et al. 2001; Winchell and Doherty 2008). Again, however, unless the distribution and habitat of hypothesized umbrella species and co-occurring species is evaluated rigorously, conservation of the umbrella or its habitat is unlikely to achieve comprehensive conservation objectives (Hitt and Frissell 2004).

### **Adaptive Management in Mitigation Plans**

Many mitigation plans include adaptive management of varying degrees of scientific rigor. For example, the East Bay Municipal Utility District Low Effect HCP ([https://ecos.fws.gov/ecp0/conservationPlan/plan?plan\\_id=3505](https://ecos.fws.gov/ecp0/conservationPlan/plan?plan_id=3505)) includes triggers for changes in management activities that relate to reproduction of several species. In most cases, the outcomes of management are assessed, but alternative management methods are not treated as formal experiments.

Ideally, the scientific information provided by adaptive management will benefit not only the plan area and species that use that area but other conservation efforts in the region, reducing the need for redundant research and, in turn, increasing the cumulative funds available for conservation of habitat (Wilhere 2002). However, existing adaptive management programs for private lands under the CESA and ESA generally were developed for reserves that were designed relatively early in the planning process as a primary mitigation measure, even when data were limited. Because reserves sometimes are designated if they meet coarse land-cover criteria, evidence that the area is viable habitat or occupied by the species may be sparse. We believe there are ecological benefits to conservation of open space. Nevertheless, without strong indications that reserves indeed are high-quality habitat and contribute to a species' viability, investments that are alternatives to rapid designation of reserves may have greater long-term value. We suggest that research on population biology, stressors, and habitat use and quality provides the best scientific data to inform reserve acquisition and management and to increase the likelihood that mitigation will meet conservation goals. Complementing mitigation planning and implementation with research, which we

reference as *adaptive conservation*, ultimately may contribute far more to the long-term viability of a species than preservation of marginal habitat that may not support reproduction and recruitment of the covered species. Although the state and federal endangered-species regulatory agencies rarely equate research with mitigation, others (e.g., Wilhere 2002) have proposed that mitigation credits be allocated for provision of information that is applicable to regional conservation.

Here, we outline how adaptive conservation in the context of a multiple-species plan to minimize and mitigate incidental take can ameliorate data gaps and increase the effectiveness of conservation efforts. Such needs are exacerbated for species that have extensive distributions and are declining across much of their range. We consider development of an adaptive conservation strategy for taxa for which local ecological knowledge and conservation options are highly variable. We introduce flexible methods for integrating multiple sources of data to optimize identification of potential locations for mitigation. These methods link research to action, including adaptive management. We consider adaptive conservation options for three types of species: an ecologically well-known species with a geographically restricted distribution, a geographically restricted species for which data are limited, and a species with a geographically extensive distribution. Although these classes of species do not encompass the full possible range of distributions and data that might be addressed in conservation plans, we believe that they are among the most common and challenging.

### **ECOLOGY WELL KNOWN, DISTRIBUTION RESTRICTED**

If a species' distribution is relatively constrained (e.g., a regional endemic), its ecology is fairly well known, and the quantity and quality of its habitat have been assessed rigorously and appear to be sufficient, then connectivity analyses may have considerable potential to inform adaptive conservation, including habitat acquisition and management. Efforts to conserve species are more likely to be effective when populations or areas that function as habitat are connected rather than isolated (Harris 1984; Hanski 1999). Connectivity is affected by topography; the built environment; vegetation composition, structure, and configuration; and ecological processes, such as fire or flows of water and nutrients. Assessment of connectivity provides information that may be relevant to identifying areas through which individuals can move among discrete populations or patches of habitat, thus facilitating gene flow and recolonization (Loss et al. 2011). High connectivity also can increase the resilience of populations to changes in land cover, land use, and climate, and minimize or facilitate the spread of non-native invasive species (Glen et al. 2013) or other undesirable environmental changes (Gray and Dickson 2015). Genetic data can be used as data inputs or to evaluate the outputs of analyses that did not include genetic data.

Different methods of assessing connectivity are useful in different situations. For example, methods based on graph theory (e.g., Urban and Keitt 2001; Bodin et al. 2006) represent an area as a set of nodes (patches of habitat or other discrete areas) connected to some extent by edges. Edges are functional links between pairs of nodes (e.g., populations in discrete patches linked by dispersal) that efficiently can represent simple connections among populations. Methods based on circuit theory (McRae 2006; McRae et al. 2008) are relatively flexible and incorporate maps of environmental heterogeneity that are known or assumed to affect animal movement. Circuit theory explicitly evaluates how topography, land cover, and other abiotic and biotic attributes affect the potential movements of individuals. Circuit-theory based methods are used to predict genetic and ecological effects

of environmental change, and to identify high-priority areas for maintaining or increasing connectivity. Such methods can be applied to diverse data, including coarse-resolution distribution maps, simple maps of habitat and nonhabitat, complex spatial data, or data on multiple species (Fleishman et al. 2017). Models that are based on circuit theory simultaneously consider all possible pathways that might connect pairs of populations. By extension, the models indicate probability of gene flow and landscape conditions that may impede genetic differentiation. Empirical validation of such estimates with genetic data is rare, but informative. For example, analyses of the genetics of giant gartersnakes (*Thamnophis gigas*), a species listed as threatened under the CESA and ESA, revealed relatively recent levels of genetic exchange (breeding) among populations (via genetic similarity) (Wood et al. 2015).

Spatially explicit, mechanistic population models (Schumaker et al. 2004; Schumaker and Brookes 2018) include mechanisms to simulate connectivity within the context of a population viability model so that connectivity is one of multiple factors that may affect probability of persistence. Spatial individual-based models such as HexSim (Schumaker and Brookes 2018) create connectivity outputs that are similar to those produced by circuit-based analyses but include greater biological realism by considering dispersal behavior and population demography and dynamics. The mechanistic simulations created by such models link the behavior and fates of individuals to their locations, supporting evaluation of the effects of environmental change on movement, population sizes, distributions, and genetic diversity (e.g., Heinrichs et al. 2019a, 2019b; Nogueira-McRae et al. 2019). Accordingly, the results of these models can project the population-level effects of barriers to movement and alternative reserve designs and be applied to examine the effects of compensatory mitigation (Barbosa et al. 2019). If there is a desire to project future occupancy given potential changes in land use or land cover, such changes can be simulated by modifying the input environmental data layers. If interactions among species, whether mutualistic or antagonistic, are known or hypothesized to affect species' distributions, then it is possible to model the interactions and resulting distributions of multiple species simultaneously. For example, HexSim models of the Northern Spotted Owl (*Strix occidentalis caurina*), a subspecies listed as threatened under the CESA and ESA, were developed to inform recovery plans and to model competitive interactions with Barred Owls (*Strix varia*) (USFWS 2011; Schumaker et al. 2014; Dunk et al 2019). These types of analyses can support defensible conservation decisions and contribute to selection of mitigation actions and locations that will increase the likelihood of species persistence.

As described below, reserve selection or other prioritization algorithms then can be used to integrate the results of distribution and connectivity analyses, and other considerations, to prioritize lands for multiple uses, including research, preservation, and economic activities compatible with conservation. These algorithms can be applied across extensive areas to optimize the quantity and connectivity of habitat for one or more species given different scenarios of environmental change (Margules and Pressey 2000; Moilanen and Kujala 2008). The outputs of any connectivity analyses can become additional data layers in iterative optimization analyses. For example, both occupancy and connectivity could be included in optimization runs. In the case of the Northern Spotted Owl, the outputs of spatially explicit, individual-based population models were used in a prioritization algorithm (Zonation; Moilanen et al 2009, 2014). The algorithm estimated and compared the dynamics of simulated populations of Northern Spotted Owls among a suite of candidate habitat networks, which varied in size and configuration, given alternative scenarios of future habitat quality and interactions with Barred Owls (Dunk et al 2019).

## ECOLOGY UNCERTAIN, DISTRIBUTION RESTRICTED

In some cases, a species' range may be known to be limited, but little information exists on the species' occupancy, habitat use, or vital rates (birth, death, immigration, and emigration). In these cases, we suggest four high-priority areas of research that can inform adaptive conservation: assessment of occupancy or habitat use, tracking the movements of individuals (often via radio or global positioning system [GPS] telemetry), demographic characterization, and assessment of habitat quality.

### Occupancy or Habitat Use

Estimation of occupancy or habitat use requires robust sampling that allows estimation of detection probabilities (MacKenzie et al. 2006). If a population is fairly open, occupancy models may estimate transient exploitation of resources or habitat use in a given location rather than consistent presence. Existing data often are insufficient to model presence, let alone occupancy, of a given rare species. Many observations of rare taxa have not been reported to publicly accessible data sources, such as the California Natural Diversity Database, so presence of the species in any given area may be underrepresented in public data. Furthermore, some records may reflect misidentification and may be biased towards locations near roads (Kadmon et al. 2004). Because inferences generally become stronger as sample sizes and the spatial extent of sampling increase, it is ideal to collect occupancy data with a spatially balanced design from as many locations over as much of the species' range as possible. Environmental DNA (eDNA) may be an effective means of establishing simple presence of terrestrial or aquatic species that are difficult to sample or cryptic (Bálint et al. 2018; Schumer et al. 2019). For example, the method has been applied to detect bull trout (*Salvelinus confluentus*), a species listed as endangered under the ESA (McKelvey et al. 2016), and endangered Gouldian finches (*Erythrura gouldiae*) in Australia (Day et al. 2019). However, environmental DNA usually does not allow one to obtain the precise coordinates or time at which a species was present, and it can be less effective in lotic than in lentic systems (Rees et al. 2014).

### Tracking Movement

Tracking multiple individuals of a species—in multiple populations, if applicable—throughout their annual or reproductive cycle provides information on occurrence (and, by extension, habitat use), the locations and attributes of home ranges, movement distances during multiple life stages, and behavior, potentially including interactions among individuals. Presence alone, especially of long-lived species, may not indicate recruitment and long-term viability. Therefore, assessment of population-level demography over multiple (often three or more) years or reproductive cycles allows for association of birth, death, emigration, and immigration rates with variation in climate, land use, and other abiotic and biotic environmental attributes.

Telemetry data can help inform assessment of connectivity. For example, it can identify barriers to movement or sources of mortality of vertebrates such as desert bighorn sheep (*Odocoileus hemionus*) and pronghorn (*Antilocapra americana*), various subspecies and populations of which are listed under the CESA and ESA and by the Navajo Nation (Fleishman et al. 2017). Moreover, telemetry data can reveal the locations and attributes of

habitat features that rarely are used, but are essential to survival and reproduction, such as nesting locations or neonatal movements of herptiles (Forsythe et al. 2004).

Advances in miniaturization are making telemetry more feasible, especially for relatively small animals. It widely is accepted that a tracking device should not exceed 3–5% of an animal's weight. Until recently, most GPS transmitters were too heavy to affix to small-bodied taxa, such as bats. However, GPS tags with weights of 1.45–180 g now are available, and field tests indicated that their accuracy is acceptable for some applications (e.g., bats, Conenna et al. 2019; giraffes, Hart et al. 2020). Another new technology, the Motus Wildlife Tracking System, receives individually identifiable signals from UHF tags within a line-of-sight distance of about 15 km (Taylor et al. 2017). There are, of course, limitations to these methods. For example, the smallest GPS tags require recapture of the animal or retrieval of the tag, and the Motus system relies on fixed receiving stations. Nevertheless, ongoing innovations almost certainly will continue to increase the practicality of tracking.

## Demography

Research on survival, reproductive success and recruitment often increases understanding of management actions that are consistent with population growth and stability. Effective conservation of any species relies on increasing values of demographic parameters that may be limiting viability. Integrated population models use information on population size, survival, and reproduction to project rates of population change and identify factors that affect those rates of change (Kéry and Schaub 2012). Mark-recapture is among the methods that provide data for demographic analyses, but is not feasible for many species, such as those that migrate across large distances or cannot survive being handled multiple times. Resighting, recapture, or recovery of marked individuals allows estimation of apparent survival (survival minus permanent emigration) if marked individuals are followed for three or more time intervals. Furthermore, mark-recapture analyses can provide estimates of stage-specific survival that, when coupled with estimates of stage-specific fecundity, can identify the age class or classes that make the greatest contribution to population growth (Crouse et al. 1987).

## Habitat Quality

It is helpful to evaluate habitat quality rigorously rather than simply assessing environmental associations with occupancy or abundance. As an example, evaluation of relations among the demography or physical condition of a target species, composition and abundance of prey, climate and other abiotic variables, and land uses (e.g., agriculture, recreation) can inform estimates of the degree to which these factors affect habitat quality and are useful for habitat evaluation in the context of reserve design and management. For instance, food availability and pre-migratory fat deposition in Northern Waterthrushes (*Seiurus noveboracensis*) varied along a moisture gradient, allowing for inference to habitat quality (Smith et al. 2010). Similarly, comparison of long-term occupancy and fledgling production provided indications of habitat quality in Eagle Owls (*Bubo bubo*) (Brambilla and Bionda 2013). Yet some elements of habitat quality, such as prey base, rarely are evaluated and documented. Instead, inferences about the quality of foraging habitat largely are anecdotal. However, conventional wisdom about land-cover types or other ecological attributes that represent the highest-quality foraging habitat is not always consistent with data on availability of prey or spatial and temporal variation in space use (Fleishman et al. 2016).

## EXTENSIVE DISTRIBUTIONS

Some unlisted species that are included in plans to mitigate incidental take under the CESA or ESA have geographically extensive distributions and are declining across much of their range, often as a result of widespread habitat loss and reductions in habitat quality (generally acknowledged as the greatest threats to species; Wilcove et al. 1998). Many such species, especially birds, are designated as species of special concern by federal or state resource agencies. For example, the Coachella Valley Multi-Species HCP, which also received a permit from California under sections 2800–2835 of the state’s Fish and Game code, included Yellow Warbler (*Setophaga petechia*), which breeds across Canada and the northern two-thirds of the United States. In these cases, acquisition and management of local reserves is unlikely to have appreciable relevance to the species’ regional or global status; opportunities to acquire areas that are known to be seasonally meaningful, such as major breeding or stopover grounds, are unusual. Migratory species may be affected by threats in either their breeding or wintering range, and when species traverse political boundaries, jurisdiction over such threats becomes more fragmented. Furthermore, the status and trends of such species often are asynchronous among regions. When potential habitat for these species, especially foraging habitat, is widespread, a considerable proportion of an ecosystem conceivably could qualify as habitat. Presence or even reproduction of the species in reserves will not necessarily indicate high-quality habitat quality, and absence or departure of the species from conservation areas will not necessarily indicate low-quality habitat.

Instead, we suggest that adaptive conservation for these species emphasize strategic acquisition of open space (large, undeveloped areas that may serve as high-quality or well-connected habitat for the listed species and other native species), potentially in locations relatively distant from the permit area, rather than attempts to conserve local areas that likely have low habitat quality or effectively do not function as habitat. Conservation of locations near the permit area where individuals sporadically are detected may not contribute meaningfully to conservation of the species, and conserving all possible locations in which the species might occur vastly overestimates habitat amount and is not feasible. By extension, ad hoc conservation of locations in which the species conceivably will occur is unlikely to contribute to regional viability. We acknowledge the challenges to acquisition by local governments that do not have jurisdiction outside the permit area, or to mitigation outside California for species that are listed under the CESA but not the ESA. Three research foci may contribute to identification of regions in which to concentrate conservation efforts: models of the full life cycle of the species, spatially extensive analyses of occupancy or abundance, and analysis of genetic structure over as large an area as possible. The first can help to identify life stages that contribute the most to survival, reproduction, and recruitment, potentially focusing conservation efforts on locations that may be distant from the area in which take is permitted. The second may identify locations, or environmental attributes, that are most closely linked with consistent occupancy or relatively high abundance. The third can delineate genetically distinct populations and, as discussed above, connectivity among populations.

## SPATIAL PRIORITIZATION

Optimization models, which often are applied to reserve design, can inform development and implementation of plans for mitigation of incidental take (e.g., USFWS and

NMFS 2016). The areas of inquiry described above can help to increase the reliability of data layers for each species included in the optimization. Of the available methods, Zonation (Moilanen 2007; Moilanen et al. 2009, 2014) is among the most applicable to mitigation plans for incidental take. Zonation is a free, publicly available method for spatial planning that hierarchically prioritizes locations for conservation—or any other land use—on the basis of desired attributes or objectives. Desired attributes may include but are not limited to stable populations of protected species or particular environmental features or land uses. These attributes may be given equal or unequal weights. Population genetics, connectivity, economic costs of acquisition or management, and stressors to species or their habitat (e.g., proximity to urban development) readily can be incorporated into the analyses. The outputs are compatible with decision-making in the context of multiple, and potentially competing, land uses. Because the optimization is straightforward to iterate, it can accommodate new data, advances in ecological understanding, and changes in social or environmental conditions, thereby informing phased acquisition of conservation areas (Di Minin et al. 2014).

Zonation optimization models are applied to grid-based data, often known or hypothesized species' distributions (including probability distributions, such as detection-weighted occupancy or abundance), or habitat locations, topography, potential for acquisition or easements, and other environmental data layers. Therefore, the process is compatible with geographic information systems (GIS) and statistical models of species' distributions. Furthermore, cloud computing, such as Google Earth Engine, has made integration of remotely sensed data in such processes much more feasible and rapid. Optimizations in Zonation rank all locations (e.g., pixels or cells within the grid) on the basis of their potential contribution to conservation or other objectives. The ranking is achieved by an iterative process of cell removal, which starts with the full study area (or part of the study area, such as areas within a given set of jurisdictional boundaries) and removes cells in the order that minimizes the marginal loss of targets at each step. The value of remaining cells to all species or other targets is re-evaluated after each removal step because the relative priority of a given cell may change after other areas are removed. The result of this process is a hierarchical ranking that can be used to identify the highest-priority locations (e.g., the most efficient conservation network) for any total area conserved.

Optimization allows one to account for aggregation, or the principle that all else being equal, fewer large conservation areas are preferable to many small conservation areas. Multiple ways of accounting for connectivity are possible. The process also allows one to analyze replacement costs (the difference between the value of the optimal solution for meeting the objectives and the value of a different solution) to compare the ecological priority or economic cost of alternative reserve configurations.

There are many examples of practical applications of optimization modeling via Zonation. For example, the Finnish government uses such optimization to support implementation of METSO, a program to conserve biological diversity in its privately owned southern forests (Mikkonen et al. 2018). For more than a decade, the Victoria (Australia) Department of Environment, Land, Water and Planning has used similar methods to inform its regulatory and investment decisions across extensive areas because it allows for objective, transparent, and repeatable ranking of the relative contribution of different areas (or actions) to management objectives. Moreover, by producing hierarchical rankings that are not dependent on explicit area or cost targets, outputs are applicable to situations in which funding or the area that will be acquired for conservation is unclear.

One might conduct an optimization analysis to identify areas with the greatest potential to contribute to the conservation goals of a given mitigation plan, which can be encoded in the analysis inputs and settings. Input data layers might represent existing and potential future development, existing preserves and conservation areas, probabilities of occupancy, connectivity, and habitat quality. Numerous settings can be adjusted to a particular context. For example, the *Core Area Zonation* cell removal rule uses the maximum (weighted) proportional representation of remaining habitat (the fraction of the total remaining habitat contained within each cell) across all species to rank cells for removal at each iteration. This removal rule seeks to maintain balanced (according to supplied weightings) habitat representation for all species at all stages of the removal process. Proportional representation is calculated with respect to the areas included in the analysis only.

Use of a generic edge-removal algorithm to prioritize structural connectivity (larger, more-connected areas of high predicted occupancy or associations with occurrence) tends to produce more-aggregated conservation networks. The edge-removal algorithm allows cells to be removed only from the edges of retained areas at each iteration<sup>3</sup>.

An iterative cell-removal process can remove existing preserves and conservation areas last. This will ensure that any new reserves complement existing conservation areas by targeting species that may not be conserved effectively within the existing areas. Removing existing preserves last also places higher priority on areas closer to those existing preserves, promoting structural connectivity of the expanded conservation network (Moilanen et al. 2014).

## CONCLUSIONS

Many mitigation decisions must be made on the basis of assumptions and inferences drawn from limited data. Explicit implementation of new and scientific research that is targeted to the mitigation context can reduce reliance on unverified and potentially erroneous information. The latter may be particularly useful when the habitat quality or other ecological benefit of a potential mitigation acquisition is uncertain. The financial feasibility of gathering and analyzing new data inevitably is a concern. We suggest that research to fill knowledge gaps likely will yield a positive return on investments in both research and land acquisition, especially in regions where the cost of mitigation lands is likely to be high.

Mitigation plans for incidental take in California and at the federal level tend to rely on reserves and actions that are as close to the permit area as possible. However, especially when species have extensive distributions and are declining across their range, research may suggest that conservation actions in other regions are likely to make a greater contribution to long-term persistence of the species. The types of research we outline above also can strengthen adaptive management plans that otherwise might primarily emphasize vegetation maintenance or manipulation (e.g., ICF 2015; CVCC 2016). For example, rather than making assumptions about the attributes and quality of foraging habitat for widely distributed species, we suggest that targeted research on the composition and abundance of prey be used to inform acquisition and subsequent adaptive management.

Although research on the ecology of covered species is not heavily emphasized in design or implementation of most mitigation plans for incidental take, we believe that such research has considerable potential to guide effective investments in acquisition and adaptive management that minimize incidental take and contribute to recovery.

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# Plants



# Plants

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1. Amargosa niterwort (*Nitrophila mohavensis*).  
Photo Credit: Naomi Fraga, California Botanical Garden
2. Pine Hill flannelbush (*Fremontodendron decumbens*).  
Photo Credit: Jeb Bjerke, CDFW
3. Pallid manzanita (*Arctostaphylos pallida*).  
Photo Credit: Nick Fullerton, CC BY 2.0
4. Santa Cruz Island bush-mallow (*Malacothamnus fasciculatus* var. *nesioticus*).  
Photo Credit: Brent Miller, CC BY-NB-ND 2.0
5. Western Joshua tree (*Yucca brevifolia*).  
Photo Credit: Jeff Sullivan, CC BY-NB-ND 2.0
6. Santa Ana River woollystar (*Eriastrum densifolium* ssp. *sanctorum*).  
Photo Credit: U.S. Fish & Wildlife Service, Pacific Southwest Region, CC BY 2.0

FULL RESEARCH ARTICLE

## Status of the Amargosa niterwort (Amaranthaceae) in California and Nevada

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The Amargosa niterwort is a narrow endemic restricted to alkali wetlands of the northern Mojave Desert in Inyo County, California and Nye County, Nevada. Groundwater pumping and subsequent hydrological alteration within the Amargosa groundwater basin has been identified as the most significant threat to the long-term persistence of the species. Parallel monitoring programs were established in California (2010) and Nevada (2014) to establish baseline trends in abundance, measured as ramet (stem) number, and to aid in determining management actions that are needed to ensure the long-term viability of populations. Monitoring in California shows significant variation in abundance between years; however, there are consistent trends, indicating that factors influencing increase or decrease in abundance is similar across the population. However, this pattern is not shared in Nevada, where following a sharp decline between 2014 and 2015 the population has remained relatively stable. Two of the three Nevada macroplots are downstream from Crystal Reservoir and are likely influenced by discharge from the reservoir including periodic water releases and leakage from the earthen dam. Significant increases and decreases in abundance within macroplots are not correlated with climate variables including precipitation, thus it is important to understand how groundwater flow and spring discharge impacts abundance of plants within and between populations.

**Key words:** alkali wetland, Amaranthaceae, Amargosa niterwort, *Nitrophila mohavensis*, Mojave Desert, rare plant monitoring

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The very narrow global range and specific habitat requirements of *Nitrophila mohavensis* Munz & J.C. Roos (Amargosa niterwort) make it vulnerable to extinction (USFWS 2007). As such, implementing research and monitoring are essential to understanding its status and to inform management actions and conservation strategies to advance recovery. Here we describe ongoing research, monitoring, and management activities that have been conducted or are in progress, including a cross-border initiative to share results and develop range-wide conservation strategies.

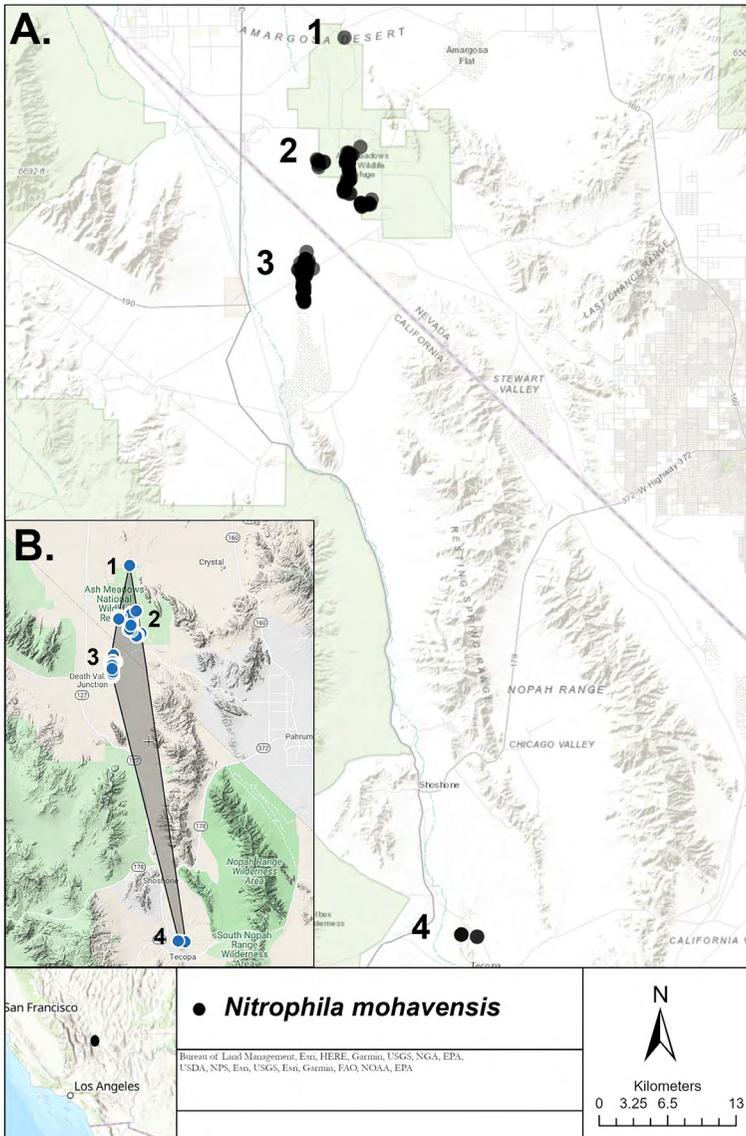
*Nitrophila mohavensis* is a narrow endemic restricted to the Amargosa River basin in the northern Mojave Desert in Inyo County, California and Nye County, Nevada (Fig. 1). It occurs on land managed by the Bureau of Land Management (BLM), Ash Meadows National Wildlife Refuge (AMNWR), and private land (USFWS 2007). It is an herbaceous rhizomatous perennial in the Amaranthaceae (amaranth family) that forms dense colonies of erect stems that are low to the ground and die back to an underground rhizome in the winter. The flowers are pink, relatively small (2–3.5 mm; Wetherwax et al. 2012), and located singly in the leaf axils. *Nitrophila mohavensis* was first described in 1955 from specimens collected at lower Carson Slough in Inyo County, California, at the time of its description *N. mohavensis* was considered “locally abundant in heavily alkaline mud” (Munz and Roos 1955). It was listed as endangered under the California Endangered Species Act in 1978, by the U.S. Fish and Wildlife Service (USFWS) under the Federal Endangered Species Act in 1985, and by the state of Nevada in 1986 due to habitat alteration from agriculture, groundwater pumping, and water diversion (USFWS 2007; CNPS 2020; NatureServe 2020).



**Figure 1.** Amargosa niterwort in flower on lower Carson Slough, Inyo County, California.

## Distribution and Abundance

There are four primary locations where *N. mohavensis* occurs: 1) Soda Springs, NV, 2) AMNWR, NV, 3) lower Carson Slough, CA, and 4) Tecopa Hot Springs, CA (Fig. 2). Based on delineations outlined by the USFWS (2020), it is currently known to occur at 14 occurrences across its range: 12 occurrences in Nevada and two in California. The lower Carson Slough occurrence in Inyo County, California is the largest known occurrence in terms of total population size (USFWS 2020); this holds true, even if all of the Nevada occurrences



**Figure 2.** A. Range-wide distribution map of Amargosa niterwort. B. Convex hull encompassing the 185-points that represent the known global distribution of these species. Population 1. Soda Spring; Population 2. Ash Meadows NWR; Population 3. Lower Carson Slough; Population 4. Tecopa Hot Springs.

are treated as one site (Table 1; Fig. 2). At the time of initial listing, *N. mohavensis* was thought to be restricted to lower Carson Slough in California, however it was documented in Tecopa Hot Springs as early 1963, based on an herbarium specimen collected by L.C. Wheeler (8409 [RSA], CCH2 2020). Mary DeDecker reported the occurrence at Tecopa Hot Springs to regulatory agencies in 1986 (CNDDDB 2020). *Nitrophila mohavensis* was first documented at AMNWR in 1984 and it has since been mapped throughout the Refuge more extensively (USFWS 2007; 2020). A previously unknown patch of *N. mohavensis* was recently documented on BLM land in Nevada in 2019 and extends the Nevada Carson Slough occurrence by 300 m west of the AMNWR boundary (Lara Kobelt, Bureau of Land Management personal communication).

**Table 1.** Known locations for Amargosa niterwort in California and Nevada. Global population estimates are from 2009 to 2017 (USFWS 2020), extent of occurrence was calculated as a part of this study.

Population	State	Site Name	Land Ownership	Global Population Estimate	Extent of occurrence (km <sup>2</sup> )
1	NV	Soda Spring	AMNRW	3,993	~5m <sup>2</sup>
2	NV	Ash Meadows NWR	AMNRW	54,299	16.02
3	CA	Lower Carson Slough	BLM	176,886	3.44
4	CA	Tecopa Hot Springs	BLM/Private	470	0.04
		Total Global Estimation		235,648	19.5

## Habitat

*Nitrophila mohavensis* is restricted to salt encrusted soils in sparsely vegetated, alkaline wetlands, in flat open areas that are exposed to full sun, between 400 m (1,300 ft) to 700 m (2,300 ft) in elevation (CCH2 2020; CNDDDB 2020). The soil has been described as finely textured silts and clay with little internal drainage (Hasselquist and Allen 2009), but also as sandy loam (57% sand), with a pH ranging from 8.4 (Johnston and Zink 2004) to 9.6 (DeFalco et al. 2017). The moisture level of the soil within occupied habitat has been described as moist to saturated, with the soil being subject to perennial moisture due to the presence of a high groundwater table (USFWS 2007; Hasselquist and Allen 2009; DeFalco et al. 2017). *Nitrophila mohavensis* forms the dominant component of the vegetation where it occurs and is associated with *Atriplex confertifolia* (Torr. & Frém.) S. Watson (shadscale, Chenopodiaceae), *Chloropyron tecopense* (Munz & J.C. Roos) Tank & J.M. Egger (Tecopa bird's beak, Orobanchaceae), *Cleomella brevipes* S. Watson (shortstalk stinkweed, Cleomaceae), *Distichlis spicata* (L.) Greene (saltgrass, Poaceae), *Nitrophila occidentalis* (Moq.) S. Watson (borax weed, Amaranthaceae), and *Suaeda nigra* (Raf.) J.F. Macbr. (bush seepweed, Chenopodiaceae, USFWS 2007; CCH2 2020; CNDDDB 2020).

Using an isotopic analysis of transpired water, Hasselquist and Allen (2009) found that *N. mohavensis* accesses water near the soil surface in the early spring and hot summer months as opposed to accessing water from greater depths (> 30 cm), indicating that groundwater upwelling, surface water from spring outflows, and precipitation may all be important sources of soil moisture. This is consistent with a separate study that found *N. mohavensis* habitat had lower soil salinity and higher soil moisture content compared with adjacent saltgrass (*D. spicata*) and mixed shrub habitat. In addition, there was no evidence of interspecific competition between *N. mohavensis* and *D. spicata* by DeFalco et al. (2017).

However, given the extensive distribution of *D. spicata* within *N. mohavensis* habitat, the potential for competition should be investigated further.

## Reproductive Biology

A population genetic study of microsatellite loci sampled 178 individuals across the range of *N. mohavensis* and found evidence of sexual and asexual reproduction (Martin et al. 2013). Asexual clonal reproduction can occur when ramets (stems) arise from a genet's (genetic individual's) extensive network of rhizomes, but evidence for multiple genotypes within dense patches of plants, suggests that clusters of ramets could consist of more than one genet (Martin et al. 2013). A total of 32 of 78 recovered multi-locus genotypes were sampled from more than one individual, providing evidence of clonal reproduction. Three genotypes were collected from 22 samples across the range of the species (a maximum distance of more than 50km), indicating that clones can disperse a relatively long distance (Martin et al. 2013). Mechanisms for dispersal have not been investigated, but stems could disperse long distance during large flood events. A combination of sexual and asexual reproduction is further substantiated by DeFalco et al. (2017), who found shifts from clonal to sexual reproduction in plants that were transplanted into adjacent saltgrass (*D. spicata*) habitat. Pollinators of *N. mohavensis* have not been documented, but ants and wind have been suggested as pollen dispersal mechanisms (Tanner et al. 2012).

## Threats

Numerous threats are known to impact or have potential to impact plants and habitat of *N. mohavensis* including groundwater pumping, hydrological alteration, off-highway vehicle incursion, road creation and maintenance, trampling by feral horses, development, and climate change (USFWS 1983, 1990, 2007). Groundwater pumping and subsequent hydrological alteration within the Amargosa groundwater basin has been identified as the most significant threat to the long-term persistence of the species (USFWS 1990). Eleven piezometers were installed in 2009 at lower Carson Slough in Inyo County, California to monitor the shallow groundwater because of the importance of maintaining high soil moisture.

In 1998 it was observed that road construction and realignment along State Line Road directly impacted plants and habitat at the lower Carson Slough occurrence in California. At this occurrence, the road is raised above ground level creating a berm-like barrier, impeding water transport on the other side of the road (south), thus altering habitat. State Line Road bisects the most abundant occurrence of *N. mohavensis* and provides an access point for off-highway vehicle incursion and thus remains a significant threat to the species.

## METHODS

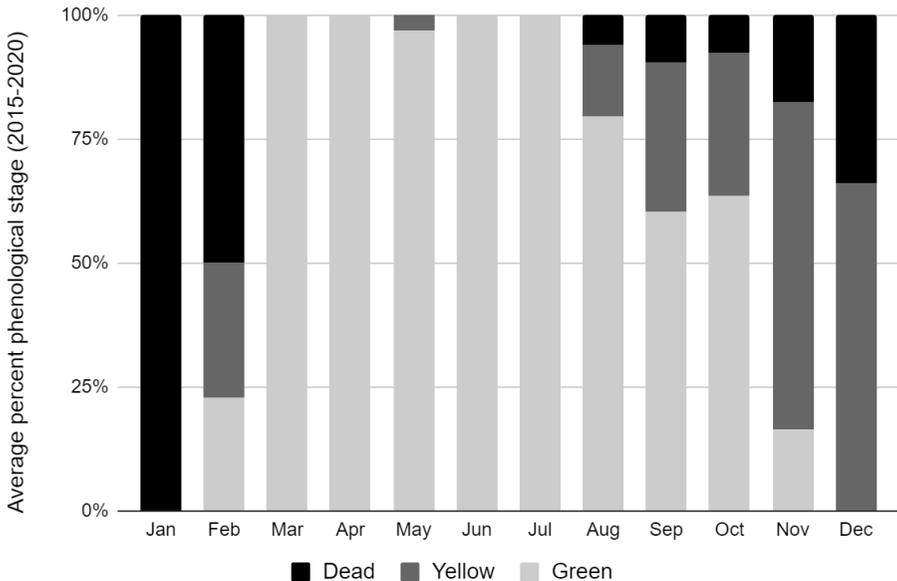
### Population Surveys

We conducted surveys for *N. mohavensis* between 2018 and 2020 at lower Carson Slough, and Tecopa Hot Springs in Inyo County, California to determine the current extent and range of the species in California as a part of a Traditional Section 6 Grant (P1886001) issued by the USFWS. Surveys took place between the months of April and July when *N. mohavensis* is most likely to be in flower and stems are green (Fig. 3). Latitude and longitude

were recorded using a GPS unit when plants were found. An effort was made to identify and map the edges of the population at lower Carson Slough to develop a more accurate map of the population boundary (Fig 2). Comprehensive surveys were also conducted at AMNWR between 2007 and 2009. The current survey effort to map *N. mohavensis* in California will continue until 2022. To establish the area of occupancy, the range-wide extent of occurrences was calculated by creating a convex hull around 185-points that represent the known global distribution of these species in ArcGIS Pro 2.4.0 ((c)2019 Esri Inc.; Fig. 2). The point dataset is based on GPS-data collected during the above population surveys in 2018, 2019, and 2020 in California and data provided by Nevada Natural Heritage Program in 2019 and Lara Kobelt in 2019 for Nevada. Extent of occurrence was calculated individually for each of the four known population areas using the same method, by creating individual convex hulls around the four areas rather than the entire group (Table 1; Fig. 2). Area of occupancy was calculated for the global range of the species using the 185-point layer and creating a 10m buffer around each point. A voucher specimen was collected at lower Carson Slough to verify the species identification and document the target species for surveys (Fraga 6371, RSA).

### Population Monitoring

*California monitoring.*—A pilot monitoring program was established by John Willoughby (retired BLM, independent) for BLM in California in 2010 at lower Carson Slough, with data collected at 23 belt transects that were established across five macroplots. CA macroplot 4 is monitored by taking a total census in a 30m x 20m area and does not have numbered belt transects (Table 2). In 2011 five belt transects were added to CA macroplot 1 and two belt transects added to CA macroplot 3 to increase sampling power, for a total of



**Figure 3.** Phenology of Amargosa niterwort from phenological states recorded in two 0.5 m x 1 m growth plots between 2015 and 2019 at lower Carson Slough in CA. The data reported are averaged percentages.

30 belt transects that have been monitored between 2011 and 2020 (Table 2). Belt transects are 0.5 meters wide and range in length from 75m (macroplots 1, 2, and 5) to 100m (macroplot 3). Monitoring did not take place in California in 2012, 2013, and 2014 due to low rainfall, except data were collected for CA macroplot 4 in 2014. In California, monitoring took place in early September in 2010 and late August in 2011, but the monitoring date was moved to July in 2015 to correspond with maximum growth (Fig. 3). Data collected in 2010 is not included in the results because fewer belt transects were measured and the data are not comparable to other years.

**Table 2.** Summary of *Nitrophila mohavensis* macroplots (\*5 in 2010; \*\*8 in 2010).

Macroplot ID	State	Protocol	Number of Transects	Transect Length (m)	Transect width (m)	Years Monitored
CA1	CA	Belt transect	10*	75	0.5	2010–2020
CA2	CA	Belt transect	5	75	0.5	2010–2020
CA3	CA	Belt transect	10**	100	0.5	2010–2020
CA4	CA	Belt transect	60	20	0.5	2010–2020
CA5	CA	Belt transect	5	75	0.5	2010–2020
CN	NV	Belt transect	10	100	0.5	2014–2020
NS	NV	Belt transect	10	100	0.5	2014–2020
CP	NV	Quadrats	24 quadrats	15	0.5	2014–2020

*Nevada monitoring.*—A complementary monitoring program was established at AMNWR in 2014 and includes three macroplots. Two macroplots (CN and NS) consist of ten 0.5 x 100 m belt transects that follow the same protocol as in California; the third macroplot (CP) measures abundance in 24 permanently marked 15 x 0.5 m quadrats (Table 2). In Nevada, monitoring takes place in June and July to correspond with peak flowering time. Data were collected from 2014 to 2020, but not in 2017 within the Nevada monitoring plots.

Because *N. mohavensis* is rhizomatous, counting individual ramets is difficult without damaging plants, therefore ramets (herein referred to as clumps) were defined as a unit that includes groups of stems that are separated by less than 2 cm from each other from the point at which they are rooted. The unit of the “clump” is used in California and Nevada to measure abundance. The total number of clumps are counted in each cell (0.5 m wide x 1 m length) along the entire length of each belt transect. Differences in abundance within macroplots using belt transects as paired sampling units between years was tested using Wilcoxon signed rank test (Table 3).

## Phenology Monitoring

Five 1 m x 0.5 m plots were established in 2012 and monitored monthly until 2019 at lower Carson Slough, Inyo County, California to increase understanding of phenology. However, plants appear to have died in three of the five plots between 2015 and 2019, so phenology is only presented from two plots. Descriptions of phenological stages were recorded, including stem color or condition (green, yellow, dormant), clump size (length and width), number of clumps, and the presence of flowers or fruits. Phenological results are presented as the average percentage of observed green, yellow or dormant clumps for each month between 2015 and 2019 (Fig. 3).

**Table 3.** A. Wilcoxon signed rank test comparing the abundance of Amargosa niterwort in two macroplots with belt transects in Nevada (NV) between two years. B. Wilcoxon signed rank test comparing the abundance of Amargosa niterwort in four macroplots in California (CA) between two years. Wilcoxon signed rank test statistics are reported. \* $P < 0.05$ , ND = no data

A.

Macroplot	2014-2015	2015-2016	2016-2018	2018-2019	2019-2020
NV CN	24	19	10	18	9
NV NS	27	9	21	ND	9

B.

Macroplot	2011-2015	2015-2016	2016-2017	2017-2018	2018-2019	2019-2020
CA1	21	8*	0*	17	10	14
CA2	4	0*	0*	6	0*	0*
CA3	10	7*	8*	21	0*	22
CA5	4	4	0*	0*	3	0*

## Climate Data

Annual cumulative precipitation, mean daily temperature, mean daily minimum temperature, mean daily maximum temperature, and 30-year normals for each of the above variables were downloaded for Lower Carson Slough in Inyo County California from the (PRISM 2020). The climate variables were also downloaded for Crystal Reservoir in Nye County, Nevada. The Nye County data provided the same results in the statistical analyses, so they were not included here. The normals are baseline datasets describing average monthly and annual conditions over the most recent three full decades and cover the period 1981–2010. These data were summarized for the growing season of *N. mohavensis*, which is consistent with the hydrological year (also known as water year), defined as precipitation from October of the prior year through September of the next year. Correlations between annual precipitation and recorded *N. mohavensis* abundance based on monitoring data were assessed using Pearson correlation coefficient (parametric) and Spearman rank correlation coefficient (non-parametric; Table 4) because it was not clear if the data were normally distributed.

## Seed Collection

Seeds were sampled at lower Carson Slough in California in 2010, 2011, and 2020 to establish long-term germplasm collections in off-site and secure storage. Collections were made according to standard methods approved by the Center for Plant Conservation (CPC 2017) with no more than 5% of a population's annual seed output collected. Individuals were selected randomly throughout the population to capture genetic diversity and maternal lines were collected and stored separately for the collection made in 2020. Individual seed samples were cleaned, processed, and checked for viability at the California Seed Bank at California Botanic Garden in Claremont, California. Viability was assessed using non-destructive methods due to limited seed quantity by visually examining seeds to determine if they were full. Seeds were dried in a desiccation chamber until they reached 13% relative humidity. After the drying period, seeds were placed into foil/plastic laminate storage pouches that were heat-sealed and placed into freezers at  $-18^{\circ}\text{C}$ . Germination trials were not

**Table 4.** Pearson's correlation coefficient ( $r$ ) and Spearman rank correlation coefficient ( $r_s$ ) values comparing climate variables and abundance in California and Nevada. \* $p < 0.05$ .

	$r$ CA Total Abundance	$r$ NV Total Abundance	$r_s$ CA Total Abundance	$r_s$ NV Total Abundance
Total Precip (cm)	0.402	-0.371	0.321	-0.086
Tmin (C)	-0.091	0.285	0.143	0.2
Tmean(C)	-0.356	0.356	-0.143	0.486
T max (C)	-0.539	0.397	-0.464	0.486

performed because seed quantities were too small (e.g, there was an average of two seeds per maternal line in the 2020 collection).

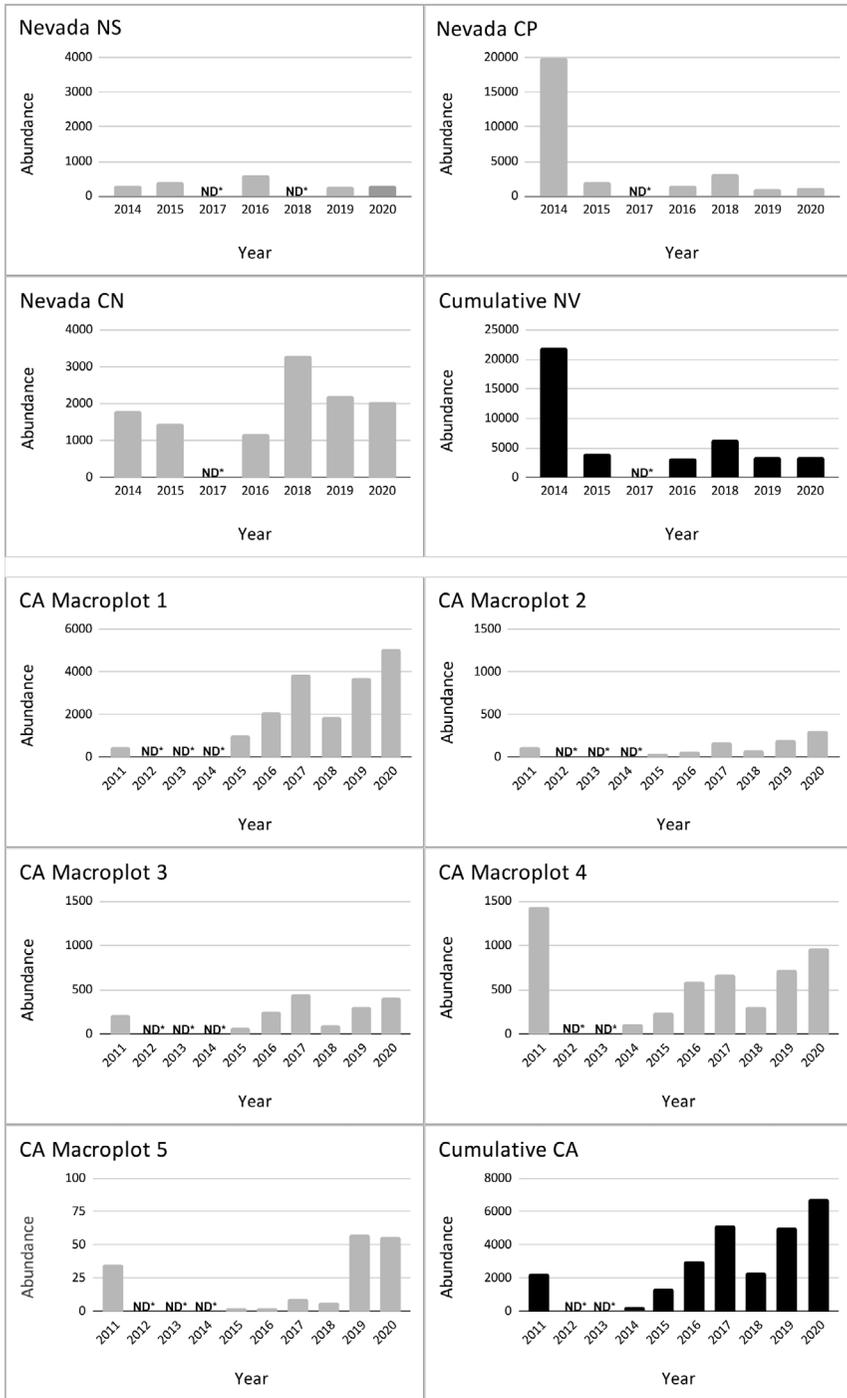
## RESULTS

### Distribution and Abundance

Based on survey data, the global extent of occurrence, or the smallest continuous area that can be drawn to encompass all known sites for *N. mohavensis*, is estimated to be 39,409 ha (394.09 km<sup>2</sup>, 97,382 acres; Fig. 2). If we consider each of the four known populations separately, the extent of occurrence is 1,950 ha (19.5 km<sup>2</sup>, 4,819 acres) when convex hulls are calculated individually for each population and the areas summed (Table 1). The area of occupancy, or the area within the extent of occurrence that is known to be occupied is estimated to be 9 hectares (0.9 km<sup>2</sup>, 222 acres). Plants on private property in Tecopa Hot Springs appear to be extirpated due to development based on surveys conducted in 2018, 2019 and 2020, although plants in Tecopa Hot Springs remain extant on land managed by BLM.

*California monitoring.*—In California, there is variation in the abundance of clumps between years within all macroplots, but the pattern of variation (increase and decrease within a macroplot) is consistent across all macroplots starting in 2015. There was a large and significant increase in abundance in CA macroplot 1 between the years of 2015 to 2016 and 2016 to 2017 (Table 3). Between 2015 and 2020 CA macroplot 1 accounted for 70–78% of all clumps counted in the CA macroplots, and thus is an important driver of the pattern of abundance for the cumulative total of clumps in all CA macroplots (Fig. 4). There was an increase in the number of clumps across all macroplots between 2015 and 2016, but there was only a significant increase in the abundance of clumps in CA macroplots 1–3 (Table 3). There was a significant increase in the number of clumps across all macroplots between 2016 and 2017 (1–5; Table 3). There was a decrease in the number of clumps across all macroplots between 2017 and 2018, but this difference was only significant in CA macroplot 5. CA macroplot 5 has the fewest number of clumps compared to all other macroplots; it is located on the south side of State Line Road, where plants are less abundant. 2020 had the highest number of clumps recorded for all macroplots across all monitored years except for macroplot 4, which was most abundant in 2011 (1,439 clumps).

*Nevada monitoring.*—In Nevada, there was a decline in *N. mohavensis* abundance over the monitoring survey period (2014–2020; Fig. 4). This decline is primarily the result of the sharp decline at CP between 2014 and 2015. Abundance remained stable over the monitoring period at other macroplots, and at CP after 2015 (Table 3; Fig. 4). Abundance trends at the three macroplots in Nevada did not follow a consistent pattern. Abundance at



**Figure 4.** Abundance of Amargosa niterwort clumps in each macroplot and cumulative abundance for NV and CA for all monitoring years. \*ND = No data

NS increased between 2015 and 2016 following the same trend as the CA macroplots, but at both CP and CN, abundance declined slightly over the same time period. In contrast to the CA macroplots, abundance at CP and CN increased in 2018 relative to 2017 and declined in 2019 (NS was not measured in 2018). In Nevada, the CN and NS macroplots where belt transects were used, did not show significant change in clump abundance (Table 3A).

### Phenology and Seed Production

Data collected during phenology monitoring indicate that plants of *N. mohavensis* begin to go dormant as early as August, but are primarily dormant between the months of November and January (Fig. 3). New growth typically begins to emerge from underground rhizomes in February and plants are green and at maximum growth in July. Flowers have been reported in the months of May, June, and July and seed collections were made on August 1, 2010 (10 seeds total from 30 sampled individuals), June 27, 2011 (51 seeds from 34 sampled individuals), and July 17, 2020 (94 seeds from 24 individuals). The seed collections in 2010 and 2011 were collected in bulk, so we could not determine how many sampled individuals contributed to seed production. Seed production appears to be relatively low, with few filled seeds being collected across all three years. There are a total of 155 seeds present in the California Seed Bank, collected from 68 sampled individuals, (average of 2.3 seeds per sampled individual).

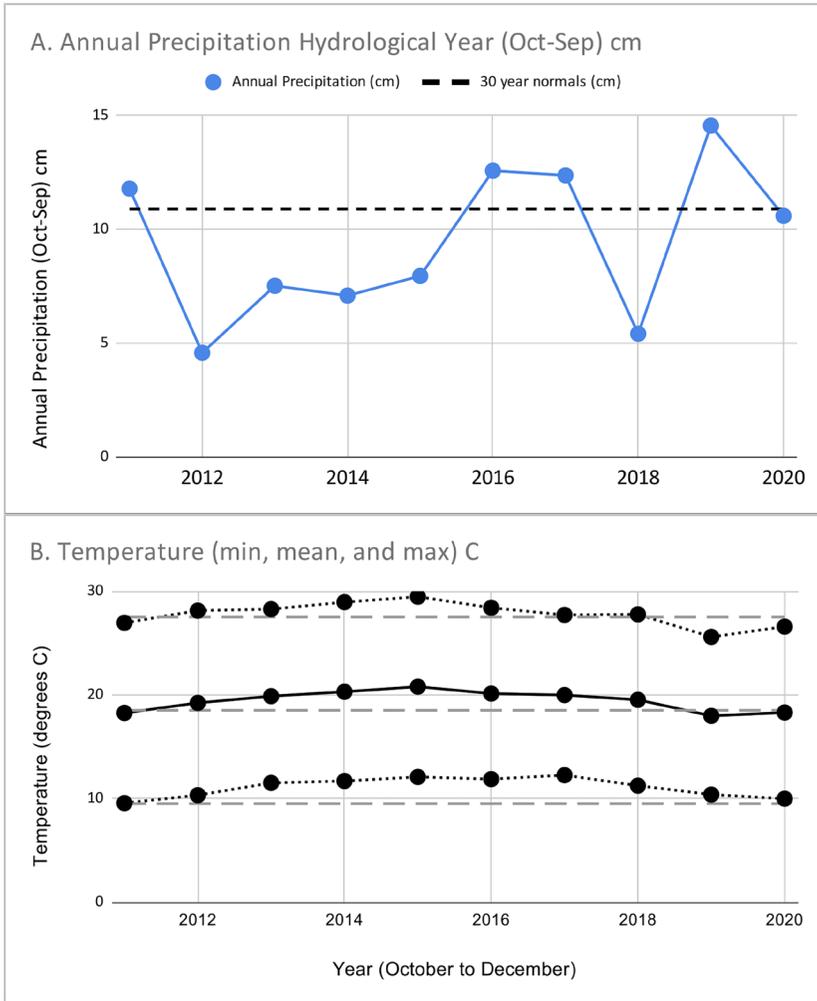
### Climate

Five of the nine monitoring years of *N. mohavensis* were drier than the 30-year normals for annual precipitation on lower Carson Slough (Fig. 5). California experienced a significant drought between 2012 and 2015; this is reflected in weather conditions reported in PRISM (2020). There was above average precipitation between 2016–2017, and in 2019. The driest year recorded since monitoring began was in 2012. Precipitation was similar to the 30-year normals in 2011 and 2020. Temperatures were above average between 2012–2018, with the first four years having below average precipitation, followed by two years of above average precipitation (2016 and 2017; Fig. 5).

Abundance was not significantly correlated with any climate variables for California or Nevada (Table 3). Abundance of *N. mohavensis* in California had a slightly positive but insignificant correlation with precipitation and a negative but insignificant correlation with mean maximum temperature. Abundance in Nevada had a slight negative correlation with precipitation, but this was also not significant (Table 3). Macroplots CP and CN in Nevada occur below Crystal Reservoir, and it is likely that water release from the dam is the dominant factor driving abundance at these macroplots. Macroplot NS in Nevada is not below Crystal Reservoir and shows an increase in abundance in years with increased precipitation and has increasing and decreasing trends that are similar to the lower Carson Slough occurrence in California.

### Threats

Prior reports have documented direct impacts to plants and habitat from off-highway vehicle (OHV) use. These threats had largely been abated or reduced due to fencing along Ash Meadows Road at lower Carson Slough (USFWS 2007) and exclusion of OHVs from AMNWR. However, recent surveys have revealed that OHV use continues to be a direct



**Figure 5.** A. Annual cumulative precipitation Oct–Sep (cm) across monitoring years between 2011 and 2020, 30-year normals (1981-2010) are included as a dashed line. B. Annual mean daily temperature (solid line) with minimum and maximum temperature (dotted lines) (°C) for the 2011 to 2020 monitoring period, 30-year normals are included as a dashed line.

threat to the *N. mohavensis* in California due to damaged and down fencing. Fencing was observed to be down on multiple instances in 2017 and 2018 and new tire tracks were noted when the fence was down (Fig. 6). Given the frequency with which the fence is subject to damage, OHV trespass continues to be a significant threat to the lower Carson Slough occurrence of *N. mohavensis*. Feral horses were also observed on lower Carson Slough in 2018 and 2019 and evidence of their tracks and droppings have been observed near occupied *N. mohavensis* habitat. Feral horses have been successfully excluded from AMNWR using fencing.

Decline in groundwater resources and alteration of hydrology are the most significant threats to *N. mohavensis* populations, and to other endemic species of plants and animals that



**Figure 6.** Off highway vehicle incursion into *Amargosa niterwort* habitat at lower Carson Slough, Inyo County, California.

occur in groundwater dependent ecosystems. The northern Amargosa Valley groundwater basin, comprised of the Amargosa River Valley at the river's headwaters in Nevada, to the California-Nevada state line is over-allocated (Zdon and Associates, Inc. 2014; Partner Engineering and Science, Inc. 2020). Groundwater usage within the northern Amargosa River basin has steadily increased over the past 25 years, and significant impacts to groundwater resources could result if right holders fully exercise their water rights (Partner Engineering and Science, Inc. 2020). Groundwater monitoring from the California State Line to Tecopa, California, indicates there is a slow but continual groundwater decline, primarily from pumping within the Nevada portion of the basin (Partner Engineering and Science, Inc. 2020). In AMNWR, management of Crystal Reservoir water levels, including periodic water releases and leakage from the earthen dam, result in inconsistent water supply to the alkali meadows below the dam. Long-term trends in groundwater withdrawal may reduce spring flow to the systems that feed the alkali habitats that support *N. mohavensis* and other endemic species in the region (Belcher et al. 2016).

## DISCUSSION

### Distribution and Abundance

At lower Carson Slough CA macroplot 1 accounts for 70% of all clumps within the monitoring plots and is located in an area with the highest density of plants. There has been an increase in the number of clumps reported from CA macroplot 1 since 2011, with a sig-

nificant increase in 2016. There is significant variation in the abundance of *N. mohavensis* clumps between years in California (Table 2). However, there are consistent trends across macroplots (increase or decrease) relative to the prior year starting in 2015, indicating that factors influencing the increase or decrease in the number of clumps is similar across the population. This pattern is not shared in Nevada, except for macroplot NS, which shares similar trends of increasing or decreasing abundance between years as California. Aside from the significant decline at macroplot CP in Nevada from 2014 to 2015, the plots in Nevada have been relatively stable with few significant changes between years. Two of the Nevada macroplots are directly downstream of Crystal Reservoir (CP and CN) and are affected by release rates of water from the dam. While it is likely that surface flow and changes in groundwater in the region are influencing changes in abundance at most of the monitored sites, the macroplots below Crystal Reservoir are buffered by a year-round source of water from the dam.

Abundance is not correlated with the climate variables we examined, and changes in abundance may have more to do with changes in subsurface groundwater flow or spring discharge. Groundwater levels are thought to be the most significant driver of *N. mohavensis* habitat quality. Data on groundwater availability near macroplots has not been analyzed together with *N. mohavensis* data, but groundwater is monitored at both AMNWR (spring discharge rates and groundwater well levels; USFWS 2020) and the lower Carson Slough (piezometers). Analysis of groundwater level impacts on *N. mohavensis* abundance and reproduction are needed to provide a greater understanding of the observed patterns of inter-annual variation in abundance and population trends. In addition, monitoring by Miller (2020) in Nevada found evidence that reproduction (flowering and/or fruiting) was strongly and significantly positively correlated with cumulative annual precipitation, and strongly and significantly negatively correlated with all temperature values. While the climate variables we examined do not appear to influence abundance significantly, it may impact important factors related to long-term population viability such as flowering and seed production. The relationship between climate and reproduction should be further investigated. No other plant traits measured in the Nevada monitoring program were significantly correlated with climate variables that were examined.

## Threats

Ameliorating threats range wide is essential for the recovery of *N. mohavensis*, particularly in California, which has the most abundant population and the most recorded threats. In the prior five-year review (USFWS 2007), it was reported that threats such as OHV incursion have been halted. However, ongoing surveys have revealed that habitat at lower Carson Slough remains vulnerable to disturbances from OHV due to its proximity to State Line Road, which bisects the population and downed fencing. Off-highway vehicle activity may cause soil compaction, damage plants and underground rhizomes, alter habitat quality, and introduce invasive plants (Switalski 2018). Ensuring that there is a consistent source of funding for fence construction and repair will be important to reducing this threat. Regular monitoring of the fence status is required to ensure that threats such as OHV and feral horses do not continue to impact plants. Further, it is likely that plants on the south side of the road have been impacted by improvements to State Line Road which is now elevated above ground level and may act as a berm-like barrier impeding water-flow from north to south. CA macroplot 5 has the fewest number of clumps that were counted within

the monitoring period. Population size of CA macroplot 5 ranged from two clumps in 2015 and 2016 to 58 clumps in 2019. An effort should be made to ensure that surface and subsurface flow of water is able to pass across the road and to map and monitor additional areas that contain plants on the south side of the road, as plants occur up to 2 km south of State Line Road (Fig. 2).

Groundwater overdraft has been identified as the single most significant threat to the long-term persistence of *N. mohavensis* (USFWS 1990; 2007; 2020; Belcher et al. 2016). Regionally, monitoring of the status of the carbonate aquifer that is the source of spring water throughout the range of *N. mohavensis* is critical to tracking the long-term effects of groundwater withdrawal on spring ecosystems. Sources of groundwater extraction that are known to affect groundwater levels within the region include agriculture in the Amargosa Valley, Nevada (e.g., alfalfa production and a dairy farm) and agriculture and residential development in Pahrump Valley, Nevada (Parker et al. 2021). Local groundwater monitoring collected within and around *N. mohavensis* populations should be analyzed together with abundance data in California and Nevada in order to improve understanding of how variation in subsurface water availability affects these populations. Inter-annual variation, including significant increases and decreases in abundance within macroplots between years are not correlated with the climate variables we examined including precipitation, thus understanding how groundwater discharge and variation in surface and subsurface flow within *N. mohavensis* habitat will be essential to understanding population trends in the future.

### **Cross-Border Initiative**

A working group for *N. mohavensis* was established in 2018 and consists of agencies and stakeholders from California and Nevada including BLM, California Botanic Garden, and the USFWS. The working group has convened regular meetings to facilitate data sharing, discussion of threats, monitoring results, priority management actions and considerations, and to evaluate future research and management objectives. Establishing communication across state boundaries is essential for the long-term conservation of a highly imperiled cross-border species like *N. mohavensis*. The habitat at lower Carson Slough on BLM land in California is an extension of the extensive wetland ecosystem included within the AMNWR, thus research objectives and management considerations in CA likely involve ecological processes and management considerations occurring at AMNWR. As a result of convening the working group, we have compiled and analyzed our combined data to increase our understanding of *N. mohavensis*. We have also identified research priorities that will aid in conservation strategies including investigating how groundwater pumping could affect salinity and water availability at occupied sites, and further investigating plant reproduction, including pollination and seed set. Additionally, a form of herbivory (webbing produced around stems) has been observed at sites in California and Nevada and requires further research to understand any associated impacts to the populations (Fig. 7). Some of this work is ongoing (e.g., reproductive biology studies), but increasing understanding of the relationship between groundwater availability and current patterns of abundance and distribution is of the highest priority for the long-term conservation of this species.

### **ACKNOWLEDGMENTS**

We would like to thank John Willoughby who designed the monitoring protocol that is currently being implemented in California and Nevada and Karen Tanner who was



**Figure 7.** Webbing observed around stems of Amargosa niterwort.

instrumental in development of the protocol for AMNWR. We also thank Jennifer Wilkening (USFWS) for her support in conception of this research, and Lara Kobelt for providing survey data and assisting with monitoring in California. Kobelt and Wilkening are founding members of the Amargosa niterwort Working Group. We thank the numerous field assistants who have collected data monitoring the California and Nevada populations. Funding was provided by the BLM, California Botanic Garden, and U.S. Fish and Wildlife Service including Traditional Section 6 Grant (P1886001). The Amargosa Conservancy assisted with data collection in growth plots between 2012–2016. We thank Cheryl Birker, Carolyn Mills, and Kim Schaefer for assistance with data collection and seed collection and processing. Patrick Donnelly reviewed an early draft of this manuscript and provided useful comments. We thank four reviewers who provided useful comments and greatly improved this manuscript.

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*Associate Editor was R. La Rosa*

# Invertebrates



# Invertebrates

Photos, previous page:

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1	2
3	4
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1. Franklin's bumble bee (*Bombus franklini*).  
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Photo Credit: Len Mazur
3. Shasta crayfish (*Pacifastacus fortis*).  
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4. Western bumble bee (*Bombus occidentalis*).  
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5. Crotch bumble bee (*Bombus crotchii*).  
Photo Credit: Stephane McKnight, Xerces Society
6. California freshwater shrimp (*Syncaris pacifica*).  
Photo Credit: Harry McGrath

## ESSAY

## A conservation conundrum: protecting bumble bees under the California Endangered Species Act

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Bumble bees (*Bombus* spp., Apidae) are important pollinators throughout North America, and across the world. Their long tongues, ability to fly in low temperatures and inclement weather, as well as their aptitude at buzz pollination (Heinrich 2004) make them second only to the honey bee as pollinators that contribute to the multi-trillion dollar agricultural industry (Delaplane and Mayer 2000; Kremen et al. 2002; Klein et al. 2007; Gallai et al. 2009). In wildlands and natural areas, they are essential as generalist pollinators of many plant families (Goulson 2010). However, there have been alarming reports of bumble bee population declines from multiple continents (Cameron and Sadd 2020).

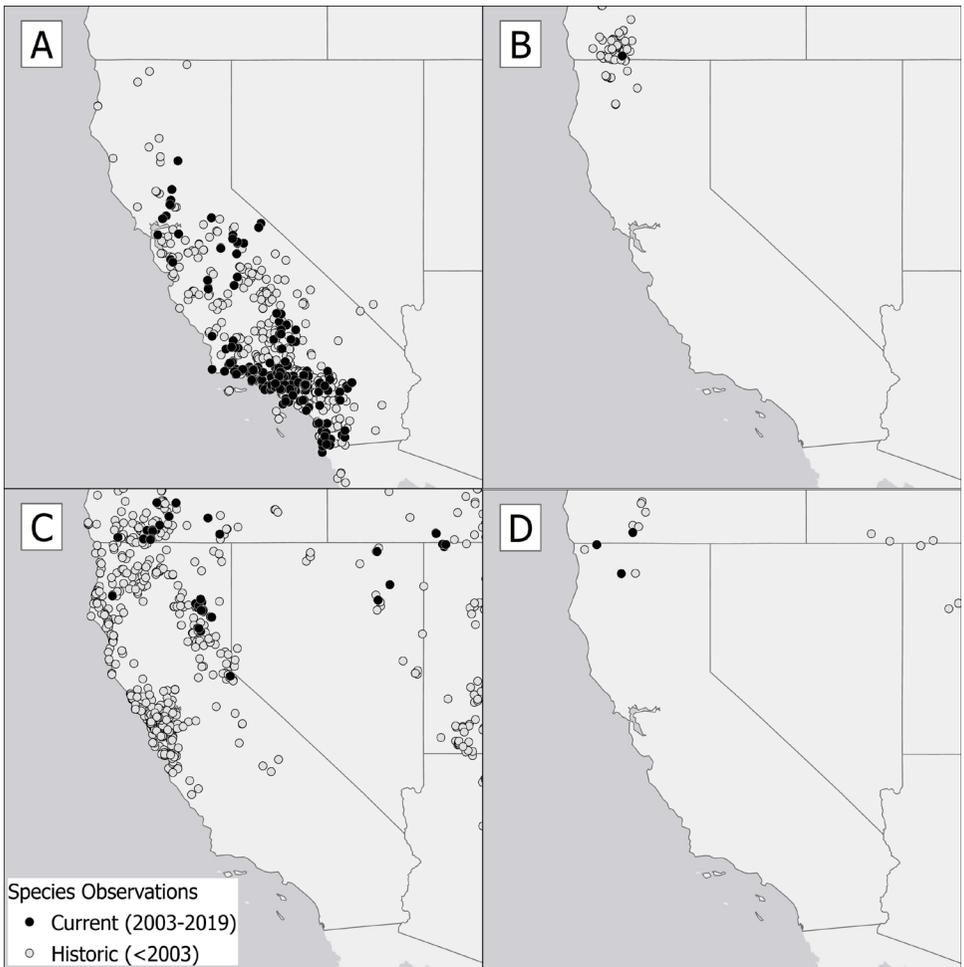
Notably, many species of bumble bees have been considered for listing as endangered species under the U.S. Endangered Species Act (ESA). The rusty patched bumble bee (*B. affinis*), native to much of eastern North America, was added in 2017 (USFWS 2017). Franklin's bumble bee (*B. franklini*), native to a small portion of southern Oregon and northern California, was Proposed Endangered in 2019 (USFWS 2019a). The western bumble bee (*B. occidentalis*) and Suckley's cuckoo bumble bee (*B. suckleyi*) are both currently under review for ESA listing by the U.S. Fish and Wildlife Service (USFWS 2016). The yellow banded bumble bee (*B. terricola*) was evaluated for inclusion on the endangered species list, but ultimately deemed not warranted for listing by the Service (USFWS 2019b).

The specific causes of bumble bee declines are largely unknown, though several key threats have been identified. Pathogen infection (Cameron et al. 2016), insecticides (Wood and Goulson 2017), habitat loss (Williams and Osborne 2009), and climate change (Kerr et al. 2015) have all been individually identified as significant factors leading to observed declines. It is likely that each of these factors also interact, creating synergistic effects and accelerating declines (Cameron and Sadd 2020).

California is home to more than half (27) of the ca. 50 bumble bee species in North America — more than any other state in the country. Two of California's species, Franklin's bumble bee and Crotch's bumble bee (*B. crotchii*), are largely endemic; when they occur outside of California, it is within a short distance from the state's boundary. According to an analysis by the International Union for the Conservation of Nature (IUCN) Bumblebee

Specialist Group, eight of California's bumble bees (~30%), including the two largely endemic species, are facing some degree of extinction risk (Hatfield et al. 2015a). Four of them are particularly imperiled, in need of rapid conservation action.

*Crotch's bumble bee*—Crotch's bumble bee historically occurred from the northern Central Valley to Baja Mexico, but has been lost from 70% of its range in California and now primarily persists in coastal southern California habitats, though also survives in a few areas around Sacramento (Fig. 1a; Hatfield et al. 2015b; NatureServe 2019; Richardson 2019; The Xerces Society et al. 2019). Crotch's bumble bee is a Species of Greatest Conservation Need (SGCN) in California (CDFW 2015), and is listed as endangered on the IUCN Red List (Hatfield et al. 2015b). SGCN status makes a species a target for conservation action, and eligible for conservation funding under the State Wildlife Action Plan.



**Figure 1.** Current (filled circles, 2003–2019) and historic (open circles, before 2003) records of A) Crotch's bumble bee, B) Franklin's bumble bee, C) the western bumble bee, and D) Suckely's cuckoo bumble bee. Data from: Richardson 2019 and The Xerces Society et al. 2019.

*Franklin's bumble bee*—Franklin's bumble bee has one of the smallest ranges of any bumble bee in the world in the Klamath-Siskiyou Mountains of southern Oregon and northern California. Its decline, which began in the late 1990s, was documented by Dr. Robbin Thorp who conducted annual surveys at historic sites and other nearby localities for this species from 1998–2017 (Thorp 1999, 2001, 2004, 2005a, 2005b). Despite extensive surveys by Dr. Thorp, the Bureau of Land Management (Code and Haney 2006), and the Service (J. Everett, U.S. Fish and Wildlife Service, personal communication), Franklin's bumble bee has not been detected since 2006 (Fig. 1b). Franklin's bumble bee is an SGCN in California (CDFW 2015).

*The western bumble bee*—The western bumble bee has experienced significant declines throughout its range, but most notably west of the Cascade/Sierra mountains, where observations are rare (Cameron et al. 2011; Hatfield et al. 2015c; Rhoades et al. 2016). Recent surveys from the Pacific Northwest Bumble Bee Atlas in Oregon, Washington, and Idaho have found that the western bumble bee's relative abundance has declined significantly throughout its former range in those states (Xerces Society et al. 2020) and has been lost from Pacific coastal areas of its historic range, experiencing an 80% decline in California (Fig. 1c; Unpublished analysis from Richardson 2019; The Xerces Society et al. 2019). These data are corroborated by a recent occupancy modeling analysis, which found that the probability of occupancy by the western bumble bee has declined by 93% over the last 21 years (Graves et al. 2020). The western bumble bee is an SGCN in California (CDFW 2015).

*Suckley's cuckoo bumble bee*—Suckley's cuckoo bumble bee is a social parasite that uses the western bumble bee as its host species; it has also been detected in the nests of other species (Thorp 1983; Williams et al. 2014). Since the western bumble bee has declined extensively, it is highly likely that this cuckoo bumble bee has experienced a parallel—and likely more serious—decline. This species is listed as Critically Endangered on the IUCN Red List (Hatfield et al. 2015d) and has not been detected in Pacific Northwest Bumble Bee Atlas surveys from Oregon, Washington, or Idaho to date (Xerces Society et al. 2020) and has been lost from 55% of its range in California (Fig. 1d; Hatfield et al. 2015d; Unpublished analysis from Richardson 2019). Suckley's cuckoo bumble bee is an SGCN in California (CDFW 2015).

Recognizing that without formal protection it was likely that these four essential pollinators faced a high risk of extinction, in 2018 the Xerces Society, Defenders of Wildlife, and the Center for Food Safety filed a petition with the California Fish and Game Commission (FGC) asking the state to grant the western, Suckley's cuckoo, Crotch's, and Franklin's bumble bees protection under the California Endangered Species Act (CESA; Hatfield et al. 2018).

Protection under CESA would mediate threats and prevent these four species from going extinct, and likely provide corollary protection to a diversity of wild species that provide pollination services to crops (Kremen et al. 2002) and natural lands throughout the state. CESA protection would: (1) explicitly protect these bumble bees from take, including capturing or killing them (but see agricultural exemption below); (2) allow for the use of Safe Harbor Agreements to encourage landowners to manage their lands to benefit bumble bees, without subjecting them to additional regulatory restrictions because of their efforts; (3) encourage farmers and ranchers to establish programs to enhance and maintain habitat for bumble bees (Voluntary Local Program); and (4) foster the development of Habitat Conservation Plans (HCPs) and Natural Community Conservation Plans (NCCPs) with public and private stakeholders.

In 2019, the California Department of Fish and Wildlife (CDFW) completed an initial review of the bumble bee petition and recommended that the FGC designate these bumble bees as Candidate Species, while CDFW conducted a more in-depth analysis of each species' status. On June 12, 2019, the FGC designated these four bumble bees as Candidate Species, affording them the full protections of listing under CESA until a final determination could be made. By early September, a lawsuit was filed against the FGC and CDFW by lawyers representing several large-scale agricultural groups, including the Almond Alliance of California, California Association of Pest Control Advisers, California Citrus Mutual, California Cotton Ginners and Growers Association, California Farm Bureau Federation, Western Agricultural Processors Association, and the Western Growers Association. The Xerces Society, Defenders of Wildlife, and Center for Food Safety joined the lawsuit as intervenors on behalf of the State, represented by Stanford Law School's Mills Legal Clinic (Superior Court of California 2019).

The California legislature passed a law in 1997 that exempts farmers from any liability associated with CESA, if and when they accidentally harm a protected (i.e. candidate, threatened, or endangered) species during the course of their routine farming practices (CA Fish & G. Code § 2087). This provision is currently set to expire in 2024, but this exemption has routinely been renewed by the legislature since its inception. In 1997, the legislature also authorized the creation of Voluntary Local Programs for farmers and ranchers that ensure they will not be penalized for accidentally harming endangered species if and when they proactively engage in conservation activities to benefit those species (CA Fish & G. Code § 2086).

Nevertheless, to establish standing in the lawsuit filed in September 2019, the consortium of agricultural groups claimed that protecting these bumble bees would interfere with their farming practices. The petitioners argued that the state of California did not have the legal authority to protect insects under CESA when they designated these four bumble bees as candidate species. They cited section 2062 of the Fish and Game Code, which states that an “‘endangered species’ means a native species or subspecies of a bird, mammal, fish, amphibian, reptile, or plant which is in serious danger of becoming extinct throughout all, or a significant portion, of its range...” (CA Fish & G. Code § 2062). However, the state legislature defined ‘fish’ broadly in the Fish and Game Code; Section 45 states that “‘Fish’ means a wild fish, mollusk, crustacean, invertebrate, amphibian, or part, spawn, or ovum of any of those animals” (CA Fish & G. Code § 45).

In the definition of “fish,” the state legislature did not qualify the type of invertebrates to be included (such as marine, freshwater, terrestrial; CA Fish & G. Code § 45). The legislature also included mollusks, crustaceans (both independently classified as invertebrates), and amphibians within its definition of “fish,” and each of those animal groups contain many species that have no association with marine habitats. Indeed, three non-marine invertebrate species are already protected under CESA, the trinity bristle snail (*Monadenia infumata setosa*; which lives on land), and the Shasta crayfish (*Pacifastacus fortis*) and California freshwater shrimp (*Syncaris pacifica*), both of which live in freshwater habitats.

Since bumble bees are unquestionably invertebrates, the FGC and CDFW, as well as the intervenors, argued that the state has the authority to protect bumble bees under CESA and that it was the legislature's intent to protect insects under CESA when the law was passed in the early 1980s. However, Judge James P. Arguelles concluded that the legislature meant only marine invertebrates when it included invertebrates in the definition of fish, stating in the final ruling that “the word ‘invertebrates’ as it appears in Section 45's definition of ‘fish’

clearly denotes invertebrates connected to a marine habitat, not insects such as bumble bees.” In February of 2021, the FGC and intervenors appealed this decision.

There is little question that protecting bumble bee diversity in the state of California benefits humans and native ecosystems, but the conservation of this resource particularly benefits the agricultural sector, which helps to feed the residents of the state. Indeed, as much as 35% of the food we eat comes from crops that are pollinated by bees (Klein et al. 2007). In agricultural systems, on average, wild pollinators contribute pollination services valued at \$3,251 per hectare to insect pollinated crops (including almonds)—the same value as that contributed by managed European honey bees (Kleijn et al. 2015). In Western North America, 50% of the wild bees contributing significantly to pollination services are bumble bees, and this service is valued at more than \$480 per hectare for insect pollinated crops (Kleijn et al. 2015). Wild bees also provide important insurance against the continued loss of managed honey bees, on which agricultural systems in California now depend (Winfree et al. 2007). Moreover, wild bees provide their pollination services for free, whereas the cost of honey bee pollination services can be expensive, often in excess of \$100/hive during the peak of almond bloom. Additionally, but not insignificantly, wild pollinators are largely responsible for maintaining plant diversity throughout California’s ecosystems, on which most wildlife species depend.

Without state protection, bumble bee populations (and other wild pollinators) are likely to continue to decline throughout California, which could lead to increased agricultural dependence on managed pollinators. Some managed pollinators have been shown to compete with native bees for resources and to spread pathogens to wild bees, accelerating declines (Graystock et al. 2013, 2016; Fürst et al. 2014; Lindström et al. 2016; Cane & Tepedino 2017). This cycle of dependence thus creates a vortex of decline that is unsustainable for agricultural systems and wild pollinators alike.

The ecosystem service of pollination is just one of the many valuable services that invertebrate animals provide. Native insects and other invertebrates play important roles in nutrient cycling and decomposition, pest control, and as food for other wildlife, like songbirds (Losey and Vaughan 2006). If CESA indeed excludes insects, not only does the law fail to protect ca. 80% of California’s animal biodiversity, but also the ecosystem services they provide that are the fabric that holds our natural systems together (Wilson 1987). Article 1 of CESA states in relation to imperiled species: “These species of fish, wildlife, and plants are of ecological, educational, historical, recreational, esthetic, economic, and scientific value to the people of this state, and the conservation, protection, and enhancement of these species and their habitat is of statewide concern,” giving broad authority to conserve species of value (CA Fish & G. Code § 2051). Excluding any insects from the law because of their taxonomic status significantly hampers CDFW and the FGC’s ability to do so. Notably, other species that are valued by Californians and facing extinction, like the monarch butterfly (*Danaus plexippus plexippus*), may therefore be ineligible for protection by a law intended to safeguard wildlife in the state. Overwintering congregations of the western population of monarch butterflies, which occur only in coastal California, have declined by more than 99% since the 1980s (Pelton et al. 2019). Without state protection, this iconic species is likely to be extirpated from California.

Despite the fact that the overwhelming majority of invertebrates are beneficial, contributing essential ecosystem services and billions of dollars to the global economy, the prevailing attitude toward them is disappointingly negative (Kellert 1993; Batt 2009), likely leading to the uncertainty surrounding their inclusion under CESA. Nevertheless, the

charismatic nature of an animal should not seal its fate. We have now spent more than a year considering whether bumble bees can be included under the law, rather than investing that time developing practical solutions to benefit bumble bees and the fields they help pollinate.

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## FULL RESEARCH ARTICLE

## Phylogenetic relationships among subclades within the Trinity bristle snail species complex, riverine barriers, and re-classification

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The Trinity bristle snail (*Monadenia setosa*) is listed as a threatened species under the California Endangered Species Act (CESA). In northern California, populations of this endemic terrestrial gastropod occur in rare, isolated, and highly fragmented locations within the greater Trinity Basin. Since 1952 when it was originally described, the taxonomic status of the Trinity bristle snail has been questioned based on unpublished information limited in geographic scope and sample size, which resulted in the taxon being reduced from species status (*M. setosa*) to subspecific status (*M. i. setosa*) within the Redwood sideband (*M. infumata*) species complex. Primary objectives of the present study were to: 1) use DNA extraction and PCR sequencing to gain insight into patterns of genetic variation and phylogenetic relationships among a larger sample of endemic populations of the Trinity bristle snail; 2) re-evaluate the systematic and taxonomic status of the species using outgroup analysis and references samples from sympatric ecologically co-occurring taxa within the genus *Monadenia*; and 3) evaluate the potential biogeographic effects of major riverine systems on genetic differentiation among relic and disjunct populations within the Trinity Basin. Results of the DNA sequence analysis using several different tree reconstruction methods revealed that subspecies of the Redwood sideband (*M. i. subcarinata*), Yellow-based sideband (*M. i. ochromphalus*), and the Trinity bristle (*M. i. setosa*) exhibited a phylogenetic signal at  $\geq 95\%$  species probability. Except for the Yellow-based sideband, molecular evidence detected the presence of several morphologically cryptic subclades within each species clade formerly undescribed by the scientific community. Syntopic<sup>1</sup> ecological relationships between subclades of the Trinity bristle snail and the Redwood sideband occurred in several areas within the geographic range of the Trinity bristle snail, which indicated that these subclades were conservatively differentiated at the subspecific level. A Bayesian coalescent tree showed that genetic variation

<sup>1</sup> Syntopy refers to the joint occurrence of two species in the same habitat at the same time, which may result in hybridization between closely related taxa or sister species. In contrast, sympatric species occur together in the same region, but do not necessarily share the same localities as syntopic species do (Futuma 2009).

among allopatric subclades of the Trinity bristle snail and the Redwood sideband were congruent with hydrological discontinuities associated with site-specific riparian stream corridors and the primary river systems within the Trinity Basin. Correlation analysis revealed a pattern of area effects, wherein sparsely bristled Trinity bristle snails were generally found to the northwest and more abundantly bristled individuals to the southeast in relation to primary river corridors that bisect the central Trinity Basin. A similar but opposite trend was observed in the directional pattern of banding. Here the most conspicuously banded individuals were found in samples distributed to the northwest while individuals with less conspicuous banding patterns occurred in a more southeasterly direction in relation to primary riverine corridors. These geographic patterns of bristles and bands appeared to reflect shallow clines that were evident in samples of both the Trinity bristle snail and the Redwood sideband. Parsimony character state reconstructions revealed that the presence of bristles and conspicuousness of bands was widespread among genetic samples, but these attributes did not provide a definitive morphological character that could be used to distinguish among co-occurring taxa.

**Key words:** biogeography, DNA sequence analysis, *Monadenia setosa*, northern California, phylogenetics, river systems, taxonomy, terrestrial gastropod, Trinity bristle snail

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## Distribution and Status

There are 284 species representing 30 families of land snails and slugs currently described as living within the geographic boundaries of California. Numerous additional species remain to be described, some recognizable only by use of modern DNA sequence analyses (Roth and Sadeghian 2006). The genus *Monadenia* consists of a diverse group of air-breathing land snails (gastropods) within the family Bradybaenidae<sup>2</sup>. Species within this genus are commonly referred to as “sidebands.” Currently there are 14 species and 19 subspecies of terrestrial snails in the genus *Monadenia* within the geographic boundary of California that are primarily distributed from south-central California, on the west side of the Sierra Nevada, into the Cascade Mountains of southwestern Oregon (Roth and Sadeghian 2006).

The Trinity bristle snail (*M. setosa*<sup>3,4</sup>) is a rare and rather large terrestrial forest-dwelling gastropod (Fig. 1<sup>5</sup>) endemic to the greater Trinity Basin of northern California. Populations occur in isolated and highly fragmented locations within central Trinity County and extreme eastern Humboldt County (Fig. 2). Geographically, populations may be found along both sides of the western-most segment of the mainstem Trinity River, east slope of the New River gorge, South Fork Trinity River, Hayfork Creek, and along the east slope of South Fork Mountain on the Trinity-Humboldt county line (Fig. 2; Table 1).

<sup>2</sup> Phylum: Mollusca, Class: Gastropoda, Order: Stylommatophora, Family: Bradybaenidae, Genus: *Monadenia*, Species: *Monadenia setosa*.

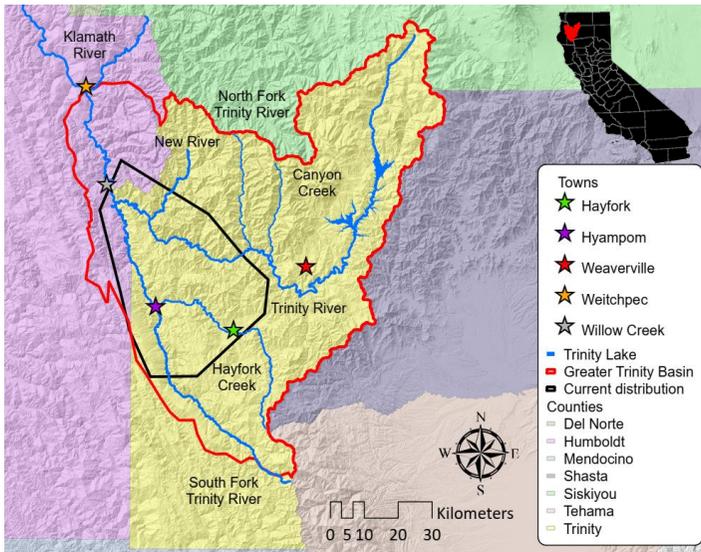
<sup>3</sup> <https://wildlife.ca.gov/Conservation/Invertebrates>

<sup>4</sup> <https://nrm.dfg.ca.gov/FileHandler.ashx?DocumentID=84011&inline>.

<sup>5</sup> <https://onlinelibrary.wiley.com/cms/asset/0ff24ac9-31b6-4999-a249-470a29709e0d/jawr12774-fig-0004-m.jpg>



**Figure 1.** A) Trinity bristle snail (*M. setosa*) on a decaying rain-soaked moss-covered tree branch at the Type locality at Swede Creek. B) dorsal and C) lateral side views of the surface of the shell showing striated surface topography of radiating impressed lines and ribs, lack of a conspicuous dull or subdued banding pattern, and (D and E) the minute hair-like bristles on the shell's thin periostracum that are brownish in color, and plump and conical in shape. Bristles may be worn off on old-aged (large) shells through abrasion but are generally visible by close examination with a hand-lens and can usually be re-hydrated on shells that are not too worn. Presence of these fine hair-like bristles covering the surface of the shell in live animals contribute to the “velvety” or “dirty” appearance when covered with specks of detritus.



**Figure 2.** Current geographic distribution of the Trinity bristle snail (*M. setosa*) in Trinity County and eastern Humboldt County, showing topographic relief and distribution of major river systems referenced to towns in the Trinity Basin of northern California.

**Table 1.** Boundary of polygon delineating the current geographic range of the Trinity bristle snail (*Monadenia setosa*) in the greater Trinity Basin.

Location	UTM-east	UTM-north
CA, Trinity County, 6.6 km NE of the town of Willow Creek, Waterman Ridge, 1.7 km S Maple Spring.	451911.8	4536580.2
CA, Humboldt County, 7.0 km of the town of Willow Creek, Madden Creek, 0.7 km W South Fork Trinity River.	447236.1	4525208.2
CA, Trinity County, 4.6 km E of the town Mad River, 0.6 km S Miller Spring, 1.2 km N Lamb Gap, South Fork Mountain Road.	461034.2	4477304.9
CA, Trinity County, 8.5 km N of the town of Forest Glen, 0.4 km S Bear Wallow Meadow.	470447.9	4477240.1
CA, Trinity County, 8 km NE of the town of Hayfork, Baker Creek, 4.1 km NW Big Creek Ranch.	490809.1	4494134.9
CA, Trinity County, 6.9 km SW of the town of Junction City, 0.5 km SW Soldier Creek.	492538.4	4502894.7
CA, Trinity County, 7.5 km NE of the town of Del Loma, 1.9 km NW Cherry Flat and French Creek.	474522.3	4521649.3
CA, Trinity County, 9.1 km NE of the town of Forest Glen, 0.8 km SE Big Flat of Naufus Creek.	473434.3	4478230.0
CA, Trinity County, 4.9 km SW of the town of Helena, south side Trinity River, 2.9 km NW Squaw Camp Spring, 0.5 km SE Eagle Creek.	487366.6	4509143.0
CA, Trinity County, 1.0 km SW town of Dailey, Bell Creek tributary.	462418.3	4527703.0

In 1972, Roth included the species on a list of rare and potentially endangered land snails (Roth 1972). This taxon was one of the first species of terrestrial gastropods proposed as Threatened under the Endangered Species Act (USFWS 1973, Federal Register, 41: 17742, 28 April 1976). In 1980, the California Department of Fish and Wildlife (CDFW) listed this species as “rare,” and in 1984, it was reclassified as “threatened” under the California Endangered Species Act (CESA; CDFW 1972). The species subsequently became a Category 2 (“C2”) Federal Candidate Species in November 1994 (Federal Register, 59: 58,982, 58,983)<sup>6</sup>; a category later eliminated.<sup>7</sup> Also in 1994, the Trinity bristle snail was included as an “Additional Species” in the Northwest Forest Plan covering late-successional and old-growth forests in the range of the northern spotted owl (*Strix occidentalis caurina*; USFS and USBLM 1994). The International Union for Conservation of Nature Red List of Threatened Species lists the Trinity bristle snail as “Vulnerable” (VU) (Groombridge 1994; Baillie and Groombridge 1996; Roth 1996)<sup>8</sup>. NatureServe assigned the Trinity bristle snail the range-wide global rank of “G2T1” for critically imperiled taxa with a high risk of extinction or elimination due to restricted range, few populations or occurrences, steep declines, and severe threats. Currently, the Trinity bristle snail is the only species of terrestrial gastropod in California listed under CESA.

<sup>6</sup> Available from: [https://ecos.fws.gov/docs/federal\\_register/fr2729.pdf](https://ecos.fws.gov/docs/federal_register/fr2729.pdf)

<sup>7</sup> C2 category defined as taxa “for which information now in the possession of the United States Fish and Wildlife Service (USFWS) indicates that proposing to list as endangered or threatened is possibly appropriate, but for which persuasive evidence on biological vulnerability and threat are not currently available to support proposed rules” (Federal Register, 59: 58,982, 58,983, 15 November 1994. In 1996 the USFWS published a notice making a final decision to eliminate the C2 List (61 Federal Register, 61:64,481, 5 Dec. 1996).

<sup>8</sup> <https://dx.doi.org/10.2305/IUCN.UK.1996.RLTS.T13666A4324507.en>.

## Background

The Trinity bristle snail was hypothesized to be a relict species of the Pleistocene epoch when climatic conditions were much cooler and forest conditions more mesic than today (Talmadge 1952; Dunk et al. 2004). Populations occur within Klamath mixed-conifer forests along riparian corridors and in adjacent upland habitats characterized by a mesic deciduous hardwood understory. Although mostly observed within moist, well-drained and shaded canyons or within riparian stream-side benches covered with a thick layer of leaf mold, isolated populations also occur in drier upland mixed conifer habitat with adequate canopy cover (Sullivan 2021). Depending on weather conditions, the Trinity bristle snail is most active from May through October, and generally observed between dusk and dawn, when the air temperature is cooler and more humid. The species is active during the day, particularly after several days of warm spring or late fall weather associated with fog and saturating rains (Sullivan 2021; Fig. 3).

In 1952, Robert R. Talmadge, a Pacific Gas and Electric employee and amateur naturalist residing at Willow Creek, Humboldt County, California, described the Trinity bristle snail as a new species (Talmadge 1952). The type locality was described based on specimens found along the lower reach of Swede Creek, Trinity County, a small tributary draining into the Trinity River along the northeast side of Highway 299 (Topotype: 40.793°N, 123.355°W). Where along Swede Creek these specimens were found was not specifically identified. Twenty-six years later Roth (1978) performed a contract study for the United States Forest Service (USFS) and surveyed adjacent tributaries to the Trinity River from Italian Creek to Manzanita Creek, all in Trinity County. This effort focused on delineating the range and habitat associations of the species (Armijo 1979). Results of this investigation showed that extant populations of the Trinity bristle snail were present within riparian vegetation in Swede Creek, and along the lower riparian drainages of Little Swede Creek and Big French Creek based on samples of empty shells. Roth and Eng (1980) published results of their 1978 field study and added the first published photographs of a shell from the Trinity bristle snail, along with figures describing external anatomy and elements of the reproductive system. They concluded that the species was rare within its range with no evidence of expansion or restriction of its geographic distribution within historical times.

In 1986, Roth reported results of a second and more extensive field investigation conducted from 1981 to 1982 (Roth 1982; Roth and Pressley 1986); which expanded the area of consideration to include Big French Creek and regions of potential suitable habitat on the south side of the Trinity River, subsidiary drainages south to Hayfork Creek, and drainage divides north of Swede Creek and Big French Creek. This effort also extended the known range of the Trinity bristle snail to the north, east, and south of its previous geographic range, including Ripstein Campground, north of Junction City (on the NE), Hawkins Creek (on the NW), Hyampom (on the SW), and Hayfork Summit (on the SE). Additional surveys were also conducted along Hayfork Creek as far south as the Natural Bridge Picnic Area (123°00'–123°30' W longitude by 40°37.5'–40°52.5' N latitude).

From 1999 to 2002, surveys conducted by the USFS (Shasta-Trinity National Forest, Survey and Manage Program) found Trinity bristle snails at several new locations within Trinity County but retained no reference samples by location for verification purposes. From 2008 to 2011 Green Diamond Resource Company surveyed the environmental study limits associated with several timber harvest plans and CDFW listed species incidental take permits along the eastern slope of South Fork Mountain near Hyampom (eastern Humboldt and



**Figure 3.** View from Hay Fork Mountain on the divide between extreme eastern Humboldt county and extreme western Trinity County looking northeast into the fog-belt of the greater Trinity Basin, which includes the bulk of the geographic distribution of the Trinity bristle snail (*M. setosa*). Moisture provided by the presence of this “fog-belt” is likely very important in providing the mesic forest conditions in association with saxicolous riparian and upland microhabitats for this species and other terrestrial gastropods found throughout the region.

western Trinity counties). These surveys also resulted in additional samples of the Trinity bristle snail, but they too lacked published systematic reference samples and photographs. The above surveys summarized all known information on the geographic range of Trinity bristle snail at that time.

Throughout most of the history of its investigation the Trinity bristle snail was considered a distinct species (Talmadge 1952). Recently the taxonomic validity of the designation “species” has been questioned based upon unpublished information narrow in geographic scope and sample size, including: 1) anecdotal and qualitative observations of several shells in which the “base was smooth and glossy rather than matte and covered with minute bristles,” which lead to the hypothesis of morphological intergrades between the Redwood sideband and the Trinity bristle snail along the western margin of the range of the Trinity bristle snail (Roth and Eng 1980; Roth and Pressley 1986); and 2) DNA-sequenced data from only two specimens sampled from throughout the greater Trinity Basin (Cordero and Lindberg 2002). From these data it was concluded that: 1) the Trinity bristle snail was a subspecies of the Redwood sideband, hence the name *M. i. setosa*, with *setosa* as the specific epithet for the type specimen; and 2) the Trinity bristle snail was part of a large interbreeding complex forming a continuum with other populations of *Monadenia* distributed throughout the greater

Trinity Basin, a conclusion incorporated into the most recent taxonomic classification of species and subspecies within the genus *Monadenia* (Roth and Sadeghian 2006).

## Objectives

Specific objectives of the current research were four-fold.

- First, update knowledge on the geographic distribution of the Trinity bristle snail in the Trinity Basin of Northern California.
- Second, sample representative locations throughout the known geographic range of the species, collect representative shells and tissue samples, and re-inventory populations at and near the type locality and other areas of potential occurrence that historically were poorly.
- Third, re-evaluate the systematic status and taxonomic classification of the Trinity bristle snail using molecular phylogenetic outgroup analyses, reference samples from co-occurring taxa, and geographically disjunct populations of Trinity bristle snails.
- Fourth, evaluate the potential biogeographic effects of hydrological and topographic discontinuities within the Trinity Basin on genetic differentiation among allopatric relic populations.

## METHODS

### Study Area

Representative samples of tissues and shells were obtained and evaluated from throughout the known geographic range of the species (Sullivan 2021). Surveys focused on geographic regions throughout the northwestern segment of the Trinity River and its tributaries in Trinity and Humboldt counties based on previous field surveys (Talmadge 1952; Walton 1963; Roth 1978; Roth and Eng 1980), including: 1) the Type Locality and areas nearby (Italian, Manzanita, Swede, Big, French, Price, Bidden, Limestone, and Little Swede creeks, an unnamed creek south of Big Bar, south side of Trinity River east of Price Creek, Trinity County); 2) areas of potential occurrence based on quantitative suitable habitat descriptions not yet surveyed; 3) populations and habitats associated with recent timber harvests; 4) isolated locations along the Trinity River, South Fork of the Trinity River, and Hayfork Creek; 5) several new locations near South Fork Mountain (Trinity Co. and Humboldt Co. lines) from Blake Mountain west of Hyampom, south to Norse Butte, and east to near Forest Glen; and 6) hypothesized contact and “hybrid” zones between the Trinity bristle snail and the Redwood sideband (Roth and Pressley 1986).

### Survey Methods

Field surveys focused on known ecological and microhabitat descriptions of “critical” and potential suitable habitat based on historical accounts (Talmadge 1952; Roth 1978; Roth and Eng 1980). Survey efforts were facilitated in many areas as shells of Trinity bristle snails often washed or drifted down from suitable habitat at higher elevations onto well-worn deer trails, catchments, or other depressions that prevented their scattering or crossing fragmented talus or boulder fields (Sullivan 2021). Live and active Trinity bristle snails were sampled during warm wet, foggy, or rainy conditions during the months of March, April,

May, September, and October. During colder winter or more arid summer months, inactive live specimens were found sealed in their excavated subterranean estivation chambers well below the ground or substrate surface (Sullivan 2020).

### Statistical and Morphological Analyses

All statistical tests were performed using the R-suite of statistical programs (v4.0.2, R Core Team 2020). Principal components analysis (PCA) using singular value decomposition<sup>9</sup> of the centered data matrix described variation, discarded redundant variables, examined the extent of association among morphological, ecological, and genetic attributes of terrestrial gastropods, and assessed the ability of attributes to explain variation among samples (Smartt and Sullivan 1990; Sullivan and Smartt 1995; Sullivan 1996, 1997, 2020). Component axes that accounted for > 1% of the total variation in “attribute space” were retained for further analysis. This method is generally preferred for numerical accuracy as resulting principal components are orthogonal, thus minimizing multicollinearity between model predictors, with the goal of identifying a smaller subset of variable components that capture the majority of variance in predictors (Everitt and Hothorn 2011). Nonparametric Spearman’s rank correlation ( $r_s$ ) 2-tailed test was used to calculate the strength and direction of the relationship between any two variables expressed as a monotonic relationship, whether linear or not (Corder and Foreman 2014). P-values were adjusted using the Benjamini-Hochberg method (Benjamini and Hochberg 2000; Machiwal and Madan 2006); levels of significance were evaluated at  $p = 0.05 = *$ ,  $0.01 = **$ , and  $0.001 = ***$ .

Abundance of periostracal bristles (henceforth called “bristles”) on the shell’s thin periostracum “skin” of live snails was ranked as: none = 1, sparse = 2, or abundant = 3. Similarly, conspicuousness of bands was ranked as: slightly conspicuous = 1, moderately conspicuous = 2, or highly conspicuous = 3. Designations of river-side (east vs. west) were ranked from: 1 = W-side Trinity River and E-side South Fork Trinity River, 2 = W-side Trinity River and W-side South Fork Trinity River, 3 = NE-side Trinity River and NW-side New River, 4 = W-side Trinity River, E-side Hayfork Cr, NE-side South Fork Trinity River, 5 = NE-side Trinity River and NE-side South Fork Trinity River, 6 = W-side Trinity River and W-side Klamath River, and 7 = W-side Trinity River and E-side Klamath River. Parallel ranking of designated watersheds and adjacent riparian stream (creek) corridors ranged 1 to 15 and 1 to 18, respectively.

### Molecular Methods

*Sampling methods.*—From 333 geo-referenced sites throughout the known range of the Trinity bristle snail a representative sample of 61 adult specimens were used in the DNA analysis. An attempt was made to obtain a sample of five specimens per location, but the exact number depended on site-specific availability. Typically, sample sizes for molecular biodiversity assessment using DNA range from five to ten individuals per species, although one or two specimens is not uncommon since these are often the only representatives available (Phillips et al. 2019). For the DNA analyses live snails were placed in ventilated plastic

<sup>9</sup> Singular Value Decomposition (SVD) is a linear algebra computational method frequently used to calculate principal components for a dataset in a more efficient and numerically robust way ([https://en.wikipedia.org/wiki/Singular\\_value\\_decomposition](https://en.wikipedia.org/wiki/Singular_value_decomposition)).

containers with screw tops and a small amount of moist leaf litter along with a collection number and site coordinates (UTM-east, UTM-north). Samples were brought back to the laboratory where each snail was rinsed in cold distilled water and tissue samples from the foot appendage taken and immediately placed in capped glass containers containing 95% ethanol. Sampling of tissue was conducted under extreme sanitary condition to prevent cross-contamination of samples. European outgroup sequences were obtained online from GenBank<sup>10</sup>.

*DNA extraction, PCR, and Post-PCR and sequencing.*—Samples were assayed at the University of California Davis Department of Animal Science Genomic Variation Laboratory without knowledge of the taxonomy or location of the geographic sample. DNA was extracted from ethanol-preserved foot tissue samples including replicates where more multiple tissues were preserved per vial. The DNA extraction procedure followed the manufacturer's protocol for the Genra Puregene DNA extraction kit. We used Polymerase Chain Reactions (PCR) to amplify the cytochrome oxidase I gene (COI) as this gene has proved useful at delimitating other snail species. Initial PCR reactions were carried out using universal LCO1490, 5-GGTCAACAAATCATAAAGATATTGG-3 and HCO2198 5-TACTTCAGGGTGACCAAAAAATCA-3 primers (Palumbi 1996; Kocher et al. 1989; GoTaq® Flexi Buffer, Promega Corporation, Fitchburg, WI), and a 2.5mM concentration of each DNTP and 25mM MgCl<sub>2</sub>. Internal primers were designed that had an additional ACA on the 3' end of LCO and TTT on the 3' end of HCO following initial sequencing of samples that did not sequence during the first round of sequencing. A touch down protocol was used at 54° C (129.2° F) for the first 4 cycles at 2 minutes with 72° C (162.6° F) extension for 2:30 minutes, followed by 52° C (125.6° F) annealing for 4 cycles with 72° C (162.6° F) extension for 2 minutes, with subsequent 26 cycles at 48° C (118.4° F) annealing for 30 seconds and 72° C (162.6° F) extension for 2 minutes, and finally a 5 minute 72° C (162.6° F) extension step followed by a 10° C (50.0° F) hold. This protocol has worked well with other invertebrates and proved reliable in this situation. Cleanup of post-PCR sequencing was performed using Agencourt Ampure Magnetic Beads (Beckman Coulter Scientific). Cycle sequencing protocol used Big-Dye Terminator 3.1 (Applied Biosystems). Sequencing was performed using ethanol precipitation followed by suspension in double distilled water and sequenced on an Applied Biosystems 3730xl DNA unit.

*Phylogenetic tree reconstruction, and molecular species delimitation.*—Sequences were aligned using the MUSCLE alignment tool (Edgar 2004), where alignment contained 83 unique samples with 656 base pairs (bp) of genetic data. Alignment was visualized using the program Mesquite v.3.61 (Maddison and Maddison 2019) and phylogenetic trees were reconstructed using three methods. First, MrBayes v. 3.2.1 (Ronquist et al. 2012), which jointly calculates both the distance and nucleotide substitution models that best fit tree reconstructions. The maximum-credibility method evaluates each of the sampled posterior trees. Each clade within the tree was given a score based on the fraction of times that it appears in the set of sampled posterior<sup>11</sup> trees (Drummond and Rambaut 2019). The product of these scores equates to the tree's score (Rambaut et al. 2018). The tree with the highest score was

<sup>10</sup> <https://www.ncbi.nlm.nih.gov/genbank/>

<sup>11</sup> A maximum clade credibility tree summarizes results of a Bayesian phylogenetic inference. Whereas a majority-rule tree combines the most common clades to yield a tree that was not sampled in the analysis, the maximum-credibility method evaluates each sampled posterior tree. Each clade within the tree is given a score based on the fraction of times that it appears in the set of sampled posterior trees, and the product of these scores is the tree's score. The tree with the highest score is then the maximum clade credibility tree.

considered the maximum clade credibility tree. A total of 20 x 106 MCMC generations were completed by the program MrBayes followed by a 50% majority rule consensus tree reconstruction. Second, the Randomized Axelerated Maximum Likelihood (RAxML) Blackbox interface program was used to efficiently infer trees for extremely large datasets, either in terms of the number of taxa or the sequence length (Liu et al. 2011; Stamatakis 2014). For RAxML the nucleotide substitution model chosen was the General Time Reversible (GTR; Tavaré 1986). Third, a Bayesian Coalescent tree was reconstructed using the program BEAST v1.7.5 where the coalescent functioned as a model of the distribution of gene divergence in a genealogy (Drummond and Rambaut 2007; Drummond and Suchard 2010; Liang et al 2015). Bayesian inference was based on the posterior probability distribution approximated by a sample of species trees generated from a Markov Chain Monte Carlo (MCMC) algorithm. The substitution model used was HKY for codons 1 and 2 and GTR with gamma rate variation for codon position 3 (Hasegawa et al. 1985). An uncorrelated relaxed clock was used for the scaled mutation rate. The MCMC chain ran for 119 x 106 generations. For all datasets, tree construction (phylogram) and labeling were done using the FigTree Drawing Tool software (v1.4.4; Rambaut 2009). Parsimony character state reconstructions were done using the Bayesian Coalescent tree reconstructed using the program BEAST for: 1) bristle abundance, 2) band conspicuousness, 3) watershed designation, 4) river-side, and 5) adjacent stream corridor designation for each genetic sample using the program Mesquite. Species were delimited based on the Bayesian General Mixed Yule Coalescent<sup>12</sup> procedure using the program bGMYC (Reid and Carstens 2012; Talavera et al. 2014), which accounts for uncertainty in the phylogeny. Generally, the program bGMYC tries to detect the shift in branching pattern from a Yule process, characteristic of intraspecific variation, to a birth-death process more characteristic of interspecies variation. The top 100 trees that had the highest posterior probability from the BEAST analyses were used to estimate the mitochondrial DNA (mtDNA) species delineations.

## RESULTS

### Geographic Distribution of DNA Samples: Morphologic and Ecological Attributes

*Correlation analysis.*—Correlation analysis indicated a significant negative relationship between the abundance of bristles and the conspicuousness of bands on the surface of shells in the individual generic samples (Table 2). The correlation between the abundance of bristles and the distribution of river-side, watershed, and riparian stream corridor was either not significant or significantly negative. Yet conspicuousness of bands showed a significant positive correlation with all three hydrological variables. As expected, there was a significant correlation between designated watersheds and adjacent riparian stream corridors given the parallel distribution and diversity in both hydrological features. Although the geographic relationship between river-side and riparian stream corridor was significant, there was no correlation between river-side and placement of watershed boundaries.

Bristle abundance was significantly correlated with UTM-east ( $r_s = 0.83$ ,  $p = 0.001$ ,  $n = 69$ ) but negatively correlated with UTM-north ( $r_s = -0.55$ ,  $p = 0.001$ ,  $n = 69$ ), suggesting a

<sup>12</sup> The GMYC method classifies branches in a gene tree as intra- or interspecific by maximizing the likelihood of a GMYC evolution model. Branching events between species are modeled with a Yule model, which assumes a constant speciation rate and no extinction (Nee, May, and Harvey 1994; Barraclough and Nee 2001), and branching events within species are modeled using a neutral coalescent process (Hudson 1990).

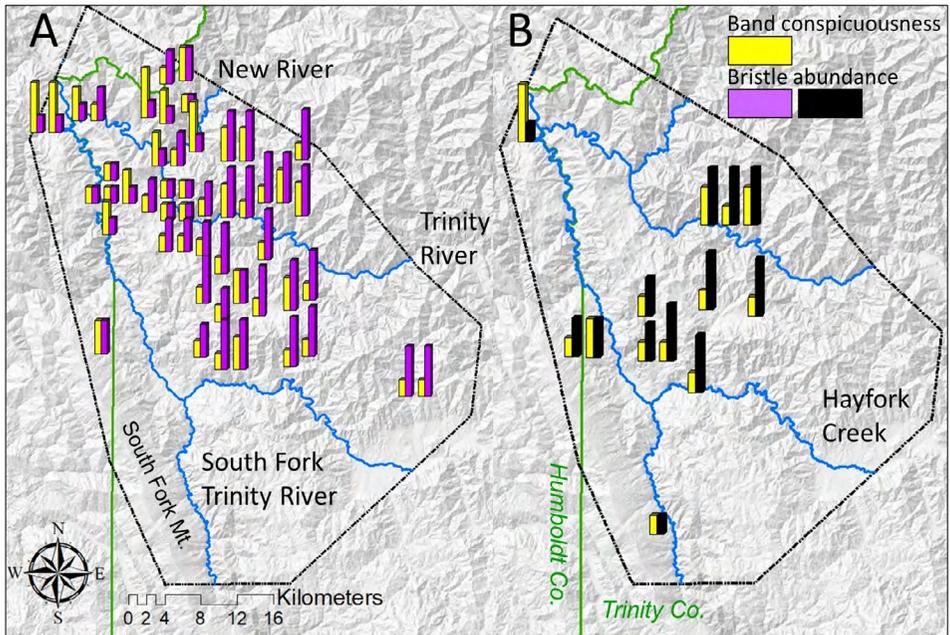
general northwest-to-southeast distribution in accordance with the opposite prevailing flow of the Trinity River, South Fork of the Trinity River, and Hayfork Creek, which bisect this section of the greater Trinity Basin. Sparsely bristled individuals were generally found to the northwest and more abundantly bristled snails to the southeast compared to mainstem river corridors (Fig. 4). In comparison, conspicuousness of bands was significant and positively correlated with UTM-north ( $r_s = 0.58$ ,  $p = 0.001$ ,  $n = 69$ ) and negatively correlated with UTM-east ( $r_s = -0.39$ ,  $p = 0.001$ ,  $n = 69$ ). This distribution pattern indicated a general northwest-to-southeast distribution, with the most conspicuous bands observed on snails to the northwest and less conspicuous bands being found on individuals to the southeast of bisecting rivers systems, just the opposite from the pattern of abundance in bristles. Importantly, genetic samples of *M. setosa* and *M. infumata* showed the same clinal trends in the morphology of the shell for both bristle abundance and band conspicuousness.

*Principal components analysis.*—Components analysis of the morphologic and geographic attributes associated with the distribution of genetic samples accounted for

**Table 2.** Spearman rank correlation coefficient ( $r_s$ ) matrix among morphological and hydrological geographic attributes associated with each location/site where genetic samples ( $n = 69$ ) were obtained within the known range of the Trinity bristle snail (*Monadenia setosa*). Correlations coefficients are below the diagonal and P-values are above the diagonal. P-values: 0.05 = \*, 0.01 = \*\*, 0.001 = \*\*\*.

Variable	Bristles	Bands	River-side	Watershed	Riparian stream corridor
Bristles	1.000	0.001	0.860	0.045	0.065
Bands	-0.380***	1.000	0.000	0.009	0.001
River-side	0.020	0.550***	1.000	0.080	0.002
Watershed	-0.240*	0.310**	0.210	1.000	0.000
Riparian stream corridor	-0.220	0.390***	0.360**	0.970***	1.000

93.5% of the variation among collection sites on the first three PC's (Table 3). Individuals sampled with comparatively abundant bristles generally were negatively distributed along PC I (51.6%) and positively distributed along PC II (21.8%). Individuals with no bristles were generally positively distributed along PC I and negatively distributed along PC II (Fig. 5A). Individuals with comparatively few bristles split the difference along both vectors and there was no overlap in the ranking of bristle abundance among the three groups. In contrast, conspicuous of bands on the surface of the shell showed considerable overlap between snails. Ranked categories of slightly and moderately conspicuous bands plotted mostly neutral or negative along PC I (Fig. 5B) compared to samples with highly conspicuous bands, which plotted positive along this vector and separate from all other samples. As indicated in plots of bristles and bands, the distribution of river-side, watershed, and riparian stream corridor plotted positive along PC I. On PC III (20.1% of the variation explained) watershed and riparian stream corridors had negative component loadings, while all other attributes loaded positive along this vector (Table 3). Vector trajectories of bristles and bands were opposite in direction as evidenced by their component loadings, which complemented the subtle patterns of "clinal" variation in shell morphology (Fig. 4).



**Figure 4.** Map and 3-D bar graphs of the geographic distribution of genetic samples of A) the Trinity bristle snail and B) the Redwood sideband in showing the abundance of bristles compared to the conspicuousness of bands found on the surface of the shell overlaid onto the hydrological distribution of major river systems and topographic diversity of the landscape in the Trinity Basin. Paired bar graphs represent ranks (1 – 3) that each snail had for bristle abundance (pink or black bars) or band conspicuousness (yellow bars); no snail had a 3:3 ranking of bristles to bands.

**Table 3.** Principal components (PC) analysis of morphological and hydrological attributes associated with locations where genetic samples ( $n = 69$ ) were obtained within the known range of the Trinity bristle snail (*Monadenia setosa*). The proportion of variance explained refers to how much of the total variance is explained by each of the PCs with respect to the whole (the sum).

Results of the analysis	PC I	PC II	PC III
Variance explained			
Variance	2.69	1.09	1
Percent variance explained	51.6%	21.8%	20.1%
Component loadings			
Abundance of bristles	-0.418	0.845	0.207
Conspicuousness of bands	0.717	-0.393	0.443
River-side	0.612	0.293	0.674
Watershed	0.820	0.264	-0.490
Riparian stream corridor	0.918	0.261	-0.263

## Phylogenetic Analysis and Tree Construction

There was nearly complete congruency between the three phylogenetic tree reconstruction methods used in the DNA analysis among outgroups and samples of Trinity bristle snails from Trinity and Humboldt counties. The cophenetic correlation coefficient<sup>13</sup> between the MrBayes tree (Fig. 6) and the RAxML tree (Fig. 7) was 81.0% and all major clades matched except for the tree backbone, which was a polytomy among the many Trinity bristle snail clades compared to a poorly supported but resolved tree produced by the RAxML reconstruction method. Among clades, 75.0% were identical except for the backbone of the RAxML tree, versus a polytomy for the backbone in the MrBayes tree. Results from BEAST tree (Fig. 8) were similar, producing an essentially identical topology as the RAxML tree, which indicated that the tree topology was robust. Topological robustness was also echoed by congruency between the three phylogenetic reconstruction methods used.

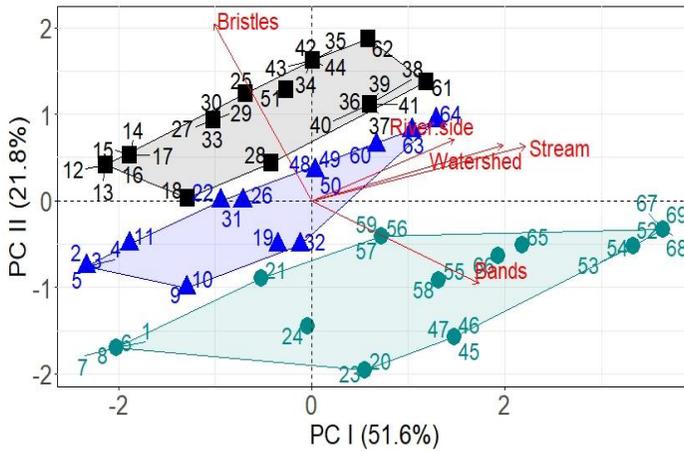
*Out-group comparisons.*—As expected, all rooted and un-rooted trees generated from the DNA sequence analysis showed that selected European out-group taxa: 1) Roman or Burgundy snail (*Helix pomata*), 2) Lapidary snail (*Helicigona lapicida*), and 3) White garden snail (*Theba pisana*) exhibited very different evolutionary relationships among themselves. This was also evident among taxa found regionally, including: 1) Church's sideband (*M. [Shastelixer] churchi*), 2) Sierra sideband (*M. mormonum*), 3) Columbia oregonian (*Cryptomastix hendersoni*), 4) Karuk hesperian (*Vespericola karokorum*), 5) Big Bar hesperian (*Vespericola pressleyi*), and 6) Beaded lancetooth (*Ancotrema sportella*). The Beaded lancetooth and species within the genus *Cryptomastix* appeared very divergent compared to other taxa within the in-group. Also, evident was a divergent sister-group relationship between subsamples of the Big Bar hesperian and the Karuk hesperian.

Rotating DNA-derived phylograms by re-rooting with any of the three European out-group species did not change any of the relationships among species or subclades within the genus *Monadenia*. All DNA-derived phylograms indicated that the genus *Monadenia* represented a monophyletic clade with samples of both the Sierra sideband and Church's sideband diverging significantly from all other taxa within the genus *Monadenia*. Evidence from each of the phylograms (Figs. 6–9) also showed that the Church's sideband shares a more recent common ancestor (less genetically divergent "sister taxon") with the *Monadenia* in-group samples compared to the Sierra sideband. Because Cordero and Lindberg (2002) used both the Sierra sideband and Church's sideband as outgroups simultaneously, their sister-group affinities compared to their "bristled" in-group clade (B-group) were unresolved (Appendix I). Nevertheless, without resolution of the phylogenetic sister-taxon relationships between these two species, Roth and Sadeghian (2006) assigned the Sierra sideband and Church's sideband to the subgenera *Corynadenia* and *Shastelixer*, respectively.

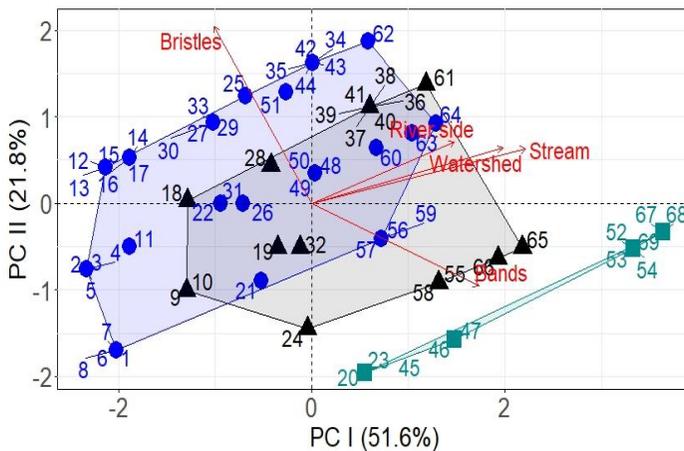
## Variation among Clades and Polytomous Subclades

*Clades and species within the in-group.*—The DNA-derived BEAST consensus tree with cartooned (triangled) clades provided the basis for delineating molecular affinities among species and subclades of large, banded forest snails within Trinity and Humboldt counties (Fig. 9). The bGMYC analysis was used as a guide to the phylogenetic delimitation of new species clades. Clades shaded with yellow had 95.0% probability of being a species

13 A measure of how faithfully a tree (phylogram) preserves the pairwise distances between the original unmodified data points.



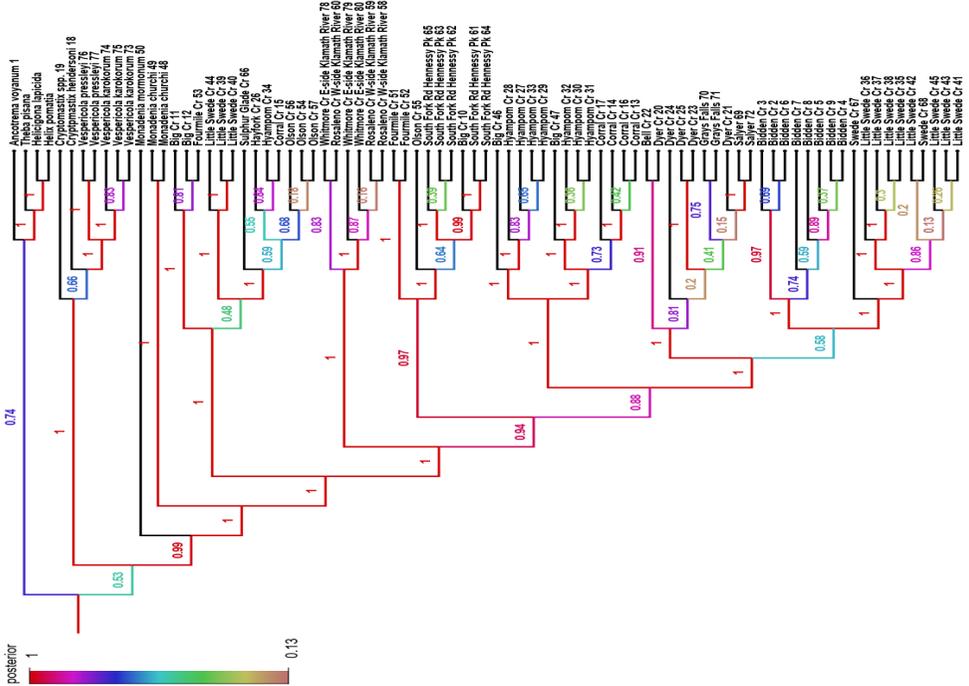
A. Bristle abundance 1 2 3



B. Band conspicuousness 1 2 3

**Figure 5.** Principal components analysis (PC) of morphological and hydrological attributes of the locations where genetic samples were obtained: A) bristles and B) bands based on: UTM-east and UTM-north directional reference coordinates, and position in relation to river-side, and designated watersheds and adjacent riparian stream corridors. Sample locations correspond to the phylograms below, including: Bidden Cr. 1, Bidden Cr. 2, Bidden Cr. 3, Bidden Cr. 4, Bidden Cr. 5, Bidden Cr. 6, Bidden Cr. 7, Bidden Cr. 8, Big Cr. 9, Big Cr. 10, Big Cr. 11, Big Cr. 12, Big Cr. 13, Corral Cr. 14, Corral Cr. 15, Corral Cr. 16, Corral Cr. 17, Corral Cr. 18, Dyer Cr. 19, Dyer Cr. 20, Bell Cr. 21, Dyer Cr. 22, Dyer Cr. 23, Dyer Cr. 24, Hayfork Cr. 25, Hyampom Cr. 26, Hyampom Cr. 27, Hyampom Cr. 28, Hyampom Cr. 29, Hyampom Cr. 30, Hyampom Cr. 31, Hyampom Cr. 32, Hyampom Cr. 33, Little Swede Cr. 34, Little Swede Cr. 35, Little Swede Cr. 36, Little Swede Cr. 37, Little Swede Cr. 38, Little Swede Cr. 39, Little Swede Cr. 40, Little Swede Cr. 41, Little Swede Cr. 42, Little Swede Cr. 43, Little Swede Cr. 44, Fourmile Cr. 45, Fourmile Cr. 46, Fourmile Cr. 47, Olson Cr. 48, Olson Cr. 49, Olson Cr. 50, Olson Cr. 51, Rosaleno Cr. W-side Klamath River 52, Rosaleno Cr. W-side Klamath River 53, Rosaleno Cr. W-side Klamath River 54, South Fork Road. Hennessy Peak 55, South Fork Road. Hennessy Peak. 56, South Fork Road Hennessy Peak. 57, South Fork Road Hennessy Peak. 58, South Fork Road Hennessy Peak. 59, Sulphur Glade Cr. 60, Swede Cr. 61, Swede Cr. 62, Salyer 63, Grays Falls 64, Grays Falls 65, Salyer 66, Whitmore Cr. E-side Klamath River 67, Whitmore Cr. E-side Klamath River 69, Whitmore Cr. E-side Klamath River 69.

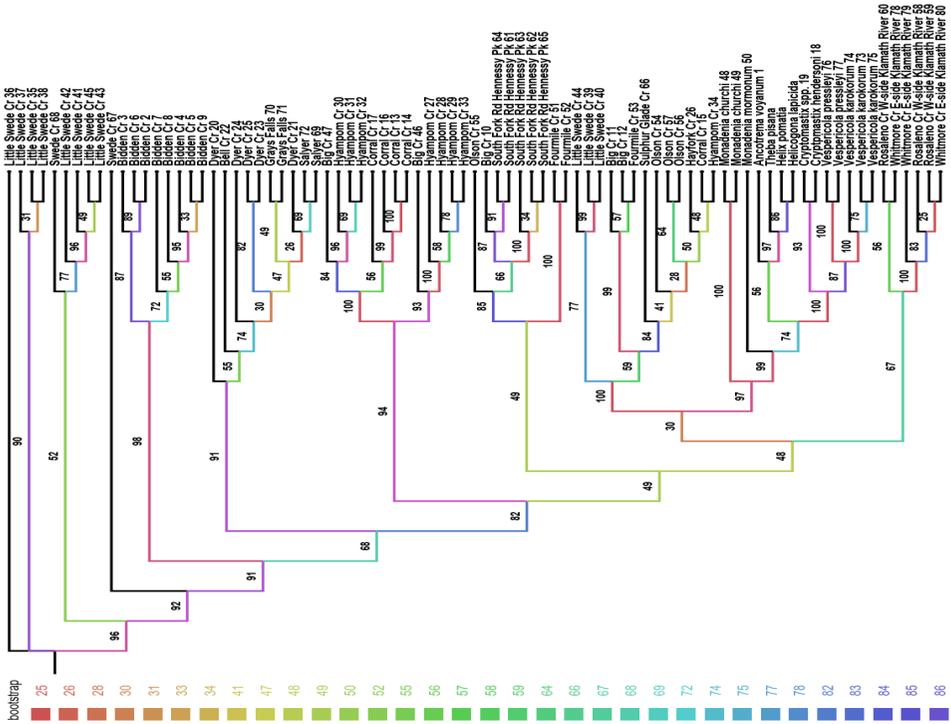




**Figure 7.** Tree generated by the Randomized Axelerated Maximum Likelihood (RAXML) Blackbox interface program using the General Time Reversible (GTR) nucleotide substitution model. Phylogram summarizes results of European outgroups and numbered genetic samples. Clades, branches, and numbers are colored by posterior probabilities for well-supported clades.

The Hyampom subclade of the Redwood sideband occurs within west-central Trinity County bounded by the central Trinity River, in the vicinity of Hayfork Creek, headwaters of South Fork Trinity River, and the eastern slope of South Fork Mountain (Hayfork, Hyampom, Corral, Olson, and Sulphur Glade creeks; Fig. 10b). The South Fork subclade includes areas within eastern Humboldt County and the west-side of the South Fork Trinity River (Big and Fourmile creeks). The Swede Creek subclade includes areas within Trinity County on the northeast-side of the central Trinity River (Swede and Little Swede creeks). Importantly, this taxon was geographically sympatric and co-occurred in the same ecological setting with the Trinity bristle snail at several locations throughout the greater Trinity Basin, which could have resulted in introgression. Because genetically and geographically distinct subclades of Redwood sideband were identified as syntopic with other similarly differentiated subclades within the Trinity bristle snail, these data are viewed as consistent with the degree of species-probability delineated by the BEAST consensus tree.

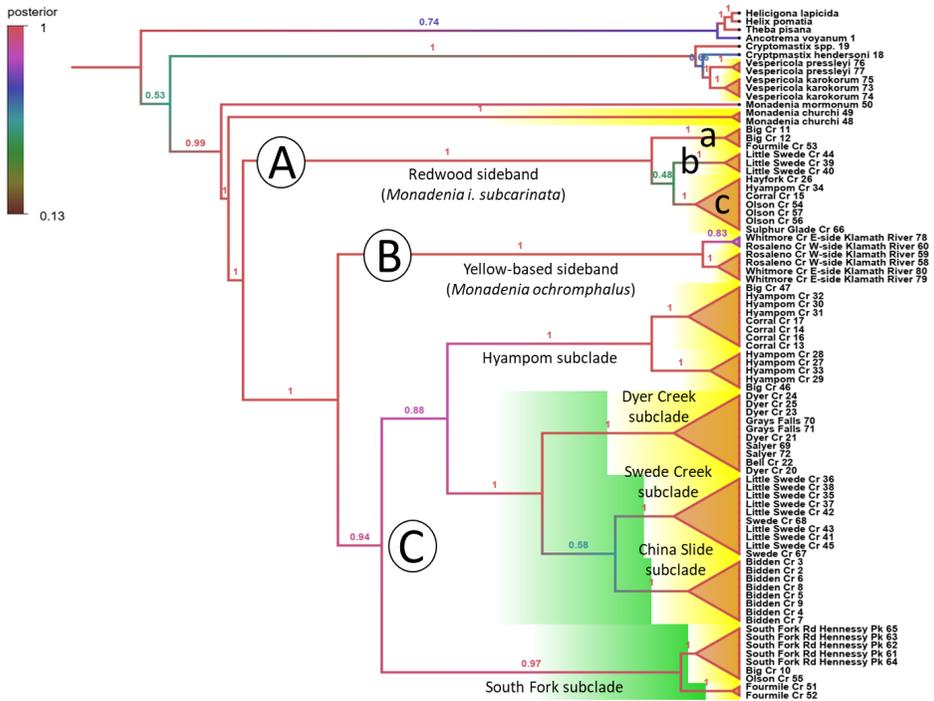
*Yellow-based sideband.*—This species has a distribution in Northern California that includes Del Norte, Humboldt, and Siskiyou counties. All DNA phylograms showed that the Yellow-based sideband (B-clade) and the Trinity bristle snail (C-clade) are sister-taxa derived from a common ancestor with the Redwood sideband. Nevertheless, all sister-taxa relationships within the genus *Monadenia* would need to be addressed for all species in a comprehensive molecular analysis to falsify this hypothesis. Within the B-clade there does



**Figure 8.** Phylogram generated by the Bayesian Coalescent Tree reconstructed from DNA sequenced data using the program BEAST summarizing genetic relationships among European outgroups and numbered genetic samples. Clades, branches, and numbers are colored by bootstrap percentages for well-supported clades.

not appear to be sufficient genetic divergence between subpopulations consistent with hydrology or geography to justify subspecies designation. Genetic samples were collected on adjacent but opposite sides of the Klamath River at Whitmore Creek and Rosaleno Creek that were within 4.0 kilometers (2.5 mi.) of each other at the extreme northeast boundary of Humboldt County with Siskiyou County. Separation between these two locations appears recent such that the Klamath River at this geographic location has not historically functioned as a significant riverine or topographic barrier to gene flow between populations on opposite sides of this headwater reach in the Klamath River system.

*Trinity bristle snail.*—For the Trinity bristle snail all DNA phylograms identified a series of allopatric polytomous subclades within the C-clade. And bristles were present in many individuals representative of each subclade whether sparse or abundant, and irrespective of age (size). These subclades consisted of five rather well-defined genetic subunits exhibiting significant geographic variation characteristic of restricted gene-flow in relation to separation by riparian stream corridors and major river systems within the greater Trinity Basin (Fig. 8 and Fig. 9).



**Figure 9.** BEAST Consensus Tree with cartooned (triangled) clades summarizing genetic relationships among European outgroups and numbered genetic samples. Branches are colored by bootstrapping. Clades shaded yellow have < 95% probability of being a species and clades shaded green have < 90% probability of being a species. Only clades that had < 95% species probability were cartooned. Samples are colored to reflect that the analysis distinguishes clades at the < 95% probability level as distinct subclades (species or subspecies). For *M. subcarinata* subclades: a = South Fork subclade, b = Swede Creek subclade, c = Hyampom subclade. Branches are colored by posterior probabilities for well-supported clades.

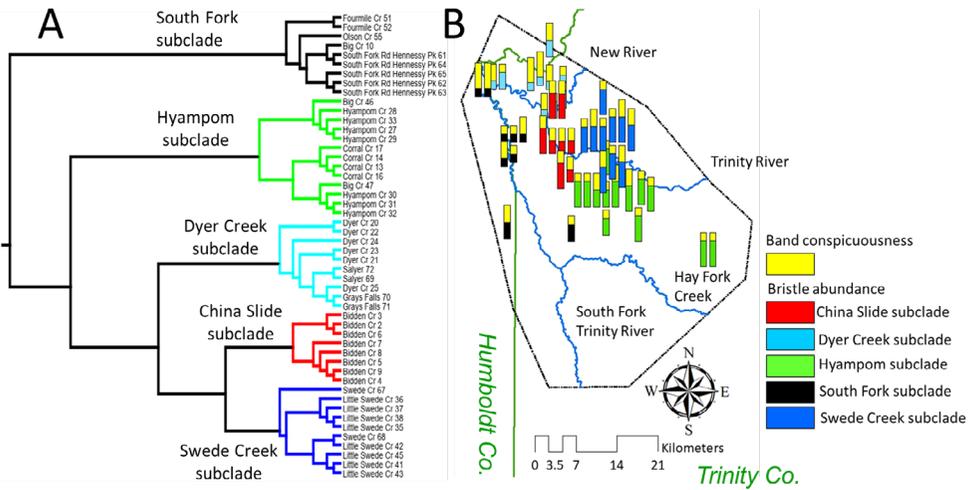
The China Slide subclade at Bidden Creek was located downriver on the opposite and west-side of the central mainstem Trinity River in the vicinity of the China Slide<sup>14</sup>. This subclade represents a homogenous site-specific genetic sample that is the “sister-taxon” to the Swede Creek subclade. The Dryer Creek subclade occurs within the northeast side of the central mainstem Trinity River and on the northwest slope of the New River gorge. It is separated from all other subclades by two major river systems from the south and east. Here there was consistent genetic separation between Hawkins Creek and Bell Creek riparian corridors, and genetic samples adjacent to the Trinity River at Grays Falls and Salyer. Genetic subunits that made up the Hyampom subclade (Hyampom Cr., Corral Cr., Big Cr.) were somewhat intermingled. Although Corral Creek samples grouped together in every phylogram, there were no apparent major geographic or hydrological barriers to geneflow, a similar pattern of genetic variation was also observed in the Redwood sideband from the Hyampom and Corral creek areas.

<sup>14</sup> Bidden Creek along Highway 299 near the China Slide and Burnt Ranch should not be confused with a different Bidden Creek that is a tributary of Corral Creek between Big Bar and Hyampom in Trinity County, which was the population monitoring site for the Roth and Pressley (1986) study.

**Table 4.** Hypothesized new species clades and subclades based on results of the genetic analyses using BEAST consensus tree, posterior probability scores, and with cartooned (triangled) bGMYC scores to arrive at a hypothesized taxonomic classification and subclade designation based on Trinity County geography and riverine barriers to gene flow within each clade based on results of the present study.

Species clade	Posterior probability score (%)	bGMYC score	Taxon (species/subclade)
A-clade	1.00		Redwood sideband ( <i>Monadenia infumata</i> ) (= <i>M. i. subcarinata</i> )
	1.00	0.95	(a) Hyampom subclade: W-side central Trinity River, NE-side South Fork Trinity River, lower Hayfork Creek.
	1.00	0.95	(b) South Fork subclade: W-side South Fork Trinity River and South Fork Mountain
	1.00	0.95	(c) Swede Creek subclade: NE-side central Trinity River
B-clade	1.00		Yellow-based sideband ( <i>Monadenia ochromphalus</i> )
C-clade	0.94		Trinity bristle snail ( <i>Monadenia setosa</i> )
	1.00	0.95	(a) China slide subclade: Trinity Co., W-side central mainstem Trinity River, vicinity of China Slide
	1.00	0.95	(b) Dyer Creek subclade: Trinity Co., NE-side central mainstem Trinity River, NW-side New River gorge
	1.00	0.95	(c) Hyampom subclade: Trinity, W-side central mainstem Trinity River, N-side central mainstem South Fork Trinity River, area surrounding Hyampom, Corral, and Big creeks
	1.00	0.95	South Fork subclade: Trinity and Humboldt Cos., W-side mainstem South Fork Trinity River, Friday Ridge Rd., Olson Creek Watershed
	1.00	0.95	(d) Swede Creek subclade: Trinity Co., NE-side central mainstem Trinity River, Type Locality at Swede Creek.

The South Fork subclade appeared to show significant geographic variation between the: 1) westside of the mainstem South Fork Trinity River before draining into the lower Trinity River near Friday Ridge Road, Humboldt County; and 2) westside of the South Fork Trinity River. Although within the Ammon Creek watershed there were two samples that were immediately adjacent to the eastside of the South Fork Trinity River and one within the Olson Creek watershed between the South Fork Trinity River and Hayfork Creek. For the most part, this pattern of genetic variation appears related to restricted gene-flow combined with both isolation by geographic distance and riverine barriers. Notably, the two specimens



**Figure 10.** A) Reduced species phylogram derived from DNA sequence data reconstructed by use of the Bayesian Coalescent Tree using the program BEAST. Subclades in the distribution map are color coded to match samples in the phylogram. B) Map of the distribution of various subclades within the C-clade of the Trinity bristle snail (*M. setosa*) showing the comparative abundance of bristles and conspicuousness of bands for each subclade throughout the current range of the species. Paired bar graphs represent ranks (1 – 3) that each snail had for bristle abundance (non-yellow bars) or band conspicuousness (yellow bars); no snail had a 3:3 ranking of bristles to bands.

from Fourmile Creek (#51 and #52) found on the westside edge of the South Fork Trinity River were clearly differentiated genetically from the co-occurring Redwood sideband (#53). The Swede Creek subclade represents the type locality for the species. Samples were obtained from both Swede Creek and Little Swede Creek, within the Italian Creek watershed. These drainages represent two adjacent but separate streams at their headwaters prior to merging downstream as a minor tributary to the central mainstem of the Trinity River. There was no significant genetic separation between these samples that could be attributed to geography (Fig. 8 and Fig. 9).

Figure 10 illustrates the biogeographic relationships among subclades within the C-clade of the Trinity Bristle species complex in relation to: 1) the abundance of bristles; 2) conspicuousness of bands; 3) distribution of major river systems flowing from the southeast to the northwest; and 4) the DNA-derived phylogram generated from the Bayesian Coalescent tree. Within each subclade the genetic samples have rather uniform abundance and conspicuousness of bristles and bands, respectively as shown by individual bar graphs. Yet the proportion of abundance and conspicuousness of these two morphological variables become somewhat intermixed in the extreme northwest section of the geographic range, even though strong genetic separation is indicated by the phylogeny.

### Parsimony Character State Reconstructions

Parsimony character state reconstructions within the C-clade showed that the strongest phylogenetic signal was associated with: 1) adjacent riparian stream corridors, 2) watershed designations, and 3) the geographic location compared to a particular side of the Trinity River, South Fork of the Trinity River, and the New River gorge (Fig. 11A, 11B, and 11C). In most

all subclades, DNA samples tied genetically to their unique hydrologic and topographic landscape. Similarly, most subclades had their most divergent genetic samples associated with geographic separation between these major riverine systems, suggesting isolation and a resulting lack of gene flow among allopatric populations. This pattern appeared particularly evident for downriver mainstem segments of the Trinity River and South Fork Trinity River as opposed to less flow inundated upriver reaches of the South Fork Trinity and its lesser Hayfork Creek tributary.

In contrast, although comparative abundance of bristles, and to a lesser extent the conspicuousness of bands, provided some additional phylogenetic signal as most taxa in clades with < 95% probability of being a species or subspecies had shell exteriors consisting of distinct bristles and slightly conspicuous bands as adults (Fig. 12A and 11B). Presence and abundance of bristles on the surface of adult shells within the C-clade was found to be highly variable. This finding in no way negates the problem of sample size in the genetic analysis but needs to be reinforced with much larger samples of adult specimens throughout the range of the species, particularly if a multivariate morphometric assessment of adult snails is to be successful. However, within subclades of the Trinity bristle snail bristle abundance was found to provide little in the way of phylogenetic information.

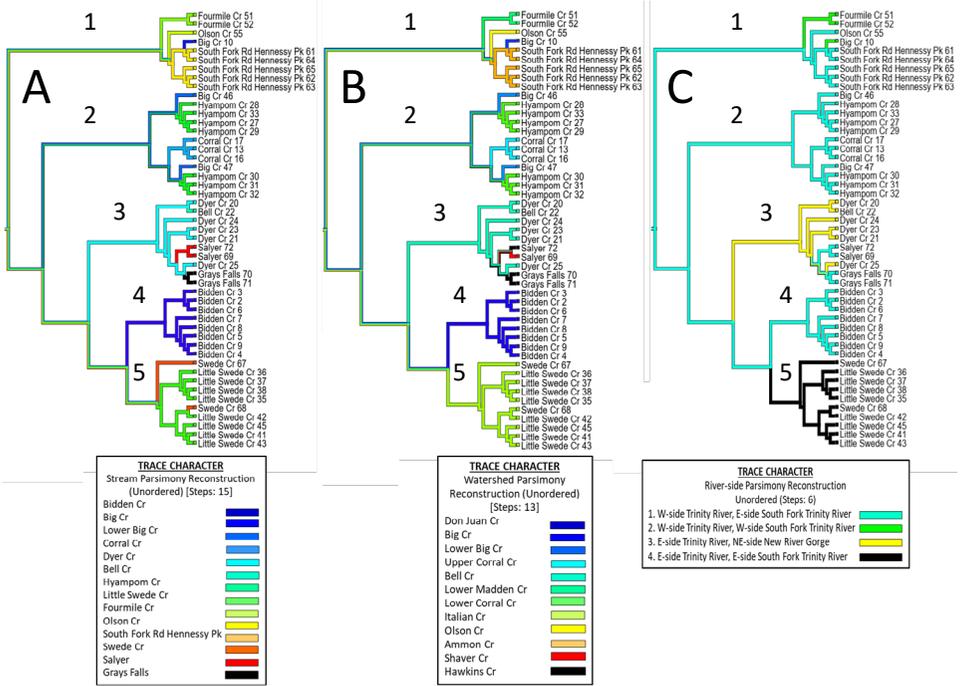
For example, although bristles were present in representatives of each subclade this was highly variable among individual snails sampled at the same location consistent with the analysis presented in Figure 10. Samples of adult Trinity bristle snails from the type locality (Swede Cr. subclade) characteristically had abundant and conspicuous bristles, whereas specimens from its sister subclade (China Slide subclade) downstream and across the central mainstem of the Trinity River had no bristles or were very sparsely bristled. Similarly, specimens from the Hyampom subclade had a high degree of samples with abundant bristles but a few snails were sparsely bristled. Within each subclade presence of sparse bristles may be a function of several factors: 1) age variation (size), 2) degree of wear to the shell surface, 3) degree of isolation, 4) genetic drift and founder effects, 5) local areas effects (selection), or 6) simply sampling error (Cain and Currey 1963; Davison and Clarke 2000; Millstein 2008, 2009).

As in bristle abundance, parsimony character state reconstructions for riparian stream corridors versus conspicuousness of banding on the surface of shells in adult specimens also appeared highly variable and provided little in the way of phylogenetic information when compared to the DNA analyses. Generally, traditional phenotypic descriptions of the banding patterns in a species are associated with large adult specimens in which presence, color, degree, and the patterning of bands are well developed, if bands are present at all. Similarly, the majority of large adult specimens with or without bristles examined here were dull in coloration, and even the largest bands around the body whorl at the edge of the shell were not well pronounced, wide, bright, or highly conspicuous. Instead, the quality of “conspicuousness” of bands was highly variable throughout the C-clade, with slightly conspicuous banding patterns being most common and highly conspicuous banding patterns uncommon.

## DISCUSSION

### Morphology

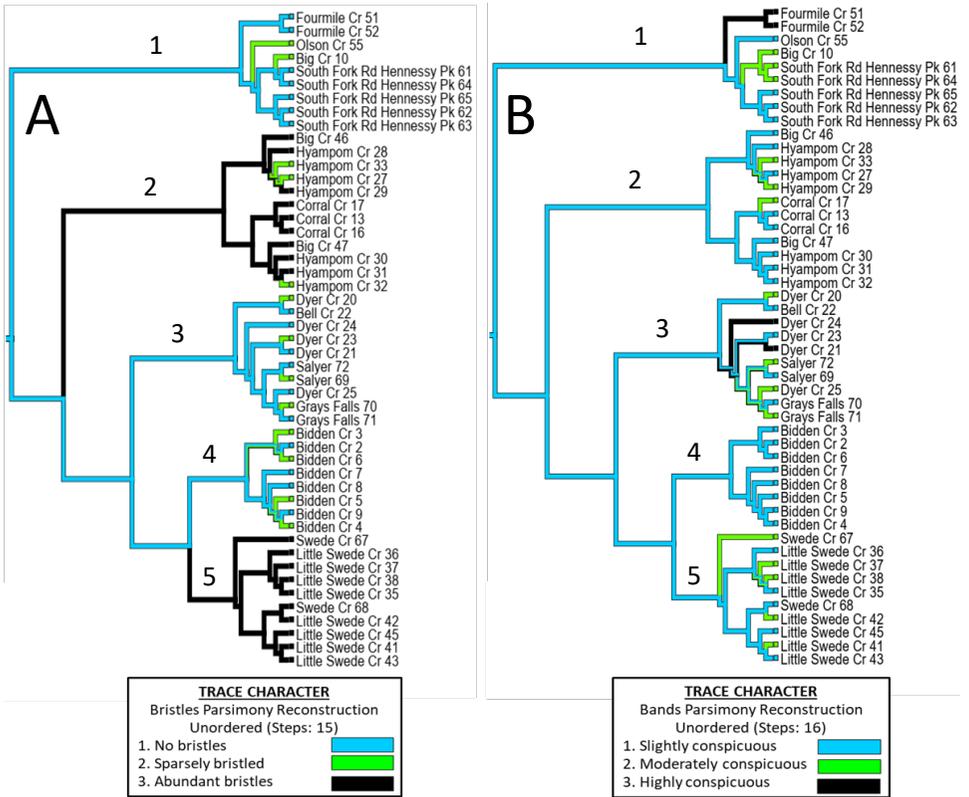
The Trinity bristle snail was originally described based on morphological criteria from specimens collected at the type locality along Swede Creek (40.793° N, 123.355°



**Figure 11.** Ranked hydrologic parsimony character state reconstructions applied to genetic samples used in the BEAST consensus tree: A) riparian stream corridor, B) watershed, and C) river-side. Subclades within the Trinity bristle snail (*M. setosa*) complex: 1 = South Fork subclade, 2 = Hyampom Creek subclade, 3 = Dyer Creek subclade, 4 = China Slide subclade, and 5 = Swede Creek subclade.

W), northeast of the Trinity River and adjacent to Highway 299, Trinity County, California (Talmadge 1952). At that time Swede Creek was the only locality for the species known to Talmadge, who did not specify where along its reach collections were made. Traditionally, differences between the Trinity bristle snail and other species within the genus *Monadenia* characteristically involved comparatively minor differences in shape, banding pattern, and proportion of the several organs of the reproductive system. For example, the shape of the mucus gland and its duct in the Trinity bristle snail appears more like that of the Redwood sideband than that of the Pacific sideband (Roth and Eng 1980). Conformation of such relationships by additional dissections and quantified morphological criteria would be consistent with results of the molecular data presented here and by Cordero and Lindberg (2002), which grouped the Trinity bristle snail into a clade with the Redwood sideband. Complicating the morphological scenario further was the observation that traditional taxa within the genus *Monadenia* (*M. setosa*, *M. infumata*, *M. fidelis subcarinata*, *M. f. klamathica*, and *M. f. ochrophalus*), which formerly appeared distinct based on the original morphological criteria, were found to be less than definitive based upon subsequent re-examination as a function of more extensive sampling (Roth 2002; Roth and Sadeghian 2006).

Evidence presented here shows that: 1) the Redwood sideband (A-clade) is the common ancestor of both the Yellow-based sideband and Trinity bristle snail; and 2) a sister-group



**Figure 12.** Ranked morphological parsimony character state reconstructions applied to genetic samples based on: A) presence or absence of bristles on the surface of the shell and B) conspicuous of banding on the surface of the shell. Subclades within the Trinity bristle snail (*M. setosa*) complex: 1 = South Fork subclade, 2 = Hyampom Creek subclade, 3 = Dyer Creek subclade, 4 = China Slide subclade, and 5 = Swede Creek subclade.

relationship exists between the Yellow-based sideband (B-clade) and the Trinity bristle snail lineage (C-clade). Yet the existing descriptive morphological criteria provides negligible insight into the geographic and evolutionary relationships among species, subclades, or subspecies. Discussions regarding use of shell morphology in phylogenetic analyses (Uit de Weerd et al. 2004; Smith and Hendricks 2013; Miller 2016), although informative at lower taxonomic resolutions, generally are not particularly useful in accurate delimitation of cryptic lineages. This is because of the high responsiveness of shell structure and banding pattern to environmental factors and similarity in local and regional adaptations in terrestrial gastropods (Goodfriend 1986; Fiorentino et al. 2008; Stankowski 2011; Cameron et al. 2013; Razkin et al. 2017).

As noted by Gladstone et al. (2019) molecular studies have recently led to the detection of many cryptic species complexes within morphologically ambiguous taxa that have previously been undescribed, the primary motivation for the Cordero and Lindberg (2002) study. Morphological assessment of taxa within the genus *Monadenia* would benefit greatly by use of genetic criteria, geometric morphometrics, and traditional morphometrics in combination with methodology such as permutational MANOVA for use in discriminate

function study designs. Such analyses are particularly important where researchers are unable to find characters that consistently differentiate one taxon from another (Anderson 2017). Organisms such as land snails are at a particularly higher risk of species misidentification and misinterpretation, in that gastropod systematics have traditionally been based almost entirely on external shell morphology (Gladstone et al. 2019).

For example, patterns of variation in shell morphology vary locally and among regions, and selection may be mediated by climate or predation (Cain and Currey 1963; Cook, 1998; Silvertown et al., 2011). Currently, there has been no published comprehensive univariate, multivariate, or cladistics analysis of shell or soft anatomy comparisons among traditional species within the genus *Monadenia*, or geographically differentiated subspecies within the genus. Also, there are no published companion keys to species, clades, subclades, or subspecies based on morphological criteria of any kind consistent with current taxonomic treatments, even though extensive new taxonomic classifications continue to be published. What is needed are genetic studies in conjunction with quantitative multivariate morphological analyses. These investigations should focus on all species and subspecies within the genus *Monadenia* as per the most recent taxonomy using a comparative approach combined with geographic information as part of the process of developing usable keys to taxa for the purpose of management and conservation.

### Sample Size and Distribution

Importantly, Cordero and Lindberg (2002) showed that their specimen #20 labeled as a Trinity bristle snail sampled at Hayfork Creek in southwest Trinity County did not group with: 1) their C-group consisting exclusively of the Redwood sideband except for one specimen (#19) labeled as a Trinity bristle snail from the type locality at Swede Creek; or 2) their D-group consisting exclusively of the Yellow-based sideband from Siskiyou County (Appendix I). Instead, their molecular data showed that the Trinity bristle snail from Hayfork Creek was the most divergent of all samples assayed in their “bristle clade” (B-group). However, no explanation was offered for this apparent “anomaly,” even though specimen #19 was assigned to their C-group along with all other samples of the Redwood sideband from Trinity and Humboldt counties.

Importantly, based on the DNA data and more extensive sampling presented here, the relationships depicted by the Cordero and Lindberg (2002) phylogram would appear to indicate sympatry between the Trinity bristle snail and the Redwood sideband at the type locality for the Trinity bristle snail (Appendix I). For example, their specimen sampled at Swede Creek may not have been a Trinity bristle snail, but a morphologically cryptic Redwood sideband as reflected in their phylogram. Given the very small geographic area covered and the indication that all four samples of the Redwood sideband were obtained from the same location in adjacent Humboldt County,<sup>15</sup> well within the range of this species in adjacent Trinity County, it is not surprising that the taxonomic boundaries of species, subspecies, and population-level taxonomy described in their account appeared “fuzzy” and largely unresolved.

<sup>15</sup> In the final report to the Roseburg Bureau of Land Management in Oregon, Cordero and Lindberg (2002) indicate that the two specimens of the Pacific sideband (= Redwood sideband); #43 and #44) were collected in Trinity County (T4N, R5E, S18 1/4SE). However, these coordinates indicate that Section 18 lies within Humboldt County, which would place these samples within the same cluster of rock pits (Lower Trinity River Rock Pits, Six Rivers National Forest) as all other samples of this taxon sampled from Humboldt County and that none of their samples were from Trinity County.

Inclusive with genetic and modern morphometric analyses are the effects of sample size and sampling distribution that are critical in evaluating the: 1) phylogenetic and biogeographic relationships among taxa, 2) taxonomic classifications, and 3) status and listing process required in the evaluation of rare and endemic species. As noted by Phillips et al. (2019), recommended sample sizes (5 – 10 individuals) currently in place likely are not sufficient because species abundance is often skewed geographically and ecologically. And sampling of  $\geq 25$  individuals may be necessary for some species exhibiting widespread distribution patterns within topographically diverse landscapes (Becker et al, 2011; Steinke and Hanner 2011). Noteworthy is the very limited comparative DNA sequence analysis of the genus *Monadenia* that resulted in the Trinity bristle snail taxonomically subsumed within the Redwood sideband lineage. This is because the analysis did not include a reassessment of Trinity bristle snails from throughout its known topographically and hydrologically diverse geographic range as only two specimens were assayed (Cordero and Lindberg 2002). Multiple samples of Trinity bristle snails at each location were not collected even though there was compelling evidence to suggest the possibility of the co-occurrence of morphologically cryptic species (Roth and Pressley 1986). Nevertheless, Cordero and Lindberg (2002) concluded that species in their “bristled” B-group correlated with the existence of setae on the shells at some time during ontogeny, which equated to five polytomous subclades (*infumata*, *setosa*, *subcarinata*, *klamathica*, and *ochromphalus*; Appendix I). Given the huge geographic area covered and the small sample sizes evaluated, it is not possible to assess any population genetic aspect of geographic variation within the B-group. And no quantitative or semi-quantitative distinction was made about the distribution, geographic variation, or abundance of setae on the shell (sparse, abundant, dense).

In contrast, results reported here suggest a pattern of area effects consisting of a general southeast-to-northwest distribution in the abundance of bristles in the direction of the prevailing flow of the mainstem Trinity River, South Fork of the Trinity River, and Hayfork Creek, which bisect this section of the Trinity Basin. Abundantly bristled individuals were generally found to the southeast whereas more sparsely bristled individuals were generally found in the northwest in relation to existing riverine corridors. The opposite trend was apparent in the conspicuousness of bands. These patterns appeared to reflect rather shallow clines among genetic samples differing in bristle and shell banding morph frequencies in both the Trinity bristle snail and the Redwood sideband. Yet the subclades in both species were abruptly differentiated by genetic, hydrologic, and geographic discontinuities. Importantly, parsimony character state reconstructions that superimposed bristle and band data onto the BEAST consensus tree clearly indicated that presence of bristles and conspicuousness of bands was widespread among genetic samples, but neither attribute appeared to function as a definitive character for any species or subclade that possessed them.

## Species and Topotype

Species are generally defined as populations that are reproductively isolated, or exclusive groups of organisms in which species boundaries in sympatry are maintained by intrinsic barriers to gene exchange (Harrison and Larson 2014). Unfortunately, morphological methods provide virtually no definitive quantitative insight as to the extent of gene flow, genetic intergradation (Mayr 1969), or the extent to which focal taxa differ phylogenetically from one another. The assumption that only small samples ( $\leq 2$ ) are needed from a “species population” to assess phylogenetic affinities, typical of modern genetic analyses

is an exaggeration prone to sampling error. This situation is particularly challenging where morphologically cryptic species co-occur and there is a lack definitive morphological characteristics to facilitate positive identification, particularly under field conditions. For example, traditionally a “topotype” in taxonomy is a specimen found at the type locality of a species to which it is thought to belong but is not necessarily of that type series. The concept of the topotype and the need to obtain adequate sample sizes for both genetic and quantitative morphological analyses is important and potentially problematic. Not only is this material required for reassessing the validity of traditionally named taxa, refinement of species descriptions with multiple datasets, and taxonomic classifications, but given that there may be syntopic morphologically cryptic species co-occurring at the type locality, not only is it important to sample from the topotype, but it is critical that samples are actually the species of interest.

### **Intergradation and Hybridization**

The South Fork clade is significant because it is an area (Don Juan Cr., Cedar Flat, and McDonald Cr. above Burnt Ranch) where Roth and Pressley (1986) found an unknown number of snails with a “*partially matte base and light scattering of bristles on the shell,*” which they interpreted as evidence of a distinct but narrow zone of secondary contact and hybridization between the Pacific sideband (= Redwood sideband) and the Trinity bristle snail. These authors also suggested that because these shell and bristle characteristics were similarly observed by USFS personnel at Clark, Hyampom, and Corral creeks, that this might represent a southern extension of the hybrid zone in and around the Burnt Ranch area. They further suggested that this “*narrow zone of apparent intergradation along the western margin of the range of the Trinity bristle snail indicates that genetic isolation from the Pacific sideband (= Redwood sideband) is not complete.*”

However, there are no published data in support of this hypothesis that would incorporate: 1) evidence of an exchange of genes between formerly allopatric species demonstrating they geographically reunited by natural introgressive hybridization, or 2) evidence of backcrossing with parental species at some time in the past or presently. Instead, the analysis of geographic variation in bristle abundance (and band conspicuousness) presented here found a predominately southeast-to-northwest decrease in bristle abundance coincidental with the direction of flow of the Trinity and South Fork Trinity Rivers, which was evident in both samples of the Trinity bristle snail and the Redwood sideband. This trend suggests a subtle “cline” in bristle abundance in the area described by Roth and Pressley (1986), where species populations likely are responding phenotypically to similar ecological conditions in syntopy. The historical and current literature is replete with quantitative evidence of examples of phenotypic responses to area effects in *Cepaea nemoralis* and other terrestrial gastropod species (Cain and Currey 1963; Jones et al. 1980; Cook, 1998; Davison and Clarke 2000; Özgo and Kinnison 2008; Silvertown et al. 2011). Similarly, DNA derived phylograms showed a lack of intermediate subunits intermingled within subclades of each species in all zones of contact (South Fork, Swede Cr., and Hyampom subclades). Instead, distinct genetic subunits tied to hydrologic and geographic discontinuities in the landscape were found in both species. Viewed collectively these data would appear to contradict the concept of genetic intergradation between the Trinity bristle snail and the Redwood sideband where they co-occur given the available evidence.

Minimal sampling detracts from the investigator's ability to address questions and estimates of the potential for gene flow and hybridization among taxa or relic populations. This situation applies to estimates of gene flow and assessment of the possibility of genetic hybrids between subclades of the Trinity bristle snail, and between this taxon and other large forest-dwelling gastropods that it may be sympatric with. Expanded analyses of nuclear genes and tracing of haplotypes would likely be required to document if hybridization has occurred between different taxa in the forms that lack the majority consensus bristle type or some other morphological criteria using both pure forms and potential intermediates in potential zones of contact. This requirement applies to estimates of gene flow and assessment of the possibility of genetic hybrids between taxa at any level among subclades of the Trinity bristle snail clade. Yet given that geographic sympatry and ecological syntopy between the Redwood sideband and several subclades within the Trinity bristle snail complex, including the type locality for the Trinity bristle snail, it would appear that these genetic units are conservatively at the subspecies level of differentiation, which in large measure is concordant with geographically disjunct riparian stream corridors and riverine systems, as shown in the BEAST coalescent tree (Fig. 7). Taken together, these data suggest further that the Trinity bristle snail is in fact a valid species as originally described by Talmadge (1952).

### Biogeographic Implications

Phylogenetic affinities among subclades within the Trinity bristle snail complex and closely related taxa appear largely concordant with mesic hydrological relationships superimposed onto the geological and topological structure of the central Trinity Basin. This observation reinforces the hypothesis that lack of gene flow between disconnected stream and river basins, in concert with topography and past (Pleistocene) geologic history, has contributed to allopatric patterns of differentiation in taxa and relictual populations of large terrestrial forest-dwelling gastropods in the greater Trinity Basin of northern California. Anecdotal to this hypothesis is the observation by Talmadge (1960) of the temporary occurrence of the Trinity bristle snails "*twenty miles downstream*" of Swede Creek where snails were presumably carried on flood flotsam drifting in the mainstem Trinity River, but this dispersal "propagule" apparently lasted only one year, as no other specimens were found or reported in follow-on surveys of the site (Roth 1978). This dispersal distance is within approximately 2 miles from the confluence of the Trinity River and the South Fork of the Trinity River near the town of Salyer and Grays Falls. According to Roth (1978), Talmadge also stated that river-born migrant Trinity bristle snails "*went ashore*" at Hawkins Bar on the flats of the floodplain of the Trinity River during the flood of 1964 but they did not persist past 1966. Hawkins Bar (SW-side of the Trinity River) is approximately 16.1 km (10 mi) by river from the confluence of the Trinity River with the South Fork Trinity River.

Similarly, although the samples from Grays Falls and Salyer were obtained close to the Trinity River, they were on opposite sides of the mainstem from upslope samples at Dyer Creek and Bell Creek. As indicated by the MrBayes maximum-credibility tree (Fig. 5A), the Dyer-Bell creek samples may have been the original genetic source (common ancestor) of the Grays Falls and Salyer (Trinity River) samples. Thus, this may be another example of downslope drift combined with cross-river colonization down river. The possibility that Trinity bristle snails and terrestrial gastropods in general are known to occasionally drift ashore downstream by way of riverine dispersal in currents of river systems suggests that dispersal and gene flow may be limited but ongoing periodically in conjunction with both

the northeast and southwest sides of the mainstems of the Trinity River, New River gorge, South Fork Trinity River, and to a lesser extent the Hayfork Creek drainage, which conveys far less volume of water compared to mainstem flows.

For example, given the accumulated effects of: 1) larger, deeper, and swifter flows, and 2) greater erosion of riverbanks and floodplains toward the mouth of rivers, each riverine system would appear to be significantly more effective as a physical barrier to over-water dispersal the further downstream compared to upstream and headwater riverscapes. Passive riverine dispersal seems far more probable in association with tributary streams of the Hyampom subclade at Hayfork Creek and Corral Creek, and the head waters of the South Fork Trinity River in combination with the lack of other strong topographic barriers. This possibility would suggest that riverine dispersal of terrestrial gastropods goes from greater to lesser probabilities along a southeast-to-northwest corridor across the central Trinity Basin into Humboldt County and beyond where the South Fork Trinity River merges with the Trinity River, and the Trinity River weds with the Klamath River at the township of Weitchpec. Strong spatial-genetic structure combined with poor dispersal capabilities suggest that: 1) down river drifting on rafts of vegetation; 2) natural landslides and massive slope failures, characteristic of the historic China Slide near the junction of the Trinity River and New River; and 3) human-mediated construction and re-distribution of rocky soils and associated leaf litter along Highway 299 between the towns of Weaverville and Willow Creek, likely facilitated dispersal by the Trinity bristle snail across river systems in the Trinity Basin historically and in recent times.

### Taxonomic Designations

*Option 1: current taxonomy.*—Based on results of the DNA sequence and qualitative morphological criteria two principal clades within the genus *Monadenia* were delineated (Cordero and Lindberg 2002; Roth and Sadeghian 2006). One clade was characterized morphologically by bristles on the surface of the shell at some time in the ontogeny of the species, which was designated as the Redwood sideband lineage consisting of three subspecies from eastern Humboldt, Siskiyou, and Trinity counties:

- Yellow-based sideband (*M. i. ochromphalus* (Berry 1937) – Counties: Del Norte, Siskiyou, and Humboldt, CA
- Trinity bristle snail (*M. i. setosa* (Talmadge 1952) – Counties: Trinity and Humboldt, CA
- Redwood sideband (*M. i. subcarinata* (Hemphill in Binney 1892) Counties: Humboldt, Trinity, CA

The second principal clade was characterized morphologically by not having bristles at any time in its ontogeny. Members of this clade were considered genetically diverse and likely contained at least two species, the most important of which was the Pacific sideband, which ranges from southeastern Alaska to southwestern Oregon and dips into California in Del Norte and likely Humboldt counties, and small sections of northwestern and north-central Siskiyou County (Roth and Sadeghian 2006). Several taxa formerly regarded as subspecies of the Pacific sideband or as separate species, were hypothesized to belong to the Redwood sideband (Roth and Sadeghian 2006). If the current taxonomy within the Trinity bristle snail C-clade is embraced, then species and their subclade designations delineated in the present study corresponds to the classification provided by Roth and Sadeghian (2006) based largely on genetic criteria. This taxonomic classification consists of 14 species and

20 subspecies of terrestrial sideband snails within the genus *Monadenia*, with the specific geographic range for each taxon delineated generally at the level of the county. There are no new morphological descriptions or keys to taxa in their checklist of land snails and slugs of California and adjacent areas in Oregon, as morphological descriptions reside within cited original descriptions sampled at the topotype. This reality includes subspecies contained within the Redwood sideband species-complex found within the greater Trinity Basin of northern California.

*Option 2: new taxonomy.*—Alternatively, the proposed new taxonomic classification includes 17 species and 24 subspecies and deviates significantly from the classification of Roth and Sadeghian (2006) in several respects (Table 5). First, the Trinity bristle snail and the Yellow-based sideband are retained as distinct species. Second, given that the Redwood sideband samples assayed were in fact the subspecies *M. i. subcarinata*, evidence provided here strongly justifies elevating this subspecies to species status as well. The traditional ranking of *M. i. subcarinata* as a subspecies of the Redwood sideband was made with respect to morphological attributes “typical” Redwood sideband specimens from farther south in California (Roth personal communication). Roth further suggested that based on the pattern of genetic divergence and speciation shown herein, that *subcarinata* is likely a “full” species. Third, genetic diversity was well-defined within disjunct subclades of the Redwood sideband (Clade-A) and the Trinity bristle snail (Clade-C), which merit subspecies status as indicated by: 1) high posterior probability and bGMYC scores delineated in the BEAST consensus tree (Fig. 8 and Fig. 9); 2) diversification within these genetic subunits concordant with geographic isolation and allopatric differentiation as a function of topographical and hydrological barriers to gene flow within the Trinity Basin; and 3) syntopic relationships among several subclades of both the Trinity bristle snail and the Redwood sideband with no apparent genetic intermingling as confirmed from the DNA phylograms. Subclade diversification within the Yellow-based sideband (Clade-B) does not warrant subspecific designation based on the sampling distribution in this study because DNA samples clustered together even though they were from opposite sides of the Klamath River near its headwaters.

The monotypic morphological condition for “bristles abundant” characteristic of the Swede Creek subclade and largely evident in the Hyampom subclade may simply be a result of genetic drift or founder effects (Cook 1998; Millstein 2008, 2009) with or without restricted gene flow between opposite sides of the central Trinity River, and which contributes to genetic patchiness on a small scale. Genetic subunits that made up the Hyampom subclade (Hyampom, Corral, Big creeks) were somewhat problematic because they were intermingled in the phylogram just like in the Redwood sideband. Although Corral Creek samples grouped or tended to group together in every phylogram all genetic units were grouped into one subspecies because they were not very divergent, and there were no major geographic barriers separating samples. Even though posterior probability and mtDNA diversity scores support splitting them up at least to the subspecies level, examination of their nuclear DNA would likely not support separating them into subspecies. So, for now genetic samples within the Hyampom subclade likely should be lumped together despite separate high posterior probability and bGMYC scores. The remaining subclades, however, all had 0.95 bGMYC as a probability score based on the BEAST consensus tree (Table 4) and were mostly allopatric in their geographic distribution in relation to topography and riverine barriers making it reasonable to designate them as separate and distinct subspecies.

**Table 5.** Proposed new taxonomic classification modified from Roth and Sadeghian (2006). Data and conclusions derived from the present study includes 17 species and 24 subspecies of sidebands within the genus *Monadenia*. Counties in California and Oregon are indicated under geographic distribution.

Species	Geographic distribution
<b>1. <i>Monadenia circumcarinata</i></b> (Stearns 1879)	Tuolumne
<b>2. <i>Monadenia mariposa</i></b> Smith 1957	Mariposa
<b>3. <i>Monadenia mormonum</i></b> (Pfeiffer 1857)	West-side Sierra Nevada
(a) <i>Monadenia m. buttoni</i> (Pilsbry 1900)	Calaveras
(b) <i>Monadenia m. cala</i> (Pilsbry 1900)	Calaveras
(c) <i>Monadenia m. hillebrandi</i> (Newcomb 1864)	Tuolumne
(d) <i>Monadenia m. hirsuta</i> (Pilsbry 1927)	Tuolumne
(e) <i>Monadenia m.</i> new subspecies “I”	Calaveras and Tuolumne
(f) <i>Monadenia m. loweana</i> Pilsbry 1927	Mariposa, Fresno
(g) <i>Monadenia m. mormonum</i> (Pfeiffer 1857)	Tehama, Placer, El Dorado, Sacramento, Amador
<b>4. <i>Monadenia tuolumneana</i></b> (Berry 1955)	Tuolumne
<b>5. <i>Monadenia yosemitensis</i></b> (Lowe 1916)	Mariposa, Madera, Fresno
<b>6. <i>Monadenia callipeplus</i></b> (Berry 1940)	Siskiyou
<b>7. <i>Monadenia chaceana</i></b> (Berry 1940)	Southern OR to northern CA
<b>8. <i>Monadenia cristulata</i></b> Berry 1940	Siskiyou
<b>9. <i>Monadenia fidelis</i></b> (Gray 1834)	Pacific coast from SE Alaska to northern CA, west of the crest of Cascade Range
(a) <i>Monadenia f. flava</i> (Hemphill in Binney 1892)	Siskiyou and southwest OR
(b) <i>Monadenia f. leonina</i> Berry 1937	Siskiyou and southern OR
(c) <i>Monadenia f. pronotis</i> Berry 1931	Del Norte
(d) <i>Monadenia f. smithiana</i> Berry 1940	Del Norte
<b>10. <i>Monadenia infumata</i></b> (Gould 1855)	Northern to central CA
(a) <i>Monadenia i. callidina</i> Berry 1940	Del Norte and Humboldt
(b) <i>Monadenia i. infumata</i> (Gould 1855)	Del Norte, Humboldt, Mendocino, Sonoma, Napa, Marin, Contra Costa, Alameda
(c) <i>Monadenia i. trinidadensis</i> Talmadge 1947	Humboldt
<b>11. <i>Monadenia marmarotis</i></b> Berry 1940	Siskiyou and Trinity
<b>12. <i>Monadenia ochromphalus</i></b> Berry 1937	Northern to central CA
<b>13. <i>Monadenia setosa</i></b> Talmadge 1952	Trinity County, eastern Humboldt County
(c) <i>Monadenia s.</i> new subspecies “China”	Trinity, W-side central Trinity River, vicinity of China landslide
(b) <i>Monadenia s.</i> new subspecies “dyer”	Trinity, NE-side central Trinity River, NW-side New River
(e) <i>Monadenia s.</i> new subspecies “hyampom”	Trinity, W-side central Trinity River, N-side central South Fork Trinity River

Table 5. continued

Species	Geographic distribution
(a) <i>Monadenia</i> s. new subspecies “setosa”	Trinity, NE-side central Trinity River, Type Locality at Swede Creek.
(d) <i>Monadenia</i> s. new subspecies “southfork”	Trinity, Humboldt, W-side South Fork Trinity River, Friday Ridge Rd.
<b>14. <i>Monadenia subcarinata</i></b> (Hemphill in Binney 1892)	Northern to central CA
(a) <i>Monadenia</i> s. new subspecies “hyampom”	Trinity, west-central Trinity county and between the central Trinity River, vicinity of Hayfork Creek, headwaters of South Fork Trinity River, and the eastern slope of South Fork Mountain
(b) <i>Monadenia</i> s. new subspecies “southfork”	Humboldt County, west-side of the South Fork Trinity River at Big Creek and Fourmile Creek
(c) <i>Monadenia</i> s. new subspecies “swede”	Trinity, northeast-side of the central Trinity River at Swede Creek and Little Swede Creek
<b>15. <i>Monadenia</i></b> , new species “m”	Del Norte
<b>16. <i>Monadenia churchi</i></b> Hanna and Smith 1933	Siskiyou, Trinity, Shasta, Tehama, Mendocino, Glenn, Lake
<b>17. <i>Monadenia troglodytes</i></b> Hanna and Smith 1933	Shasta
(a) <i>Monadenia t. troglodytes</i> Hanna and Smith 1933	Shasta, Late Pleistocene – Early Holocene
(b) <i>Monadenia t. wintu</i> Roth 1981	Shasta

## Conclusions

In general, taxonomy, distribution, critical microhabitat requirements, and habitat-area relationships of endemic populations of terrestrial gastropods are poorly known within the California-Cascades Province. This phenomenon is directly associated with isolation due to topographic and physiographic<sup>16</sup> diversity in the landscape in combination with low vagility, small, fragmented populations, and restricted gene flow. These environmental attributes function to facilitate allopatric differentiation leading to elevated levels of species diversity, as well as extinction. There are ~117 special status species of gastropods on the CDFW 2020 Special Animals List (CDFW 2020). Of these, ~ 66.7% are terrestrial snails. Surprisingly, the only saltwater, freshwater, or terrestrial species listed (threatened or endangered) under CESA is the Trinity bristle snail, even though very little is known about the distribution, systematics and ecology, life-history, and genetics of other rare and endemic species in northern California. Currently, the only other endemic terrestrial snail listed in California is the Morro shoulderband or Banded dune snail (*Helminthoglypta walkeriana*), which is listed as Endangered under the ESA (USFWS 1973). Herein, this study provides: 1) a proactive quantitative approach to species and subspecies assessment based on scientifically credible genetic, systematic, biogeographic, and taxonomic information aimed at facilitating effective management and conservation of State-listed species under CESA; and 2) a template for addressing future taxonomic assessments and status reviews

<sup>16</sup> A distinct type of landscape, landform, geology, and evolutionary history.

of special status species of other terrestrial gastropods as part of CDFW's commitment to the California Biodiversity Initiative.

As noted by Barnosky et al. (2011), the ongoing rapid decline in biodiversity has been called the sixth mass extinction, and the need to reverse this trend has never been more important. Globally, terrestrial gastropods are one of the most diverse groups of land animals (Lydeard et al. 2004) ever to occupy the Planet, with ~24,000 described species inhabiting a large range of habitats (Nicolai and Ansart 2017). Of these 1,105 species worldwide are on the IUCN red list as extinct, critically endangered, endangered, or vulnerable ([www.iucnredlist.org](http://www.iucnredlist.org); Nicolai and Ansart 2017). Mollusks as a group have suffered the highest number of extinctions of any other taxonomic assemblage. Forty-two percent ( $n = 693$ ) of recorded extinctions of animal species since 1500 were mollusks; 99% of which included both terrestrial and freshwater species (Seddon 1998; Lydeard et al. 2004). Many species of mollusks listed by the IUCN are in the "Data Deficient" category (IUCN 2001). This means that information is either absent or lacking regarding the current geographic range, rate of population decline, degree of threat, or that current critical habitat is unknown (Dunk et al. 2004). The Trinity bristle snail is no exception, as are most other taxa on the Special Animal List for CDFW. Continued lack of priority planning at delineating biological diversity within terrestrial mollusks limits funding and our ability to gauge the extent of degradation to biological and ecological ecosystems throughout the State.

Terrestrial gastropods as a group play an integral role in facilitating natural ecosystem function. They aid in decomposition, nutrient cycling and soil-building of forest, woodland, and grassland vegetation through foraging (Mason 1970a and 1970b; Jennings and Barkham 1979; Richter 1979; Nystrand and Granstrom 1997; Prather et al. 2013). And most species are major components in mulch and litter communities essential in maintaining soil fertility (Swift et al. 1979). It was estimated that terrestrial mollusks active on the floor of northern boreal forests accounted > 6% of the recycled energy in those forests (Hawkins et al. 1977; Dunk et al. 2004). Terrestrial gastropods provide food and essential nutrients to other wildlife (South 1980; Churchfield 1984; Frest and Johannes 1995; Sullivan 1995; Martin 2000; Nyffeler and Symondson 2001) and contribute greatly to plant community structure and diversity (Hulme 1996; Peters 2007). Many terrestrial gastropods are intermediate hosts of terrestrial parasites and some function as vectors for pathogens (Ball et al. 2001, Barker 2002; Cowie 2011; Rowley et al. 1987; Graeff-Teixeira 2007). Agriculturally, many species are serious pests that lead to crop damage and the spread of pesticide residue, while negatively impacting native habitats and endemic biodiversity.

Notably, both aquatic and terrestrial gastropods are potentially valuable indicators of climate change but the physiological responses in this group to changing climate are not well understood. With climate change terrestrial ectotherms are expected to be especially vulnerable to changes in temperature and regional water regimes owing to extreme weather events, particularly in temperate regions (Nicolai and Ansart 2017). Understanding the effect of climate change on soil fertility in forest, woodland, and grassland ecosystems of northern California in relation to the role played by communities of terrestrial gastropods is a preamble to safeguarding soil function (Swift et al. 1979). This need is particularly relevant given that as a group terrestrial gastropods constitute ~ 66.7% of all taxa on the California Special Animals List, a point that needs to be continually emphasized to resource agencies mandated with the responsibility of managing regional biodiversity consistent with the California's Biodiversity Initiative.

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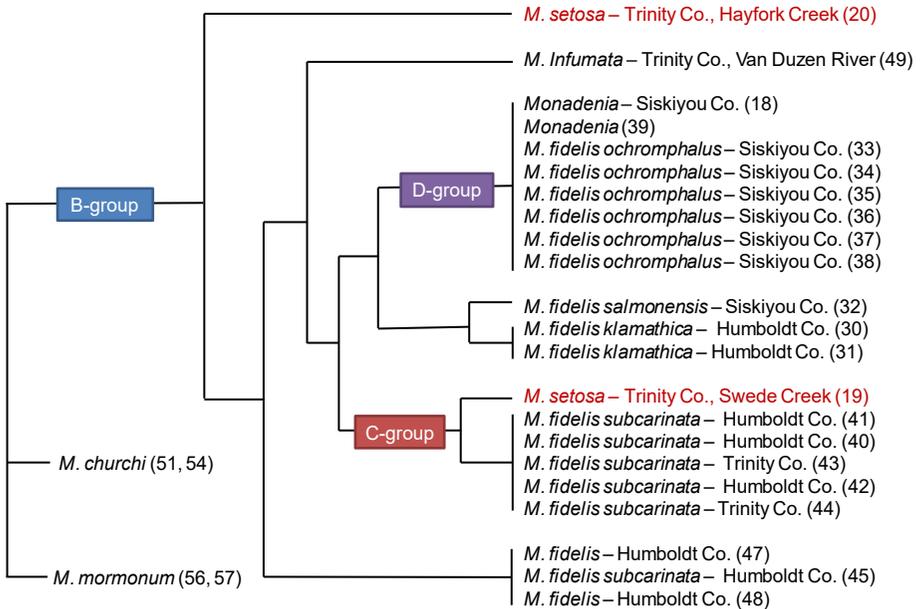
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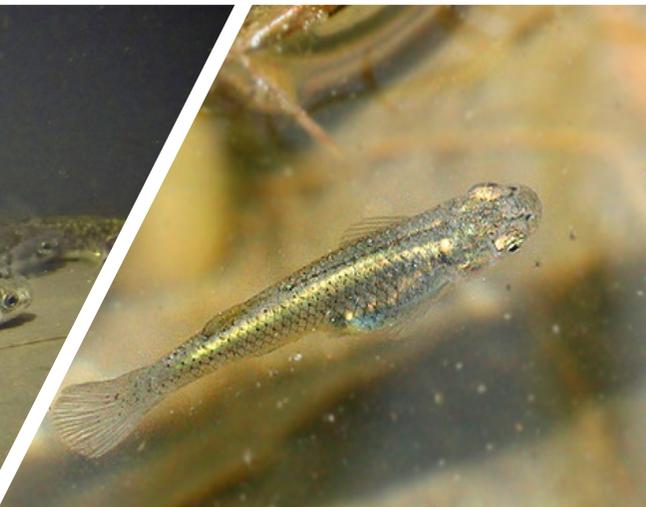
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**Appendix I.** Reconstruction of the Cordero and Lindberg (2002) phylogram showing hypothesized relationships among species and clades within the genus *Monadenia* that included samples from various counties in northern California (Siskiyou, Humboldt, and Trinity). Numbers in parentheses identify individual samples. Their Group B denotes the “*bristled clade in which bristles on the shells were present some time during ontogeny*”. Their C-group consists exclusively of *M. f. subcarinata* except for the one specimen (#19) labeled as *M. setosa* from the type locality at Swede Creek. Their D-group consists exclusively of *M. f. ochromphalus* from Siskiyou County. The species name *M. fidelis* (Pacific sideband) used in their phylogram has been changed to *M. infumata* (Redwood sideband; Roth and Sadeghian 2006). This reconstruction corresponds to their Attachment 4 (*Monadenia* Trees, D. Phylogram [Tree 33] 16S+COI, mtDNA).



# Fish



# Fish

## Photos, previous page:

Photo captions and credits from left to right, top to bottom

1	2
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5	6

1. Chinook salmon (*Oncorhynchus tshawytscha*).  
Photo Credit: Pacific Northwest National Laboratory, CC BY-NC-SA 2.0
2. Coho salmon (*Oncorhynchus kisutch*).  
Photo Credit: Mike Dean, CDFW
3. Razorback sucker (*Xyrauchen texanus*).  
Photo Credit: Bureau of Reclamation, CC BY-SA 2.0
4. Unarmored threespine stickleback (*Gasterosteus aculeatus williamsoni*).  
Photo Credit: Alexandre Roux, CC BY-NC-ND 2.0
5. Lost River sucker (*Deltistes luxatus*).  
Photo Credit: U.S. Fish & Wildlife Service, Fish and Aquatic Conservation, CC PDM 1.0
6. Desert pupfish (*Cyprinodon macularius*).  
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## REVIEW PAPER

## The value of long-term monitoring of the San Francisco Estuary for Delta Smelt and Longfin Smelt

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**Key words:** California Endangered Species Act, Delta Smelt, Endangered Species Act, *Hypomesus transpacificus*, Longfin Smelt, long term monitoring, Sacramento-San Joaquin Delta, San Francisco Estuary, *Spirinchus thaleichthys*

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Long-term ecological studies are an important tool for understanding ecosystem change over time and can be valuable for distinguishing short-term fluctuations from long-term population trajectories (Wolfe et al. 1987; Bograd et al. 2003; Likens 2012; Lindenmayer et al. 2012; Hughes et al. 2017). Such studies are imperative to understanding the causes of population decline, such as habitat loss, harvest, invasive species, pollution, and both natural and human-caused environmental change (Kimmerer et al. 2001; Kimmerer 2002; Sommer et al. 2007; Moyle et al. 2016). In this way, long-term ecological studies are critical for providing scientific data to assess population status and make evidence-based decisions to protect and recover imperiled species. Long-term ecological studies are rare due to the logistical challenges in maintaining survey consistency, changing or lack of legal mandates, high maintenance and operational costs, and political will to continue when populations continue to decline. However, listings of species as threatened or endangered under the California Endangered Species Act (CESA) and federal Endangered Species Act (ESA) can result in legal mandates to maintain, improve, or develop long-term monitoring studies to better understand how species respond to management activities. Here we provide a historical perspective of long-term fish monitoring studies conducted in the San Francisco Estuary, their evolution in response to data and management needs, how their valuable datasets have identified multiple periods of ecosystem regime change, and their role in protecting two native osmerids, Delta Smelt (*Hypomesus transpacificus*) and Longfin Smelt (*Spirinchus thaleichthys*). Prior reviews of long-term monitoring efforts in the San Francisco Estuary have focused on individual survey effectiveness, overall management priorities, and how to improve specific survey elements (Honey et al. 2004).

## THE SAN FRANCISCO ESTUARY

The San Francisco Estuary (Estuary) is the largest estuary in the western United States. The Estuary's watershed extends from the Sierra Nevada mountains to the Golden Gate Bridge and drains an area that includes almost 40% of California<sup>1</sup>. Water primarily enters the Estuary through two major rivers, the Sacramento and San Joaquin, and their tributaries (Fig. 1). These rivers converge and drain to San Francisco Bay. The area upstream of this convergence is typically composed of fresh or slightly brackish water and is referred to as the Sacramento-San Joaquin Delta<sup>2</sup> (Delta). Over a century ago, land management practices fundamentally changed the landscape of the Delta (SFEI-ASC 2014). The historic Delta was an expansive wetland that provided diverse and dynamic habitat types. The Delta today consists of deep channels, diked wetlands for agriculture, and levees for flood protection (SFEI-ASC 2014). Downstream of the convergence of the Sacramento and San Joaquin Rivers water enters the Suisun region, which provides low salinity and marsh habitat. Salinity in the Suisun region and the Delta tends to increase in the late summer and fall and, to a greater extent, during periods of drought. Downstream of the Suisun region, the salinity continues to increase as the water moves through San Pablo Bay and Central Bay, which connects to the Pacific Ocean. The South Bay is a shallow lobe that extends from Central Bay and receives infrequent episodic freshwater inflow from local tributaries.

The timing and amount of freshwater flow into the Estuary has substantially changed as a result of providing water for urban use to over 27-million Californians<sup>3</sup> and irrigation for a \$50-billion-dollar agricultural industry<sup>4</sup>. Inflow to the Estuary is managed by a system of upstream reservoirs and water diversions along the rivers and within the Delta. These water conveyance systems are operated by the U.S. Bureau of Reclamation's (USBR) Central Valley Project (CVP) and the California Department of Water Resources' (DWR) State Water Project (SWP). Unimpaired flow is the inflow that would pass through the Estuary without these upstream dams and diversions and it can be drastically different from the actual inflow that reaches the Delta (SWRCB 2010). Outflow is the amount of water that passes through the Estuary and into San Francisco Bay. Most of the difference between inflow and outflow is due to two large CVP and SWP water facilities located in the southern end of the Delta, the Bill Jones Pumping Plant and the Harvey O. Banks Pumping Plant, which export a sizeable proportion of the water entering the Delta (Figs. 1 and 2). Since 1987, the volume of water exported through these facilities has ranged from 3.7–7.4 billion m<sup>3</sup> (3–6-million acre-feet) per year, which at times has been as much as 50% of Delta inflow (Fig. 2c; Cloern and Jassby 2012; Hutton et al. 2017a,b). The volume of water exported by the facilities is often sufficient to reverse the net flow of the Old and Middle rivers (Fig. 1). When this occurs, fish are entrained in this backwards flow, transporting them into the pumping facilities (Arthur et al. 1996; Kimmerer 2008; Grimaldo et al. 2009; Smith et al. 2020).

### THE BEGINNING: MONITORING STRIPED BASS IN A CHANGING ESTUARY (1940–1970)

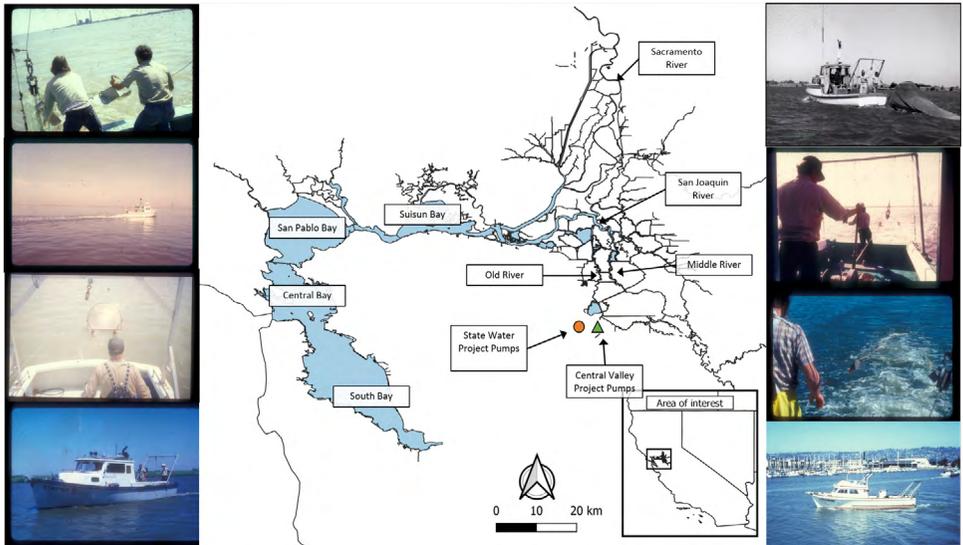
The Estuary is home to a plethora of resident and anadromous fish species and has supported lucrative commercial and sport fishing industries. The CVP's Bill Jones Pumping

<sup>1</sup> <https://www.sfestuary.org/our-estuary/about-the-estuary/>

<sup>2</sup> <https://data.cnra.ca.gov/dataset/legal-delta-boundary>

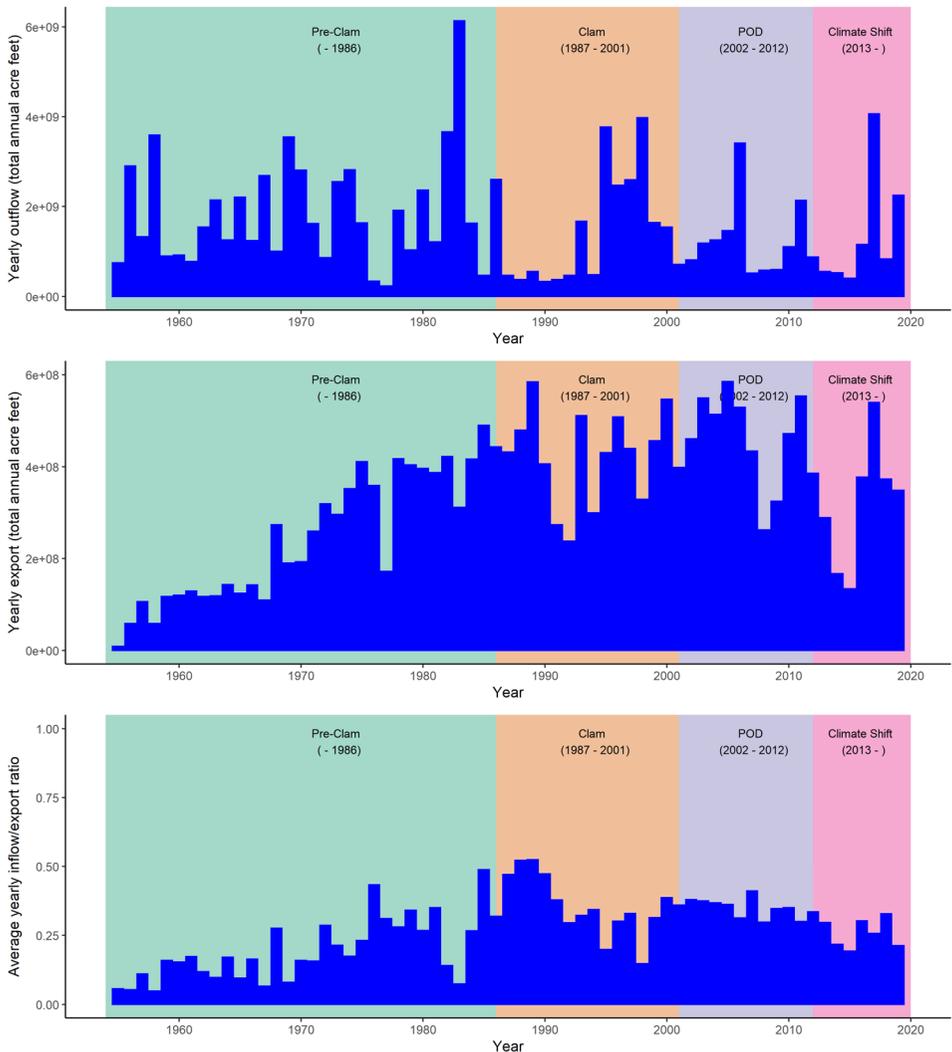
<sup>3</sup> <https://water.ca.gov/Programs/State-Water-Project>

<sup>4</sup> <https://www.cdffa.ca.gov/statistics/>



**Figure 1.** Map of the San Francisco Estuary, the study area encompassed by the long-term surveys, with State Water Project and Central Valley Project pumping stations denoted.

Plant was constructed from 1947–1951, and in 1950 the United States Fish and Wildlife Service (USFWS) released a report exploring the potential effects of the CVP on fishery resources in the Estuary (Erkkila et al. 1950). This report estimated that in the years prior to the CVP, the annual commercial catch of Chinook Salmon (*Oncorhynchus tshawytscha*) stemming from the Estuary was around 2,540,117 kg (5,600,000 lbs). In the 1940s, the California Striped Bass (*Morone saxatilis*) sport fishery produced an estimated annual catch of around 6,000,000 pounds, most of which originated in Delta waters and tributaries. The report concluded that operation of the CVP would have detrimental effects on both Chinook Salmon and Striped Bass populations. This report noted that Chinook Salmon entered the Delta from upstream spawning areas and were seasonally dominant from February to May, while Striped Bass spawned in the Delta in the spring and the largest concentration of eggs were collected in the San Joaquin River, Old River, and Middle River, highlighting the historic importance of the central and south Delta as a Striped Bass nursery. Striped Bass eggs are pelagic, so both eggs and larvae are particularly vulnerable to flow. Juvenile Striped Bass were seasonally dominant from June through September, aligning with the projected period of peak CVP demands. Recommendations were made to divert fish from the intake into a collection facility, and to conduct additional studies to better understand the ecology of the system, the effects of an altered environment, and how to protect fish (Erkkila et al. 1950). In this vein, the Bill Jones Pumping Plant was equipped with a louvre system to direct fish into the Tracy Fish Collection Facility, where fish are counted, placed in transport trucks, and returned to the Delta. The louvres rely on behavioral changes in fish swimming to direct fish into the fish facility, therefore passive organisms such as eggs and larval fish generally move past the louvre system uncounted, along with a fraction of juveniles and adults of some species (Brown et al. 1996; Morinaka 2013). The Tracy Fish Collection Facility has been salvaging fish since 1957 and is considered the oldest long-term monitoring program in the Estuary.



**Figure 2.** Daily outflow and export (acre feet/second) within the Delta region. Daily estimates from the Department of Water Resources Dayflow (<https://water.ca.gov/Programs/Environmental-Services/Compliance-Monitoring-And-Assessment/Dayflow-Data>).

Further development of the water conveyance system continued through the 1950s and 1960s. During this time, DWR supported an unmandated contract with the California Department of Fish and Game (CDFG, now the California Department of Fish and Wildlife, CDFW) to monitor and develop information needed to support water project construction (Herrgesell 2012). One such study, the Summer Towntnet Survey (STN)<sup>5</sup>, was initiated in 1959 to sample juvenile life stages of pelagic fish from June through August and provide an index of Striped Bass recruitment. The water bond passed the legislature in 1961, which led to

<sup>5</sup> <https://wildlife.ca.gov/Conservation/Delta/Towntnet-Survey>

contracted joint studies between CDFW and DWR. The objectives of these studies were to investigate the effects of the Delta Water Project on fish and wildlife resources, and to make recommendations on project plans and means of compensation for fish losses. Importantly, the plans called for coordination with other interested Federal, State, and local agencies. This partnership, and funding for monitoring, became further cemented in legislation with the 1963 Davis-Dolwig Act, which mandated close coordination between DWR, CDFW, and other appropriate agencies for the preservation and enhancement of fish and wildlife (Herrgesell 2012). This laid the groundwork for the 1967 initiation of the Fall Midwater Trawl Survey (FMWT)<sup>6</sup>, which samples sub-adult life stages of pelagic fish from September through December, and the Egg and Larval Survey, which was used to describe patterns in Striped Bass hatching and rearing. The STN, FMWT, and Egg and Larval Survey conducted pelagic trawls that primarily focused on the legal Delta and downstream into San Pablo Bay (Fig. 3; Turner and Chadwick 1972; Chadwick et al. 1977; Stevens et al. 1985). Since their inception, these surveys have collected data on all fish species collected, in line with recommendations to better understand the ecosystem. The Egg and Larval Survey ceased in 1994, but the STN and FMWT continue to this day. Over time, these early coordination efforts gave rise to the legislative basis and formation of the Interagency Ecological Program (IEP), a multi-agency consortium that to this day continues to provide, integrate, and analyze data pertaining to the Estuary and the water that flows through it. Construction of a second large pumping facility in the southern Delta, the SWP's Harvey O. Banks Pumping Plant, was completed in 1969 and upgraded to increase capacity in 1987. Similar to operations at the CVP, a louvre system is used to direct fish from the intake to the Skinner Delta Fish Protective Facility, which has been salvaging fish since 1968. CDFW works with USBR and DWR to conduct sampling of entrained fish at these facilities and provide loss estimates<sup>7</sup>. The fish facilities have undergone numerous changes over the years, many to improve the salvage of listed salmonids. Salvaged fish have been routinely identified to species since July 1992 (Brown et al. 1996; Morinaka 2013). Delta Smelt salvage data was collected prior to July 1992 but is considered less reliable than data collected after this date (IEP MAST 2015).

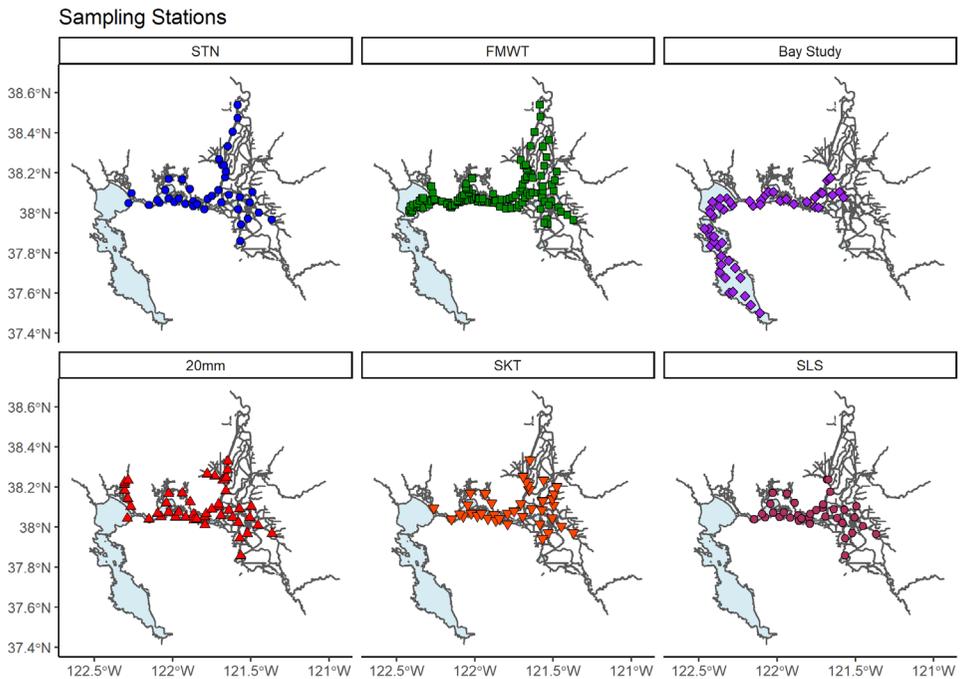
### **DECLINES, DROUGHTS, AND LEGISLATION: SHIFTING FOCUS TO DELTA SMELT AND LONGFIN SMELT (1970–1990)**

The California Endangered Species Act (CESA) was signed into law in 1970, and the federal Endangered Species Act (ESA) was signed into law in 1973. These laws provide legal backing to protect and recover species at risk of extinction. In the Estuary, these laws set the legal framework for protecting species in decline, independent of their economic value. This framework slowly started to shift the analytic goals of long terms monitoring datasets in the Estuary. The fact that the STN and FMWT collected data on all species encountered was critical to the continuation of these programs as management needs changed. In 1980, the San Francisco Bay Study (BS)<sup>8</sup> was initiated with the thought that reduced-outflow effects could be occurring downstream of the Delta and influencing organism populations in ways beyond direct entrainment. The BS conducts monthly surveys year-round to facilitate our understanding of the effects of reduced freshwater flow on pelagic and demersal fish and

<sup>6</sup> <https://wildlife.ca.gov/Conservation/Delta/Fall-Midwater-Trawl>

<sup>7</sup> <https://wildlife.ca.gov/Conservation/Delta/Salvage-Monitoring>

<sup>8</sup> <https://www.wildlife.ca.gov/Conservation/Delta/Bay-Study>



**Figure 3.** The six long-term surveys detailing sampling stations in the San Francisco Estuary.

mobile crustaceans. This survey extends through the Delta and downstream into San Pablo Bay, Central Bay, and South Bay. The BS currently uses two sampling gears: a demersal otter trawl (OT) and pelagic midwater trawl (MWT) to target different habitats and generate catch and associated environmental data to evaluate population changes based on outflow, which is used to inform water board decisions. Data from the STN, FMWT, and BS monitoring programs are used to produce annual indices of relative abundance for multiple species (Table 1). These calculated abundance indices were not designed to produce population estimates, but they do provide a repeatable, consistent measure of population trends over time (USFWS 1993). The resulting trends in the abundance indices highlighted the precipitous decline of two native fish species: Delta Smelt and Longfin Smelt; (Fig. 4a-c, Fig. 5a-c).

Delta Smelt were historically one of the most common pelagic species in the upper San Francisco Estuary (Erkkila et al. 1950). They are small (<100 mm) euryhaline pelagic fish that are endemic to the upper Estuary and were first described in 1963 (McAllister 1963). Delta Smelt are primarily an annual species, although some individuals live a second year (Moyle et al. 1992). Historically, Longfin Smelt were even more abundant and more widely distributed throughout the Estuary (Rosenfield and Baxter 2007). Longfin Smelt are slightly larger (<150 mm) than Delta Smelt, live two to three years, and utilize a wider range of salinity with a distribution from the San Francisco Estuary to Alaska (Moyle 2002). The abundance of Delta Smelt in the STN and FMWT precipitously declined in 1979 and 1981 respectively (Fig. 4a-b; Sweetnam and Stevens 1993; USFWS 1993). This coincides with an unusually high salvage event of Delta Smelt adults and juveniles in 1981 (IEP MAST 2015). Longfin Smelt exhibited strong year-classes in 1967 and 1969 before declining to

**Table 1.** CESA species targeted by the CDFW monitoring programs and associated index equations. All project data is available through the CDFW Region 3 webpage: <https://wildlife.ca.gov/Regions/3>.

Monitoring Program	Target Listed Species and Life Stage	Index Calculated for Other Species	Survey Period	Season	Mean±StDev Fork Length of Listed Species	Index Calculation	Notes
Summer Townet	Juvenile and Adult Delta Smelt	Age-0 Striped Bass	1959-Present	June-August	31±7 mm	$SI_k = \sum_j n_j * W_j / 1000$	The STN Delta Smelt Annual Index is the average value of the first two survey indices (SI).
Fall Midwater Trawl	Adult Delta Smelt & Juvenile and Adult Longfin Smelt	American Shad, Threadfin Shad, Age-0 Striped Bass	1967-Present	September-December	Delta Smelt: 60±9 mm Longfin Smelt: 66±19 mm	$SI_k = \sum_j \{ (n_j / S_j) * W_j \}$	The annual FMWT abundance index is the sum of the four monthly survey indices (SI <sub>k</sub> ) of each year (FMWT). Each monthly survey index is the sum of 14 separate area (a) indices.
San Francisco Bay Study	Juvenile and Adult Delta Smelt and Longfin Smelt	37 fish, 5 crab, and 7 shrimp species	1980-Present	Year-round	Delta Smelt: 52±9 mm Longfin Smelt: 49±7 mm	$SI_k = Avg(sn_{j,b}) * W_b$	The annual BS MWT and OT abundance indices are the average of monthly indices (Delta Smelt: June-October; Longfin Smelt: May-October). Each monthly index is the sum of five region indices (SI <sub>k</sub> ).

Table 1. continued

Monitoring Program	Target Listed Species and Life Stage	Index Calculated for Other Species	Survey Period	Season	Mean±StDev Fork Length of Listed Species	Index Calculation	Notes
20-mm Survey	Post larval-Juvenile Delta Smelt	None	1995-Present	March-July	21±10 mm	$SI_k = (10^{(\log(\log(\log_{10} sn_{j,a} + 1) / \log(10)))}) - 1$	The annual 20-mm abundance index is the sum of four survey indices ( $SI_k$ ): the two surveys before and the two surveys after young-of-the-year Delta Smelt (<60mm) reach an average length of 20mm.
Spring Kodiak Trawl	Adult Delta Smelt	None	2002-Present	January-May	65±8 mm	$SI_k = \sum (sn_{j,a} / S_a)$	The annual SKT index is the sum of the January through April survey indices ( $SI_k$ ). Each monthly index is the sum of three separate area (a) indices.
Smelt Larva Survey	Larval Longfin Smelt	None	2009-Present	January-March	7±2 mm	None	

Where:  $SI_k$  = Survey Index of survey  $k$ .

$n_j$  = Number of DS collected at Station  $j$ .

$W_j$  = STN volumetric weighting factor for Station  $j$ .

$n_a$  = Number of Smelt collected at Station  $j$  within Area  $a$ .

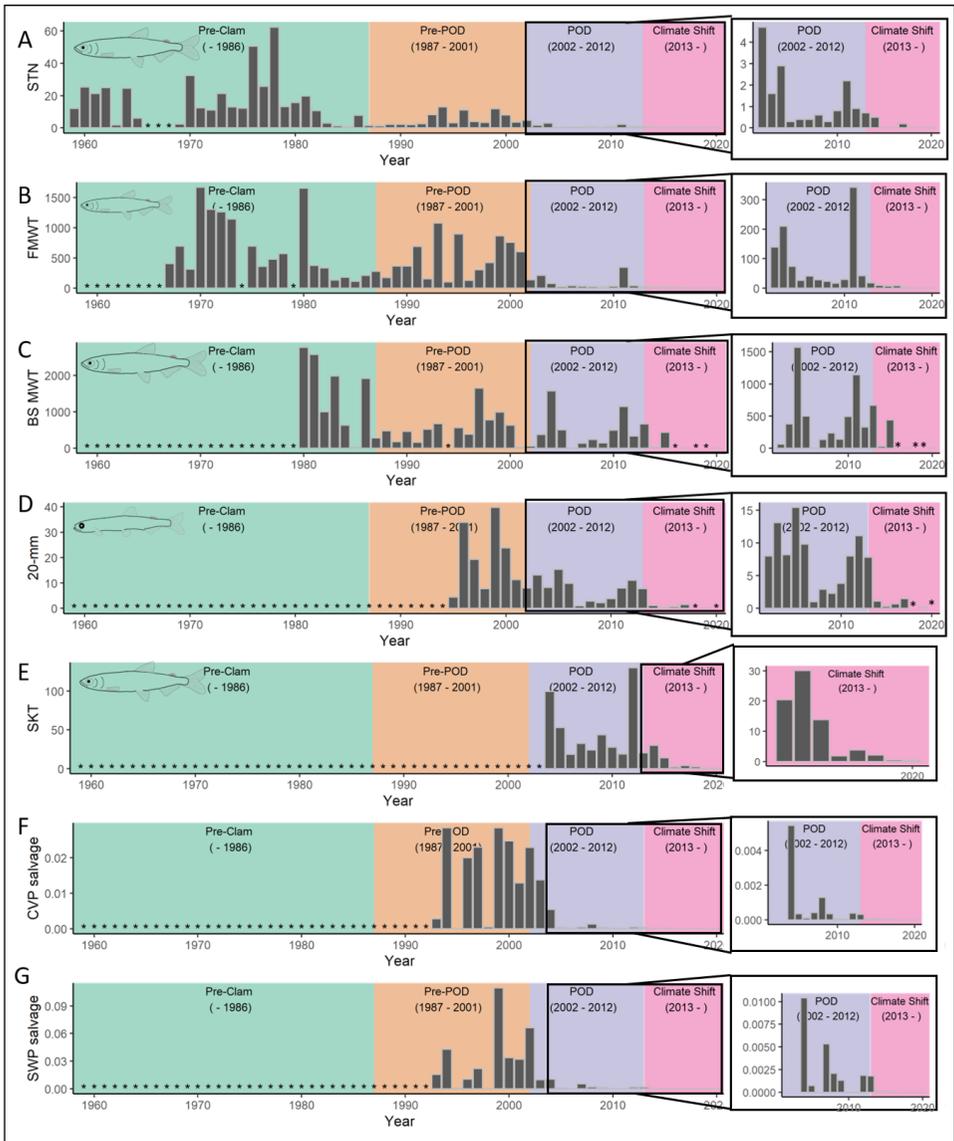
$S_a$  = Total number of stations within Area  $a$ .

$W_a$  = FMWT volumetric weighting factor for Area  $a$ .

$sn_{j,a}$  = Standardized number of Delta Smelt caught at station  $j$  per 10,000 cubic meters.

$sn_{j,b}$  = Standardized number of Smelt caught at Station  $j$  within Region  $b$  per 10,000 cubic meters.

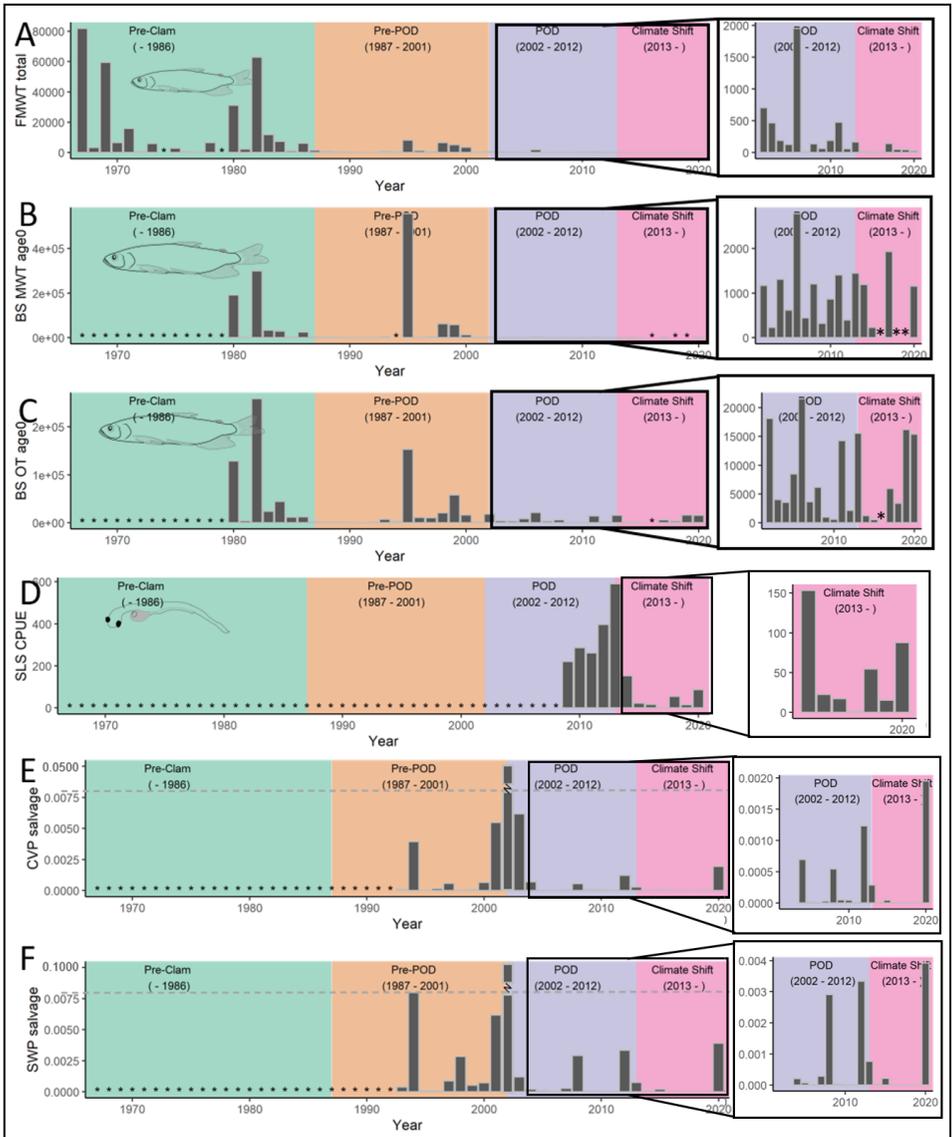
$W_b$  = BS volumetric (MWT) or area (OT) weighting factor for Region  $b$ .



**Figure 4.** Delta Smelt annual abundance indices for long-term surveys as well as the SWP and CVP pump salvages (calculated in catch per acre foot). Years in which no index value could be calculated are noted with an asterisk.

low numbers through 1979, then bouncing back in 1980 and 1982 in the FMWT (Fig. 5a). The population abundance of both species remained low through the prolonged drought from 1987-1992.

During the 1987 drought, the overbite clam (*Potamocorbula amurensis*) was unintentionally introduced, likely from ship ballast water, and quickly became the most abundant benthic filter feeding invertebrate in the upper Estuary (Kimmerer et al. 1994). The overbite clam is a voracious consumer of phytoplankton and early instar zooplankton and has led to



**Figure 5.** Longfin Smelt annual abundance indices for long-term surveys as well as the SLS catch per 1,000 m<sup>3</sup> and the SWP and CVP pump salvages (calculated in catch per acre foot). Years in which no index value could be calculated are noted with an asterisk.

reduced food abundance for smelt and other fishes in the low-salinity region of the upper Estuary (Feyer et al. 2003; Winder and Jassby 2011). The onset of drought and the introduction of the overbite clam marks the first regime shift observed since the initiation of monitoring, where a change in the fundamental dynamics of the ecosystem likely occurred that reduced the carrying capacity (Mac Nally et. al. 2014; Brown et al. 2016). The regime shift was driven by the clam invasion and facilitated by the drought, but other factors likely contributed such as ammonia loading, and changes in water inflows and exports (Brown et

al. 2016). This regime shift was only identified when the abundance indices (calculated by STN, FMWT, and BS monitoring data) of species that recruit in the upper Estuary failed to regularly reach pre-clam levels (Kimmerer 2009; Figs. 4a-c, 5a-c).

In response to the observed declines in common Delta fish species, the United States Fish and Wildlife Service (USFWS) initiated status assessments in 1987 for the federal listing of Delta Smelt while CDFW conducted status assessments to inform the Fish and Game Commission on the state listing: one in 1990 (Stevens et al. 1990) which resulted in multi-agency coordination on a targeted Delta Smelt study, and one in 1993 (Sweetnam and Stevens 1993) which incorporated the results of that targeted study. CDFW found that the listing of Delta Smelt was warranted, and the population declines detected in the long-term monitoring data sets were pivotal in this finding (Sweetnam and Stevens 1993). Subsequently, Delta Smelt was listed as a threatened species under FESA on March 5, 1993 and under CESA on December 9, 1993. Reduced freshwater flows into the estuary were identified as one of the primary causes of decline (Moyle et al. 1992; USFWS 1993). Reduced inflows were associated with lower zooplankton production and retention of larvae and juveniles in the deep and narrow channels in the confluence region of the Sacramento and San Joaquin rivers, presumed to be poor habitat for many species of fish (USFWS 1995). A secondary cause was the loss of individuals by entrainment into small agricultural diversions within the Delta and by the large pumps of the SWP and CVP in the south Delta (Moyle et al. 1992). In addition, the decline was attributed to direct (toxicity to fish) and indirect (toxicity to food web) effects from changing contaminant types and loads in the Delta and Estuary (Moyle et al. 1992; Sweetnam and Stevens 1993; USFWS 1996).

### **ADAPTIVE MANAGEMENT: LISTINGS LEAD TO NEW MONITORING PROGRAMS (1990–2000)**

As required by the federal listing of Delta Smelt, the USFWS released a series of Biological Opinions (BO) regarding the effects of CVP and SWP operations on Delta Smelt and Delta Smelt critical habitat, two of which were intended to each last one year. The 1993 BO implemented flow criteria proposed by USBR and DWR. The 1994 BO continued to implement these export reductions and incorporated Delta Smelt distribution data collected by the monitoring programs to aid in real-time water management decisions. Ongoing studies conducted by the IEP, including the CDFW long-term monitoring studies, were used to assess the effects of the 1993 and 1994 operations (USFWS 1995). In 1994, the State of California and the Federal Government agreed, through the “Principles of Agreement”, to comply with a set of Bay-Delta standards intended to “provide ecosystem protection for the Bay-Delta Estuary”. These standards were outlined in a draft Water Quality Control Plan released by the State Water Resources Control Board (SWRCB) (USFWS 1995). The 1995 BO stated that combined CVP and SWP operations, modified per the Principles of Agreement, draft Water Quality Control Plan, and winter-run Chinook Salmon BO, were not likely to jeopardize the continued existence of Delta Smelt (USFWS 1995). In 1999, the SWRCB released Water Right Decision 1641, which stated that the SWRCB is to be informed of impacts on fisheries relative to outflow and exports, assigned responsible parties to help meet the objectives of the 1995 Bay-Delta plan, and stated that ongoing monitoring activities recommended by CDFW, USFWS, and the National Marine Fisheries Service (NMFS) should continue (SWRCB 1999). These legal documents largely regulated protective actions for Delta Smelt in the following years.

The California Water Policy Council and the Federal Ecosystem Directorate (together as “CALFED”) were tasked with deciding how to implement these protective measures. One component of the CALFED process was the formation of an interagency Delta Smelt Working Group, who was tasked with regularly evaluating biological data and developing recommendations for protective actions. To aid in developing these recommendations, the 1995 BO called for the continuation of CDFW monitoring programs and required the establishment of a new monitoring program to assess larval Delta Smelt abundance and distribution in the north Delta (USFWS 1995). In 1996, the USFWS released the Recovery Plan for Sacramento-San Joaquin Delta Native Fishes (USFWS 1996). The intent of the recovery plan was to outline reasonable actions that were deemed necessary to protect the Delta ecosystem, with the ultimate goal of delisting Delta Smelt and restoring populations of other species in decline. In part, the plan called for an increase in monitoring, specifically to better understand Delta Smelt, and designated CDFW, CVP, and SWP as the responsible parties. The plan also contained objectives and criteria to measure recovery using catch data from FMWT (USFWS 1996). This plan, and the FMWT recovery criteria, are still in effect today.

The 1995 BO and the 1996 Recovery Plan led to the development of three new CDFW monitoring programs designed to specifically target larval, post-larval, and spawning adult Delta Smelt. In 1995 the Egg and Larval Survey, transitioned from targeting Striped Bass to targeting Delta Smelt, and became the North Bay Aqueduct Larval Fish Survey<sup>9</sup>. The new survey design monitored the abundance and distribution of larval Delta Smelt in the North Delta, where spawning was thought to occur, and evaluated larval entrainment to a pumping facility in Barker Slough, also in the North Delta. The 20-mm Survey<sup>10</sup> was initiated in 1995 to track the distribution and abundance of post-larval and juvenile life stages and was used as a real-time tool to inform daily CVP and SWP operations from March to July (Fig. 3). The gear was designed to fully retain a Delta Smelt 20 mm in size (Mitchell et al. 2019), which is the size when Delta Smelt have an inflated air bladder and are retained and readily identifiable at the fish salvage facilities associated with the SWP and CVP. To track the distribution of adult Delta Smelt and assist with water management decisions, beginning in 1991 an extension of the FMWT sampled into the spring, January-March (Spring Midwater Trawl). Net evaluation studies conducted in 1995 found that a Kodiak trawl net, which is towed at the surface between two vessels, was the most effective gear for detecting and retaining adult Delta Smelt; thus, in 2002 the springtime sampling gear was changed from an obliquely towed midwater trawl net to a surface towed Kodiak net and became the Spring Kodiak Trawl Survey (SKT)<sup>11</sup> (Sweetnam 1994; CDFG unpublished data; Souza 2002). The SKT samples throughout the spawning season (January-May) to determine the distribution and abundance of adult Delta Smelt. In addition, each Delta Smelt is dissected in the field and examined for gonadal development to identify and rapidly report the onset of spawning. Until 2008, when take became a greater concern, supplemental SKT sampling targeted regions believed to support Delta Smelt spawning habitat to enhance the understanding of maturity status. The 20-mm Survey and the SKT both calculate indices of relative abundance for different life stages of Delta Smelt (Fig. 4d-e). Both surveys were designed to monitor population trends of Delta Smelt, and these trends mirror those observed in STN and FMWT, but neither survey was designed to produce a population

<sup>9</sup> <https://wildlife.ca.gov/Conservation/Delta/North-Bay-Aqueduct>

<sup>10</sup> <https://www.wildlife.ca.gov/Conservation/Delta/20mm-Survey>

<sup>11</sup> <https://www.wildlife.ca.gov/Conservation/Delta/Spring-Kodiak-Trawl>

estimate. When aligned with STN and FMWT, these four monitoring programs track the abundance and distribution of critical points in the Delta Smelt lifecycle (Table 1). This combination of new and long-standing monitoring programs enabled decision makers to assess the real-time risk water operations posed to the species, and enabled researchers to gain a deeper understanding of each life stage and track the success of recovery actions by continuing to monitor population trends over time

### **THE PELAGIC ORGANISM DECLINE: DETECTING AND ADAPTING TO A NEW NORMAL (2000–2005)**

The Delta Smelt indices (Fig. 4a-e), and Longfin Smelt indices (Fig. 5a-c) showed a further decline beginning in the early 2000s along with a decline in other pelagic fishes; by 2004 this broader phenomenon was recognized as the Pelagic Organism Decline (POD; Sommer et al. 2007; Thomson et al. 2010). The POD marks the second regime shift, where, again, monitoring data showed a step-decline in Delta Smelt and Longfin Smelt abundance indices in the upper estuary. This decline was likely the result of another reduction in carrying capacity, despite some management actions already in place to protect Delta Smelt. This led to numerous studies attempting to identify the cause of the POD to improve future management. No one factor was identified, but many biotic and abiotic factors could have had a synergistic effect, including, but not limited to, an upstream shift of the low salinity zone, water exports, increased water clarity, and declines in prey availability (Sommer et al. 2007; McNally et al. 2010; Thomson et al. 2010). Additionally, Delta Smelt and Longfin Smelt salvage (individuals per acre-foot of water) was highest leading up to the POD regime (Figs. 4f-g and 5e-f). Recent modeling efforts indicate that since the 1993 listing of Delta Smelt, the highest levels of adult and post-larval entrainment occurred between 2000 and 2004, coinciding with the onset of the POD (Smith 2019; Smith et al. 2020).

In response to the POD, monitoring efforts continued to increase for Delta Smelt and Longfin Smelt, and modifications to existing surveys were implemented to increase knowledge of each species' ecology. For example, in 2005 the STN and FMWT began collecting meso-zooplankton samples, and FMWT also began collecting mysid samples, to examine potential food limitation for fish and habitat productivity. Pelagic fish collected by all monitoring programs were retained for diet and condition studies, and Delta Smelt were analyzed for condition, growth, life history diversity, and health (e.g., Hammock et al. 2015; Hobbs et al. 2019; Teh et al. 2020). Additionally, in 2005 the North Bay Aqueduct Larval Fish Survey transformed into the Delta Smelt Larval Survey, which expanded larval monitoring to assess the abundance and distribution of larval Delta Smelt in the upper Estuary, entrainment risk at CVP and SWP, and investigated catch efficiencies at different portions of the water column.

### **CONTINUING TO ADAPT: NEW RULES, DEEPER DECLINES (2005–PRESENT)**

In 2005, the State of California Resources Agency, DWR, and CDFW developed the Delta Smelt Action Plan to identify a 14-point program to address the decline in fish and zooplankton populations (California Resources Agency 2005). Shortly after the release of this plan, the California legislature directed the California Resources Agency to report on proposed actions to address the POD and stabilize the estuary ecosystem (California Re-

sources Agency 2007). This described a suite of ongoing and new actions, with coordination and funding among State and Federal agencies, to pursue flow management, habitat restoration, and monitoring activities, with DWR and CDFW continuing to take steps to address the decline of pelagic organisms. The decline in both species during the POD led to petitions to up-list Delta Smelt from threatened to endangered under CESA and FESA and re-evaluate the status of Longfin Smelt for listing under CESA and FESA. The status review for Delta Smelt highlighted the fact that index values from STN and FMWT were persistently low in the 2000s, “despite sometimes-favorable springtime environmental conditions and intensive management efforts attributable in part to prior endangered species act listings (CDFG 2008).” As a result, Delta Smelt was up-listed from threatened to endangered under CESA on March 4<sup>th</sup>, 2009, but was found warranted-but-precluded from federal up-listing. To date, Delta Smelt remain federally threatened (USFWS 2010). Longfin Smelt was added to CESA as threatened on March 5<sup>th</sup>, 2009 based on their continued decline, despite some years of beneficial freshwater outflows (CDFG 2009). The review leading to the listing pointed to water diversion operations (e.g., entrainment and mortality at export facilities and habitat modifications related to salinity), invasive species impacts to prey availability, water pollution, predators, a commercial bait fishery, and scientific collections as threats to the Longfin Smelt population (CDFG 2009). Meanwhile USFWS found Longfin Smelt was not warranted for federal listing at the time (USFWS 2009); however, in 2012 the USFWS found that the federal listing of the Bay-Delta distinct population segment of Longfin Smelt was warranted for listing, but, as in the case of Delta Smelt, it was precluded by higher priority actions (USFWS 2012).

The continued decline of both species under favorable conditions during some seasons or in some years indicated the need for additional information to inform future management actions. Therefore, following the 2008 up-listing of Delta Smelt and 2009 listing of Longfin Smelt under CESA, additional sampling sites were added in the North Delta to the STN (8 stations added in 2011; Contreras et al. 2011), FMWT (6 stations added in 2009 and 2010), and 20-mm (6 stations added in 2008) monitoring programs to better capture potential Delta Smelt habitat. This importance of this region was discovered when adults in spawning condition were regularly collected in the North Delta during supplemental SKT surveys from years prior. To maintain comparability through time, catch from these sites is not incorporated in the annual index calculations, but the data is included in the annual indices reports, and abundance trends at North Delta sites are similar to abundance trends at historic sites (Tempel 2017; White and Baxter, in review). In 2009 the Delta Smelt Larval Survey was transformed into the Smelt Larva Survey (SLS)<sup>12</sup> to monitor the distribution of larval Longfin Smelt in the upper Estuary. This survey targets small larvae (<11 mm in length), samples from January through March, and was designed to provide near-real-time distribution data for agency managers to assess the risk of entrainment to larval Longfin Smelt at the CVP and SWP. Unlike the other monitoring programs, abundance indices are not calculated using SLS data because the survey focuses sampling to the upper Estuary to directly inform entrainment risk and is unlikely to capture the center of distribution of Longfin Smelt larvae in wetter years, which shifts downstream into San Pablo Bay and the South Bay (Baxter 1999; Lewis et al. 2020; Grimaldo et al. 2020).

Legally, the FESA and CESA listings implemented additional requirements to continue operations at the CVP and SWP. In 2008, the USFWS released a Biological Opinion (BO)

<sup>12</sup> <https://wildlife.ca.gov/Conservation/Delta/Smelt-Larva-Survey>

on the Long-Term Operational Criteria and Plan (OCAP) for the coordinated operation of the CVP and SWP, which determined that continued operation of the water projects was likely to jeopardize the continued existence of Delta Smelt and adversely modify critical habitat. To minimize the effects of water operations, a series of alternative actions were established, termed Reasonable and Prudent Alternatives (RPA's). These RPA's implemented restrictions on exports at SWP and CVP by managing negative flows through Old and Middle River (OMR; Fig. 1), the main pathway leading to the South Delta pumping facilities, to protect adult Delta Smelt during spawning migrations and larvae-juveniles while hatching and rearing in the Delta. The RPA's also targeted improvements to habitat conditions for growth and rearing of sub-adult Delta Smelt by increasing Delta outflow in fall and called for intertidal and subtidal habitat restoration in the Delta and Suisun Marsh. In 2009, along with listing Longfin Smelt, CDFW issued an Incidental Take Permit (ITP) for Longfin Smelt to DWR, authorizing continued operations of the SWP (CDFG 2009). The ITP implemented restrictions on OMR flows to minimize entrainment of adult and larval Longfin Smelt during the spawning and rearing period, improve downstream transport of larvae, and increase Longfin Smelt habitat quality and quantity (CDFG 2009). The BO and ITP required the SLS, 20-mm Survey, and SKT to provide real-time distribution data, and required the calculation of FMWT indices to set salvage limits. To evaluate any necessary export restrictions, the Smelt Working Group (SWG)<sup>13</sup>, which consisted of experts in Delta Smelt and Longfin Smelt biology from the USFWS, USBR, U.S. Environmental Protection Agency, California Department of Natural Resources, DWR, NOAA Fisheries, and CDFW, was tasked with convening on a weekly basis from December through June to evaluate data on catch patterns, flow, turbidity, salvage, and other parameters, to assess the distribution of smelt in the Delta and predict the timing of their movements, and to provide an entrainment risk assessment of different life stages at varying levels of negative Old and Middle River flow. Based on these data, the SWG sent OMR flow recommendations to the USFWS (for Delta Smelt) or CDFW (for Longfin Smelt) to minimize risk of smelt entrainment. The USFWS or CDFW would then report their final decision to the Water Operations Management Team (WOMT). Reis et al. (2019) investigated the effects of these environmental regulations on water exports and found that Delta Smelt RPA's limited exports on only 8% of days from 2011 through 2018 (~275 days total). Of these reductions, 4% of days were constrained to meet Fall X2 requirements, 4% of days were constrained to meet OMR flow recommendations, and an additional 2% of days were voluntary reductions that may have been implemented to avoid excessive take. When Delta Smelt salvage is standardized to the FMWT index (sum of adult and juvenile salvage/FMWT), the standard value has been relatively low since 2009 (Moyle et al. 2016). Furthermore, Smith et al. (2020), using advanced modelling techniques, found far reduced post-larval entrainment since 2004. This indicates that flow management in the POD regime had been successful at reducing entrainment, however; the observed low levels of entrainment could also reflect the declining population (Blumenshine et al. 2000).

The 2008 BO required DWR and USBR to provide sufficient Delta outflow to maintain monthly average X2 (the 2 parts per thousand salinity isohaline as measured in kilometers upstream of the Golden Gate Bridge) no greater than 74 km in wet years and no greater than 81 km in normal years for the months of September and October. In addition, in wet or above normal years all inflow to SWP and CVP reservoirs in November would be released to provide additional outflow from the Delta to augment the fall flow Action (USFWS 2008).

<sup>13</sup> [https://www.fws.gov/sfbaydelta/cvp-swp/smelt\\_working\\_group.cfm](https://www.fws.gov/sfbaydelta/cvp-swp/smelt_working_group.cfm)

The intent of these actions was to expand and improve Delta Smelt habitat and potentially increase productivity. Criteria for the fall flow actions were easily achieved in 2011 due to high flows from early rains and ample reservoir storage. The 2011 FMWT abundance index for Delta Smelt increased substantially relative to all previous years since the POD signaling this management action was successful (Fig. 4b; Brown et al. 2014; FLOAT-MAST 2020). The criteria for the fall flow actions were also met in 2017 and 2019, but less wet conditions, and the drought years preceding the 2017 action, resulted in low summer flows and an early end to the actions in order to maintain reservoir storage. The FMWT Delta Smelt index did not substantially increase due to the 2017 or 2019 action (Fig. 4b; Brown et al. 2014; FLOAT-MAST 2020). High flows in 2011, 2017, and 2019 may have also contributed to moderate increases in the FMWT Longfin Smelt index (Fig. 5a).

Following the 2008 BO, the 2009 ITP, and the wet year of 2011 and corresponding implementation of the fall flow action, several long-term monitoring programs observed increases in many of the 2010 and 2011 Delta Smelt and Longfin Smelt indices (Figs. 4a-e and 5a-c). Unfortunately, an extensive drought period began in 2012; habitat conditions began to decline further, and the abundance indices of both smelt species plummeted to record lows and have not recovered (Figs. 4a-e and 5a-c). This period may mark the most recent regime shift, and notably brings Delta Smelt to the brink of detection limits of the long-term monitoring programs (Fig. 4). As with the previous regime changes, the drop in abundance indices were likely driven by multiple stressors including low food resources (Sommer et al. 2007; Winder and Jassby 2011; Moyle et al. 2016), a climatic shift toward warmer temperatures and lower flows (Brown et al. 2013, Castillo et al. 2018; Fig. 2), and an already diminished population following the POD (Thomson et al. 2010). This most recent climate shift period (2013–present) for Delta Smelt is marked by an abrupt drop in abundance indices (Fig. 4). Both the minor increase and steep decline in Delta Smelt indices were observed across all life stages. The depth of this decline is marked by the first recorded zero-index by STN in 2015, followed by FMWT's first zero-index in 2018 (Fig. 4a-b). Notably, all index values showed a very minimal response to the wet years of 2017 and 2019, suggesting the population was unable to rebound following improved flow conditions; however, high summer and fall temperatures in those years may have prevented a rebound (FLOAT-MAST 2020). Motivated by the 2008 BO and the continued decline of Delta Smelt, in 2011 USBR and the IEP began a large-scale synthesis study that incorporated long-term monitoring data to explore the importance of fall low-salinity habitat ("FLASH") for Delta Smelt (Brown et al. 2014). The report found interpretation of Delta Smelt response was difficult to explain without context of preceding conditions. IEP responded with an analysis inclusive of the entire life cycle, applying a 4-season approach to update conceptual models of factors affecting Delta Smelt (IEP MAST 2015). The results of the MAST would inform further actions. In 2016, the California Natural Resources Agency published the Delta Smelt Resiliency Strategy<sup>14</sup> that outlined a suite of actions intended to benefit Delta Smelt, including, but not limited to, promoting food production, augmenting outflow in the summer of 2016 and spring and summer of 2017 and 2018, and restoring habitat; some of these measures, including Delta Smelt response, were to be evaluated by the long-term monitoring data. The final regime for Longfin Smelt is marked by declines in the FMWT index and SLS catch-per-unit-effort (CPUE), which suggests that the current regime shift may have negatively impacted upstream habitat for Longfin Smelt use (Fig. 5).

<sup>14</sup> <https://resources.ca.gov/Initiatives/Delta-Smelt-Resiliency-Strategy>

## LOOKING FORWARD: A DELTA DIVIDED

In 2016, USBR and DWR requested the Reinitiation of Consultation on the Coordinated Long-Term Operation of the CVP and SWP, partly due to low numbers of Delta Smelt in salvage at CVP and SWP since the initiation of the 2008 BO (USFWS 2019). As a result, the USFWS released the 2019 Biological Opinion which removed the 2008 declaration of jeopardy for Delta Smelt, removed the RPA's under the USFWS 2008 BO, and relaxed additional protective measures to minimize entrainment of Delta Smelt, which would also affect entrainment of Longfin Smelt. In 2020, CDFW took unprecedented action to protect Delta Smelt and Longfin Smelt by not issuing a consistency determination for the 2019 USFWS BO, rather, CDFW through its issuance of Incidental Take Permit No. 2081-2019-066-00 for Long-Term Operation of the State Water Project in the Sacramento San Joaquin Delta implemented more protective measures for both smelt species (CDFW 2020). The 2020 ITP upholds many of the protective measures outlined in the 2008 BO and implements several new measures such as expanded monitoring for Longfin Smelt, additional monitoring for larval fish entrainment at SWP, and the development of a Longfin Smelt science program. Importantly, the ITP states "For the purposes of the ITP, where the terms and conditions in the federal authorization are less protective of the covered species or otherwise confluence with this ITP, the conditions of approval set forth in this ITP shall control." This effectively established different rules for export operations between the CVP and SWP in the south Delta. Despite these new state regulations, unusually high salvage of Longfin Smelt juveniles occurred in 2020 compared to other years since the POD, emphasizing the need to implement the larval entrainment monitoring program under the ITP (Fig. 5). Both the 2019 BO and 2020 ITP are currently under litigation, thus at the time of this writing it remains uncertain how smelt will be managed in the coming years.

The upper San Francisco Estuary is a prime example of the balance between anthropogenic modifications and the protection of natural resources. CESA and FESA protections put in place in the Estuary have provided some legal backing to help protect imperiled species. For Delta Smelt and Longfin Smelt, the implementation of these protections have greatly stimulated research and synthesis, which in turn have greatly expanded our knowledge about both species and the Estuary as a whole, but thus far have failed to protect these smelts from the threat of extinction. The CDFW long-term monitoring datasets have continued in large part thanks to the CESA and FESA listings of both smelt species and other related regulatory needs, and these regulations were in turn brought about because of trends detected in the data collected by the long-term monitoring surveys. These data have also been used to identify population drivers and turning points in the Estuary (Thomson et al. 2010) and have provided the foundation for a wealth of knowledge on a many other species (e.g., Orsi 1999; Baxter et al. 2010; Brown et al. 2014; IEP 2015). There are many competing factors to consider when implementing protections for a species, and in the case of the Estuary, full protection as required under the ESA and CESA is not always possible. Major declines occurred during drought periods, which further challenges recovery.

The opportunity to meet conditions needed for recovery of listed smelt are becoming increasingly challenging in the face of climate change and will require continued collaboration among agencies and stakeholders. Multiple agencies are working together to balance the water needs of Californians with the water needs of fish and wildlife in the Estuary, but the resources of the Estuary are stretched thin, leaving the system vulnerable to incom-

ing stressors (Mahardja et al. 2020), and although protections have been put in place, the abundance of these two species have continued to decline. Given the current status of both smelt species, their short life spans, the changing climate, and the myriad factors that can cause recruitment failure in any given year, recovery of these smelt in the San Francisco Estuary will require management and conservation actions over many generations in order to create environmental conditions that can rebuild population resilience. Accomplishing this will require an unprecedented level of commitment and collaboration among all regulatory and management agencies, water districts, and the public, as well as the continuation and adaptation of current long-term monitoring.

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## ESSAY

## Protecting a displaced species in an altered river: a case study of the endangered Sacramento River winter-run Chinook Salmon

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Endangered Sacramento River winter-run Chinook Salmon (*Oncorhynchus tshawytscha*) exist as a single population that spawns in the Sacramento River downstream of Shasta Dam near Redding, CA. Displaced from their historical habitat after dam construction circa 1940, their survival depends on cold water released from Shasta Reservoir. Managing and recovering the species is further complicated by their anadromous life history, habitat loss and degradation, large-scale water supply management, and climate change. The California Department of Fish and Wildlife and other resource agencies coordinate closely to protect the species from extinction, confronting challenges with collaborative restoration and science-driven management. As climate change brings more frequent droughts, warmer weather, and increased variability in precipitation, Sacramento River winter-run Chinook Salmon recovery will require greater collaboration and a shift to more holistic restoration actions that promote and maintain the diversity and resilience of the species and its habitats.

**Key words:** California, CESA, Chinook Salmon, climate change, displaced species, endangered species, recovery, reintroduction, restoration, winter-run

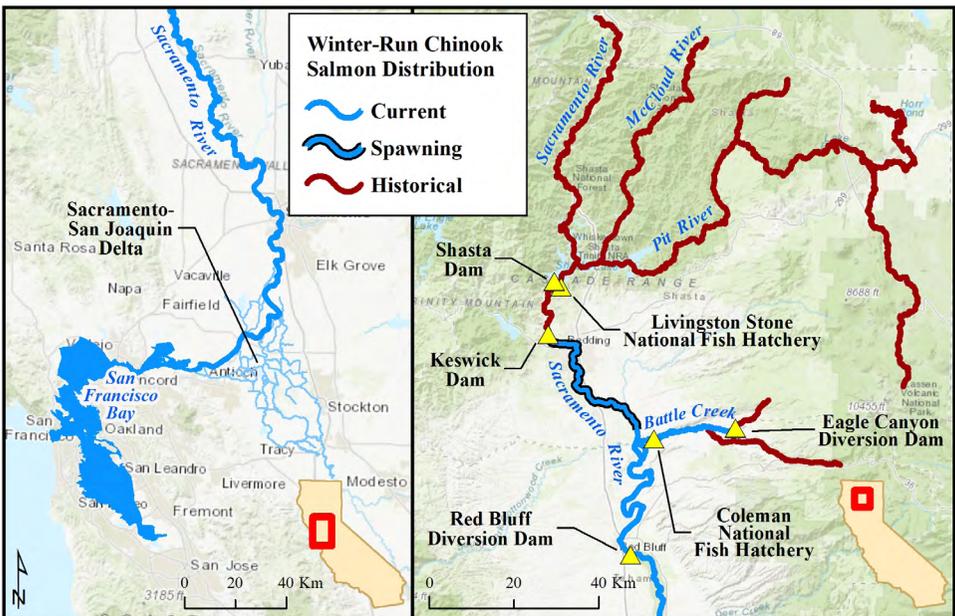
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Threatened and endangered species management is frequently complicated by ongoing anthropogenic impacts, such as continued habitat modification, pollution, and interactions with invasive species. Chinook Salmon (*Oncorhynchus tshawytscha*) in the Central Valley of California (Central Valley), which include four distinct runs named for the season when adults return to freshwater to spawn, share these threats. Their status and management are stymied by diverse anadromous life histories, harvest, large ranges, and highly altered, fragmented river systems. Climate change and extreme year-to-year variability in precipitation create additional challenges. Resource agencies, including the California Department of Fish and Wildlife (CDFW), National Marine Fisheries Service (NMFS), U.S. Fish and Wildlife Service (USFWS), State Water Resources Control Board (SWRCB), California Department of Water Resources (CDWR), and the U.S. Bureau of Reclamation (USBR), confront these challenges with applied science, adaptive management, and interagency collaboration to

protect and enhance Central Valley salmon populations and their habitats. Yet for every successful recovery action, new challenges emerge for California salmon.

This struggle is particularly noteworthy for Sacramento River winter-run Chinook Salmon (winter-run Chinook Salmon), which have experienced both some of the greatest challenges and some of the greatest recovery successes. Winter-run Chinook Salmon historically spawned in the upper reaches of Sacramento River tributaries, including the McCloud, Pit, and Upper Sacramento rivers, and Battle Creek (Figure 1). These cold, spring-fed streams provided vital habitat for this species whose life history is otherwise poorly suited for the hot, dry summers of the Central Valley (Yoshiyama et al. 2001). In fact, the life history strategy of winter-run Chinook Salmon, which begin migration in winter but delay spawning until summer, is unique within the range of Chinook Salmon (Hallock and Fisher 1985; Moyle 2002). When USBR constructed Shasta Dam (1938–1945) and Keswick Dam (1941–1950) for the Central Valley Project, they blocked access to almost all historical spawning habitat and the ecoregion where winter-run Chinook Salmon evolved. A single population remains, spawning in the mainstem Sacramento River between Balls Ferry Bridge near Anderson, California (river km 444), and Keswick Dam (river km 486) (Figure 1), and its survival depends on an altered hydrologic regime and careful water management (NMFS 2014).

Winter-run Chinook Salmon, like all Pacific salmon (*Oncorhynchus* spp.), are anadromous, meaning they are born in freshwater before spending most of their lives in saltwater and returning to freshwater to spawn. Adult winter-run Chinook Salmon leave the Pacific Ocean and enter San Francisco Bay beginning in December, continuing upstream through July (Moyle 2002). Over 90% of winter-run Chinook Salmon spawn at age three; however, some will return as two-year-old “grilse” or at age four or five (USFWS 2013; Satterthwaite



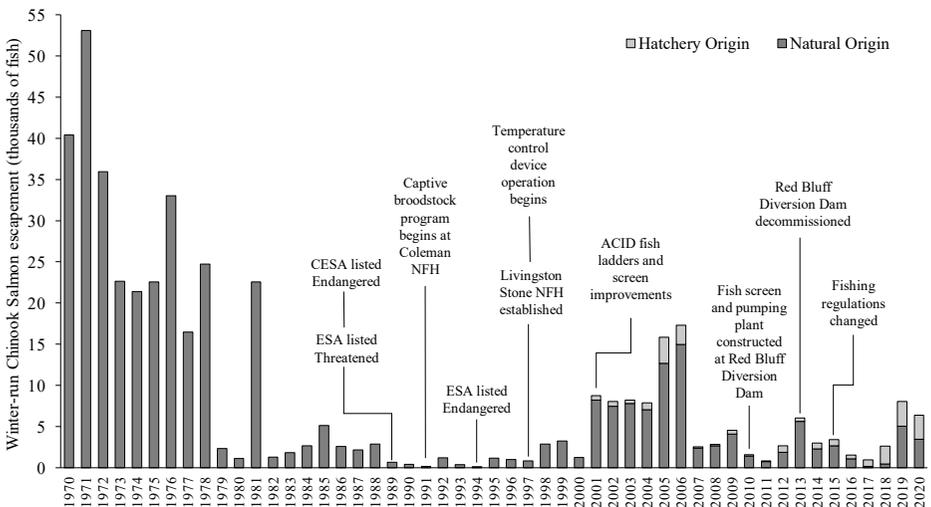
**Figure 1.** Map of historical and existing winter-run Chinook Salmon habitat in the Sacramento River basin and estuary. Data Sources: Yoshiyama et al. 2001; NMFS 2014

et al. 2017). Spawning and egg incubation occur during the warmest time of the year and require gravel-bedded stream reaches with cold, clean water to support developing embryos from April through October (NMFS 2014). Juveniles rear and migrate downstream from July through March, reaching the Sacramento-San Joaquin Delta (Delta) from December through May (Figure 1) (NMFS 2014). Residence time in the Delta is variable, and juveniles may spend one to four months rearing prior to entering San Francisco Bay on their way to the Pacific Ocean (del Rosario et al. 2013). Because, in part, of this complex life history with long migrations and variable habitat needs at different life stages, winter-run Chinook Salmon have been the object of considerable research and more monitoring than many other imperiled species.

## Unlikely Success Before Decline And Listing

Although far removed from their native range in the high-elevation, volcanic, spring-fed streams near Mt. Lassen and Mt. Shasta, winter-run Chinook Salmon initially did surprisingly well in the Sacramento River. After completion of Shasta and Keswick dams, they began spawning in the mainstem river below Keswick Dam, where the water released from Shasta Reservoir provided cool temperatures throughout the summer. Tens of thousands of winter-run Chinook Salmon returned to the Sacramento River for several generations, but the population began declining in the 1970s (Hallock and Fisher 1985), and annual escapement (i.e., the number of returning spawners each year) remained under a few thousand fish through the 1980s and 1990s (Figure 2). Escapement declined from nearly 120,000 spawners in 1969 (Moyle 2002) to a low of 144 spawners in 1994 (Azat 2020).

The primary factors driving the decline in winter-run Chinook Salmon since the 1970s are large dams, agricultural diversions, warm water temperatures, and habitat loss and degradation (NMFS 2014). Maintaining adequate river temperatures throughout the



**Figure 2.** Sacramento River winter-run Chinook Salmon escapement (i.e., number of adult spawners) from 1970 to 2020 and notable recovery actions. NfH=National Fish Hatchery; ACID=Anderson Cottonwood Irrigation District

spawning and incubation period requires deliberate management of the cold-water pool behind Shasta Dam, and the population has been highly reliant on hatchery production in some years when cold water is limited. Changes in the riverine and estuarine habitat affecting the species include major ecological shifts in the Delta ecosystem (Mahardja et al. 2017), increased water exports from the state and federal water projects, entrainment into water supply diversions, high predation rates (Moyle 2002), degraded water quality, and meteorological and hydrological changes (Williams et al. 2020). Furthermore, over 95% of historical floodplain habitat in the Central Valley, which is important for juvenile rearing and could have buffered other ecological shifts, has been leveed and drained for flood control or conversion to agriculture (Herbold et al. 2018). In what was historically an expansive estuary and nursery for rearing Chinook Salmon, only 3% of historical tidal wetland remains in the Delta, mostly as thin strips along the margins of levees (Whipple et al. 2012).

In 1989, after almost two decades of population decline, the California Fish and Game Commission listed winter-run Chinook Salmon as endangered under CESA (CA Fish and Game Commission 1989), and NMFS listed them as threatened under ESA (50 C.F.R. 17.11). The winter-run Chinook Salmon evolutionarily significant unit (ESU) was the first “distinct population segment” to be listed under the ESA, prior to a formal definition of ESU and subsequent NMFS policy development (Waples 1995). NMFS uses the ESU concept to determine whether a population or group of populations will be considered a “species” for the purposes of ESA (Waples 1991), and CDFW has generally adopted the same definition for making listing decisions for California salmon under CESA (e.g., CDFG 1998, 2002). In 1994, NMFS “up-listed” winter-run Chinook Salmon to endangered under the ESA, based on the continued decline of the species, weak expected returns, and continuing threats to the population (59 C.F.R. 440). Under the direction of NMFS, the Sacramento River Winter-Run Chinook Salmon Recovery Team published the first Recovery Plan in 1996 (NMFS 1996), which evaluated impacts and prioritized restoration actions to recover the species.

Because they exist as a single, isolated population, winter-run Chinook Salmon are at greater risk of extinction. For the ESU to be resilient, it needs multiple spatially and genetically diverse populations that maintain long-term demographic processes and evolutionary potential (McElhany et al. 2000). It must also have redundancy, meaning there should be at least two or three viable independent populations that do not share the same extinction risks (Lindley et al. 2007). Assuming extinction risks are not correlated between populations, redundancy ensures that two populations are unlikely to go extinct simultaneously (McElhany et al. 2000). Without multiple independent populations, a single catastrophic event, such as an eruption of Mt. Lassen, a prolonged drought, or failure to successfully manage cold water storage in Shasta Reservoir, could result in extinction of the ESU (Lindley et al. 2007).

### **Protecting and Recovering Winter-run Chinook Salmon**

Recovery actions for winter-run Chinook Salmon in the Sacramento River have been implemented through interagency collaboration, cooperative agreements, and regulatory actions by NMFS through the ESA (e.g., Biological Opinions) and the SWRCB, under its Water Code §275 authority to amend water rights permits and licenses to protect public trust uses (e.g., Order 90-5). NMFS Biological Opinions (NMFS 2009, 2019) and, more recently, a CDFW Incidental Take Permit (CDFW 2020), limit losses of juvenile winter-run Chinook Salmon to state and federal water project pumping facilities in the south Delta using operational triggers tied to forecasted juvenile abundance. Regulatory and permitting actions

under CESA, which authorize and limit losses incidental to otherwise lawful projects and activities, offer additional protections to winter-run Chinook Salmon in the river and estuary.

Flow and temperature management actions in the Sacramento River, which both threaten and sustain spawning winter-run Chinook Salmon and their eggs, occur at federally operated water projects. Consequently, preemption of state law by federal law limits CDFW's regulatory and enforcement authority under CESA. Interagency and stakeholder cooperation remains essential to protecting and recovering winter-run Chinook Salmon. Both informal and formal working groups evaluate restoration options, plan water management, and coordinate actions for monitoring and managing the species. For example, the Sacramento River Temperature Task Group assists USBR in meeting requirements of Order 90-5 (SWRCB 1990) and includes representatives from state and federal agencies who meet as often as weekly to plan water temperature management during the winter-run Chinook Salmon spawning and incubation period. Another interagency team of scientists reviews best available science and monitoring data to produce an annual Juvenile Production Estimate, a forecast of winter-run Chinook Salmon juvenile abundance in the Delta that is used to set limits for allowable losses at the state and federal water projects (O'Farrell et al. 2018; NMFS 2020). This collaborative, inclusive, science-driven approach to species protection and management has, so far, proven effective at responding to threats and protecting the population from extinction.

## Recovery Actions

Since listing, most species recovery actions have focused on managing habitat and increasing natural production and survival in the Sacramento River and Delta. Actions include rehabilitating spawning and rearing habitat, establishing a conservation hatchery program, improving passage at barriers, screening water diversions, reducing losses during water project operations, managing river temperatures, and limiting ocean harvest (Figure 2).

Because the population was so small at the time of listing, artificial propagation was implemented quickly, and the USFWS began a captive broodstock program for winter-run Chinook Salmon at Coleman National Fish Hatchery on Battle Creek in 1991. The captive broodstock provided a reserve for genetic diversity and produced a modest number of juveniles to supplement natural production. The program was successful in producing fish to augment the natural population and maintaining a minimum level of genetic diversity. Unfortunately, most hatchery-origin winter-run Chinook Salmon returned to the hatchery or lower Battle Creek and did not contribute to the mainstem population. To encourage fish to instead return to the Sacramento River, the winter-run Chinook Salmon broodstock program was moved to its current location below Shasta Dam in 1997, with the establishment of Livingston Stone National Fish Hatchery (LSNFH).

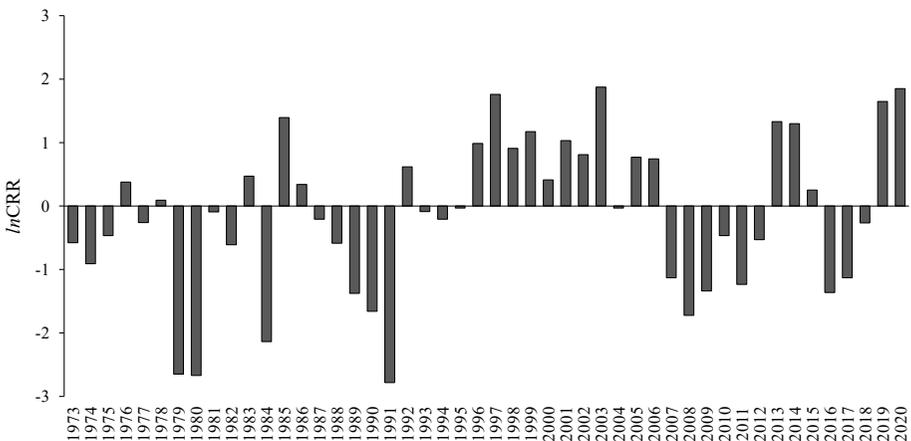
Another high priority recovery action was improving water temperature management during the winter-run Chinook Salmon spawning and incubation season. Order 90-5 required the USBR to install a Temperature Control Device (TCD) in Shasta Reservoir and to operate Keswick and Shasta Dams to maintain water temperatures to sustain all runs of salmon in the Sacramento River (SWRCB 1990). The TCD was first operational in 1997, the same year LSNFH was established. It allows dam managers to selectively withdraw water from a range of reservoir depths to meet water temperature targets in the Sacramento River.

The decade following TCD installation saw promising population growth for winter-run Chinook Salmon. The natural log-transformed cohort replacement rate ( $\ln\text{CRR}$ ) (Equation

1), an annual indicator of population growth from one generation to the next, was positive for 9 of 10 years between 1997 and 2006 (Figure 3). For a stable population, one would expect a  $\ln CRR$  greater than zero in, on average, half of all years. The rapid population growth was a promising sign for recovery, and the increasing trend in escapement from one generation ( $N_{t-3}$ ) to the next ( $N_t$ ) from 1996 to 2003 is the longest consistent trend, positive or negative, in the period of record. These calculations assume an average generation time of 3 years (USFWS 2013; Satterthwaite et al. 2017).

$$\text{Equation 1: } \ln(CRR) = \ln\left(\frac{N_t}{N_{t-3}}\right) \text{ where } N \text{ is the number of adult spawners in year } t$$

Additional recovery actions, many of which were required by the NMFS 2009 Biological Opinion for operation of the state and federal water projects (NMFS 2009), aimed to improve habitat conditions and increase species resilience. These actions included managing water temperature and Shasta Reservoir storage to protect fish, enhancing spawning and rearing habitat in the Sacramento River, screening diversions, removing passage barriers, increasing access to historical floodplains, limiting losses of juveniles at the state and federal pumping facilities, and improving facilities and operations at LSNFH. Many of these projects have been completed (e.g., installing fish screens and improving passage at Red Bluff Diversion Dam and Anderson-Cottonwood Irrigation District Dam, reconnecting side-channels to provide juvenile rearing habitat), and others are in progress (e.g., improving connectivity and juvenile rearing habitat in the Yolo Bypass). Notably, NMFS also required that USBR and CDWR take steps to reintroduce winter-run Chinook Salmon to historical habitat above Shasta Dam and in Battle Creek (NMFS 2009), which would create two additional populations to increase resilience of the ESU and support species recovery (NMFS 2014).



**Figure 3.** Natural log transformed cohort replacement rates, where  $\ln(CRR) = \ln\left(\frac{N_t}{N_{t-3}}\right)$ , and  $N$  is the number of spawners in year  $t$ .

## Responding to Crises

With better managed water temperatures and habitat enhancements (e.g., adding spawning gravel below Keswick Dam), winter-run Chinook Salmon escapement in 2005 and 2006 reached numbers not seen since the 1970s; however, the perceived recovery was brief, as warm and unproductive ocean conditions off the California coast reduced survival of young salmon. All Pacific salmon runs suffered (Lindley et al. 2009), and winter-run Chinook Salmon escapement in 2007–2012 was as low as it had been in the late 1990s (Figure 2).

This period of poor ocean conditions was followed by a prolonged statewide drought from 2012–2016, which limited Sacramento River flows and the amount of cold water available for temperature management. In anticipation of poor survival of eggs and juveniles in the river, LSNFH tripled hatchery production in 2014 and 2015, while also reinitiating the captive broodstock program to create a genetic reserve. Winter-run Chinook Salmon experienced one genetic bottleneck in the 1990s (Lindley et al. 2007), and a second catastrophic loss could be more difficult to overcome. Despite efforts to forecast water temperatures and manage the cold-water pool, dam operators lost temperature control below Shasta Dam in August 2014, just as spawning completed and several months before all juveniles would emerge from the gravel (USBR 2015). The drought continued through the winter, and the cold-water pool the following year was even more limited. To extend the period of temperature control in 2015, fisheries resource agencies, water resource agencies, and water contractors negotiated and managed to a temporary temperature management target that was warmer and closer to Keswick Dam (NMFS 2015). Despite these efforts, an estimated 95% of eggs in the river in 2014 and 2015 perished due to elevated water temperatures (Voss and Poytress 2017).

The temperature management target to protect winter-run Chinook Salmon eggs was 13.3°C (56°F), which is based on thermal tolerance estimates from controlled laboratory experiments (McCullough 1999; NMFS 2009). However, egg mortality in 2015 was much higher than predicted by the survival models, which assumed that temperatures lower than 13.3°C do not decrease survival. Martin et al. (2016) investigated this discrepancy and found that thermal tolerance in the river is approximately 3°C lower than in the laboratory, and egg and embryo mortality in the Sacramento River increases exponentially as water temperatures rise above 12°C (53.5°F). Lower inter-gravel flow velocities in the river limit oxygen supply and result in lower survival than estimated by the laboratory experiments (Martin et al. 2016). In response, NMFS revised temperature management targets to a daily average of 12°C at the compliance point, when achievable, and a secondary target of 13.3°C as part of a tiered approach to reduce temperature related egg mortality (NMFS 2019).

As winter-run Chinook Salmon struggled during the drought, CDFW and NMFS also took actions to reduce angling and harvest impacts on the species. In 2015 and 2016, CDFW recommended an emergency fishing closure from April through July for approximately 9 km of the Sacramento River upstream of Redding, CA, to prevent incidental harm to holding and spawning winter-run Chinook Salmon by anglers engaging in a very popular Rainbow Trout (*O. mykiss*) sport fishery in that river reach. The Fish and Game Commission adopted each of these temporary closures and, in 2017, permanently closed the area to fishing between 1 April and 31 July, pending information showing winter-run Chinook Salmon populations had improved to a level at which that reach could be reopened to fishing (CA Fish and Game Commission 2017).

In 2015, 2016, and 2017, CDFW, stakeholders, and the Pacific Fishery Management

Council (PFMC), which manages ocean fisheries off the coast of California, Oregon, and Washington, recommended protective measures beyond those required under the ESA (B. Kormos, CDFW, personal communication). Because winter-run Chinook Salmon ocean distribution is generally farther south than more abundant fall-run Chinook Salmon (Satterthwaite et al. 2013), the recommended actions limited commercial and recreational ocean fisheries from San Francisco, CA, south to minimize contact with winter-run Chinook Salmon. In 2017, the PFMC and stakeholders recommended, and NMFS adopted, a new ocean harvest control rule to protect winter-run Chinook Salmon and to be more responsive to sudden reductions in production. The rule reduces the allowable impact rate based on projected escapement absent fishing. Reductions to the impact rate are typically achieved through size limits and time-area closures, allowing for minimal ocean fishing opportunities at low abundances (PFMC 2017).

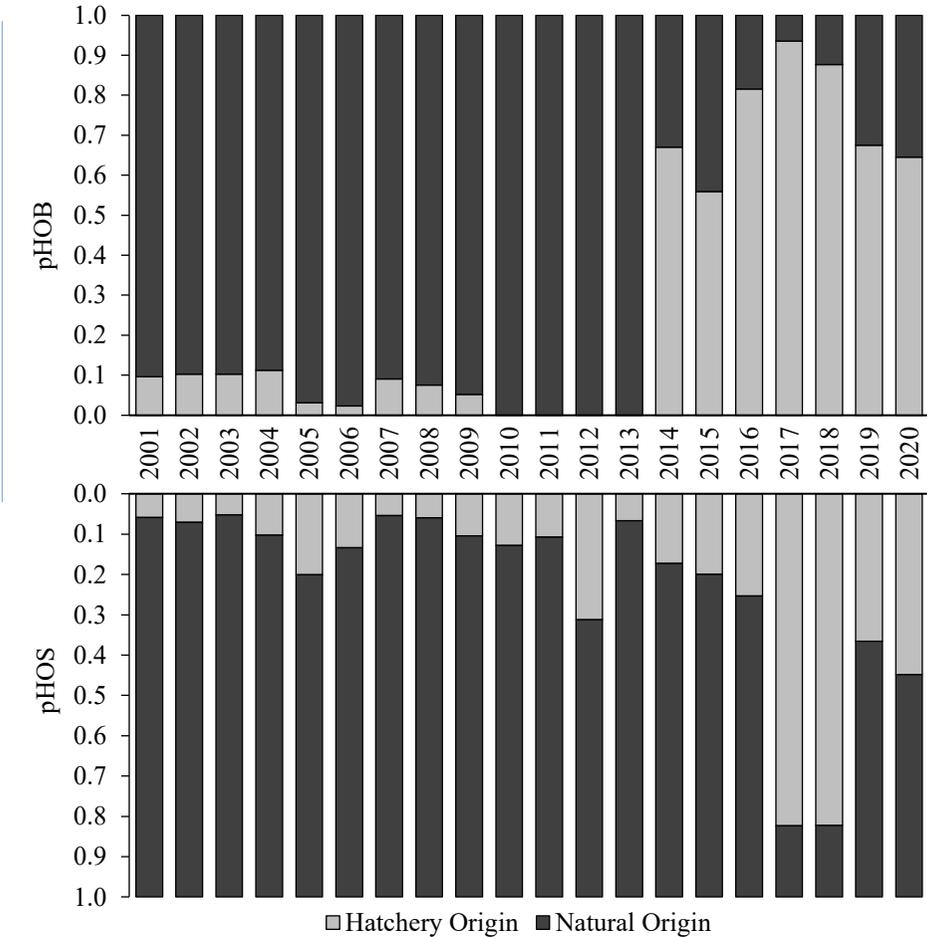
### **Opportunity for Reintroduction**

When captive broodstock at LSNFH sexually matured in 2017, the drought was ending, and hatchery production had returned to typical levels. Juveniles produced from captive broodstock provided an opportunity to begin small-scale pilot reintroduction into historical habitat in Battle Creek. In early 2018, tagged and marked winter-run Chinook Salmon were released into North Fork Battle Creek. The first fish returned in 2019 as two-year-old grilse. In 2020, more than 700 adults from the 2018 release returned to Battle Creek, and USFWS documented successful natural reproduction during juvenile monitoring (USFWS 2020). The return and successful spawning of these first winter-run Chinook Salmon in Battle Creek is a promising sign for success of the full-scale reintroduction, after completion of fish passage and flow actions in North Fork Battle Creek as part of the Battle Creek Salmon and Steelhead Restoration Project (Kier Associates 1999; ICF International 2016). The return of winter-run Chinook Salmon to Battle Creek marks an important milestone in the long-term collaborative efforts to achieve the recovery objectives of population diversity and redundancy (NMFS 2014).

### **Emerging Challenges**

Tripling hatchery production at LSNFH reduced the immediate impact of the drought on juvenile production, but it presented different risks to the population. Increasing hatchery production from approximately 200,000 to 600,000 juveniles could lead to hatchery domestication (Araki et al. 2008) or reduce effective population size (Hedrick et al. 1995). When the juveniles from 2014 and 2015 brood years returned to the Sacramento River as adults, the proportion of hatchery-origin winter-run Chinook Salmon comprising the spawning population was three times higher than before the drought (Figure 4). To limit potential impacts of hatchery domestication selection on the natural population, the proportion of hatchery-origin spawners (pHOS) in the mainstem population should be less than 0.15 (Lindley et al. 2007). In 2017 and 2018, pHOS exceeded 0.8, which puts the population at a higher risk of extinction (Lindley et al. 2007).

Despite high proportions of hatchery spawners in the population, there are reasons to be optimistic. LSNFH uses conservation hatchery practices for propagation, carefully monitoring effective population size and using genetic testing to prevent introgression with other runs or using second-generation hatchery fish as broodstock (USFWS 2013). Also, pHOS and the proportion of hatchery-origin fish in hatchery broodstock (pHOB) have



**Figure 4.** Proportion of hatchery broodstock of hatchery origin (pHOB) (top) and proportion of in-river spawners of hatchery origin (pHOS) (bottom) from 2001 to 2020. To minimize risk of hatchery domestication, the population should have less than 0.15 pHOS (Lindley et al. 2007) and pHOB should be near zero. Data Sources: USFWS 2001, 2002, 2013; K. Gooding, USFWS, personal communication

been decreasing since 2017 (Figure 4). Perhaps most notably, data collected during carcass surveys suggest that escapement in 2017 and 2018 would have been among the five lowest on record without hatchery contribution. In 2017, only an estimated 151 of 975 (15.5%) winter-run Chinook Salmon spawners were of natural origin (Figure 2). Progeny of the additional hatchery spawners that year contributed to the large number of spawners in 2019 and 2020, which are among the highest escapements in the last fifteen years (Azat 2020; D. Killam, CDFW, unpublished data).

To evaluate whether the high pHOS after the drought had population level effects, researchers at NMFS and the University of California, Santa Cruz, used genetic markers to assess reproductive success of hatchery-origin winter-run Chinook Salmon and their progeny.

They found no evidence of survival differences between the offspring of hatchery-origin and wild parents, which indicates that domestication selection is either not occurring or is very weak in the population (Thompson 2018). This suggests that the short-term, emergency hatchery interventions taken during the drought were successful in conserving genetic diversity without reducing the overall fitness of the population. These findings contradict other monitoring that found evidence of reduced fitness in hatchery-origin winter-run Chinook Salmon in 2016 (Blankenship et al. 2019); however, the large numbers of returning adults in 2019 and 2020 suggest that any reduction in individual fitness did not have population level effects.

### **The Future – Managing For Resilience**

Winter-run Chinook Salmon recovery actions have mostly focused on improving survival and responding to individual threats. While they have so far prevented extinction, species recovery in an era of climate change demands more holistic restoration actions to increase resiliency. Climate change is likely the greatest challenge for salmonids in California, particularly winter-run Chinook Salmon (Moyle et al. 2011). California's hydrology is expected to experience more variability, including more years of drought and greater swings between wet and dry conditions (Swain et al. 2018). Emerging science also suggests that North America is currently experiencing a "mega drought," a severe and persistent period of dry conditions (Mount 2020; Williams et al. 2020).

At the southern extent of Pacific salmon distribution, Central Valley Chinook Salmon evolved in a highly variable climate which included extended periods of drought and variable ocean conditions. Their resilience in such a dynamic environment is largely a result of habitat heterogeneity and life history diversity between and within populations (Herbold et al. 2018). Year-round cold water in high-elevation streams, extensive floodplains, and diverse and productive estuarine habitats buffered against temperature fluctuations and multi-decade droughts, which were common before 1850 but rare during the last century of dam building and water diversion (Herbold et al. 2018). Large dams that blocked access to rivers, simplified habitat, and reduced genetic and phenotypic diversity now limit the adaptive capacity of winter-run Chinook Salmon, making the species highly vulnerable to the effects of climate change (Crozier et al. 2019).

Water resources management, particularly temperature management, on the Sacramento River will continue to be essential for protecting winter-run Chinook Salmon and is expected to be more difficult in the future due to climate change (Crozier et al. 2019). The framework for effectively managing temperature exists in the annual temperature planning process (SWRCB 1990; NMFS 2009) and the ability to manipulate release temperature from Shasta Dam with the TCD; however, operational flexibility for water deliveries from Shasta Reservoir could provide additional survival benefits to winter-run Chinook Salmon during drier years. For example, recent analysis of historical data suggests that early season water temperatures affect spawn timing of winter-run Chinook Salmon, such that cooler temperatures in April and May result in earlier spawning peaks (Dusek Jennings and Hendrix 2020). If this is true, managed releases of cold water from Shasta Dam in the springtime could be used to encourage earlier spawning and increase egg survival in years when cold water is expected to be limited in the fall. It is important to consider, though, how this might impact later migrating winter-run Chinook Salmon and reduce phenotypic diversity in the population (e.g., Sturrock et al. 2019). Additionally, Chinook Salmon embryos are most

sensitive to temperature later in development when oxygen demand is greater (Martin et al. 2020); therefore, a strategy of releasing cold water early in the season might not decrease overall egg mortality. More study is warranted to evaluate the implications of such a strategy on winter-run and other native fish in the Sacramento River basin.

Maintaining phenotypic and life history diversity, in concert with restoring habitat complexity and reconnecting migratory corridors, is essential to the resilience of Chinook Salmon in California (Herbold et al. 2018). Habitat heterogeneity not only distributes risk (Lindley et al. 2007; Schindler et al. 2015), but it also increases species resilience to habitat and climate change at the landscape scale (Brennan et al. 2019). Strontium isotope ratios in the otoliths of winter-run Chinook Salmon adults indicate that juvenile rearing strategies are more diverse than previously recognized. Approximately half of the adults sampled in 2007–2009 had reared as juveniles in tributaries to the Sacramento River and other non-natal habitats, many of which were not known to be demographically important to winter-run Chinook Salmon (Phillis et al. 2018). This finding supports previous observations of winter-run Chinook Salmon juveniles rearing in both large (PSMFC 2014) and small intermittent (Maslin 1996) tributaries to the Sacramento River. The variation in expression and success of different rearing strategies in winter-run Chinook Salmon is uncertain, but research on Central Valley fall-run Chinook Salmon indicates that successful rearing and migration strategies vary with hydrologic regime (Sturrock et al. 2015). Regulated stream flows (Sturrock et al. 2019) and warmer and drier hydrologic regimes (Munsch et al. 2019) can truncate migration size and timing, weakening the “portfolio effect” of phenotypic diversity that helps stabilize salmon populations. Maintaining and supporting diverse life history strategies within the population could increase winter-run Chinook Salmon resilience to climate change; however, actions favoring a narrow range of phenotypes could have negative evolutionary and demographic consequences (Sturrock et al. 2019).

Similarly, management actions to support the recovery of a single species can have implications for other species of concern (e.g., Alexander et al. 2018). For example, conserving cold water for winter-run Chinook Salmon spawning and egg incubation reduces environmental flows available to support outmigration of juvenile Central Valley spring-run Chinook salmon, which are also at risk of extinction (Moyle et al. 2011) and listed as threatened under both the ESA and CESA. Federally threatened Green Sturgeon (*Acipenser medirostris*) embryos have a higher optimal temperature range than winter-run Chinook Salmon, and temperature management for winter-run Chinook Salmon in the Sacramento River has the potential to harm their development (Van Eenennaam et al. 2005; Poletto et al. 2018; Zarri et al. 2019). These types of trade-offs could be reduced by a transition from single-species management toward ecosystem-based management that supports multiple species and focuses on restoring physical and biological river processes (Poff et al. 1997), increasing habitat diversity, and reconnecting migratory corridors to historical spawning and rearing habitats (Herbold et al. 2018), and integrating management actions to maximize restoration benefits (Munsch et al. 2020). These types of landscape-scale restoration actions would increase habitat heterogeneity in time and space and increase the resilience of many native species, including Chinook Salmon, to climate change.

Winter-run Chinook Salmon are particularly vulnerable to climate change because they currently rely on managed cold-water releases from Shasta Reservoir. To maintain self-sustaining populations in a warming climate, winter-run Chinook Salmon need access to historical habitat in high-elevation, cold, spring-fed streams. Establishing an additional

population in Battle Creek will provide population redundancy and reduce the extinction risk of the ESU (McElhany et al. 2003; Lindley et al. 2007; NMFS 2014). Given the greater likelihood of severe and persistent drought in the future (Swain et al. 2018), the existing population in the mainstem Sacramento River will likely require more frequent and greater hatchery intervention unless changes are made to water management or access is provided above Shasta Dam. Additional studies are necessary to determine how to best limit negative hatchery effects and the feasibility of reintroduction above Shasta Dam, such as the McCloud River reintroduction pilot project (CNRA 2017).

Winter-run Chinook Salmon management provides a unique case study in successful interagency cooperation to protect a species from extinction. Recovering the species to the point where they no longer need protection under the ESA and CESA will require continued collaboration to grow the population and expand their range by reconnecting historical habitat and establishing populations in streams that are less vulnerable to climate change. Effective management of winter-run Chinook Salmon during this time requires employing science advancements to improve life-stage monitoring (Johnson et al. 2017), adaptively managing reintroduction programs (ICF International 2016), and taking actions to maximize and preserve species and habitat diversity (Herbold et al. 2018). Continued commitment to collaborative stewardship, science-driven management, and holistic, landscape-scale restoration can, together, recover this iconic Central Valley species.

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## FULL RESEARCH ARTICLE

## Assessing the distribution and abundance of larval Longfin Smelt: What can a larval monitoring program tell us about the distribution of a rare species?

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Following its listing as threatened under the California Endangered Species Act in 2009, Longfin Smelt (*Spirinchus thaleichthys*) became a focus of resource managers in the San Francisco Estuary. Water exports were identified as one of the factors affecting Longfin Smelt abundance, and managers were challenged with balancing freshwater flows through the Sacramento-San Joaquin River Delta between human and ecosystem needs. This balance becomes especially challenging during the winter and spring when Longfin Smelt are spawning. Resource managers identified that the impact associated with entrainment of larval Longfin Smelt in the winter was uncertain, and to understand and manage this risk, new data was needed. In 2009 the Smelt Larva Survey was implemented and has since sampled newly hatched larvae from January–March. Here, I analyze this data and ask specific questions regarding distribution and densities of the larvae throughout five regions of the Upper Estuary – Napa River, Suisun, Confluence, Northern Delta, and Southern Delta – with the goal of understanding the spatial and temporal patterns of larval distribution since 2009. I found that larvae were most prevalent in the Suisun, Confluence, and Northern Delta regions, and less common in the Southern Delta and Napa River regions. Larval Longfin Smelt densities changed following a recent drought and record low population abundances. Median per-station averaged densities ranged from 154 to 274 fish per 1,000 m<sup>3</sup> between 2009 and 2013 but declined to 1 to 65 fish per 1,000 m<sup>3</sup> from 2014 to 2019. This survey data demonstrates that Longfin Smelt reproductive output has declined since their listing in 2009 and that their distribution into the Southern Delta is low relative to the rest of the Upper Estuary. These results reaffirm the species' continued decline since its listing, and that improving the abundance of spawning adults is one of the many important steps needed for long-term recovery and resilience.

**Key words:** California Endangered Species Act, larval fish, Longfin Smelt, San Francisco Estuary, smelt larva survey

Longfin Smelt (*Spirinchus thaleichthys*) was once one of the most abundant fish species in the San Francisco Estuary (Estuary) (Rosenfield and Baxter 2007). This small, anadromous fish with a one-to-three-year lifespan, migrates into low salinity and freshwater habitats during the late fall and early winter to spawn (Baxter 1999; Merz et al. 2013; Moulton 1974). Longfin Smelt are generally mature and ready to spawn by age two (CDFG 2009a). Once hatched, most young of the year Longfin Smelt rear in the Upper Estuary in important nursery areas (Hobbs et al. 2006) prior to emigrating downstream into more saline habitats, where they stay until adulthood (Rosenfield and Baxter 2007). Over time, the species declined to record low levels in the Estuary, leading to its listing as Threatened under the California Endangered Species Act (CESA) in 2009 (CDFG 2009a). During the status review for the listing process, the California Department of Fish and Game [now the California Department of Fish and Wildlife (CDFW)] identified that one of the important factors affecting Longfin Smelt abundance was loss associated with water diversions, chiefly from the State Water Project (SWP) and the Central Valley Project (CVP), which are located in the southern extent of the Sacramento-San Joaquin River Delta (Delta) (CDFG 2009a).

The SWP is designed to export approximately 4.2 million acre-feet of water per year and deliver it south for agricultural, municipal, and industrial needs<sup>1</sup>. The Banks Pumping Plant is the main export facility for the SWP and is located approximately 12 km northwest of the town of Tracy, CA. The Banks Pumping Plant has a rated diversion capacity of 10,300 cfs and is the majority of the export capacity between the two facilities. Similarly, the Jones Pumping Plant is the key export facility for the CVP and exports an average of 5.6 million acre-feet of water for agricultural, municipal, and industrial needs<sup>2</sup>. The Jones Pumping Plant is located approximately 3 km east of the Banks Pumping Plant and has a rated diversion capacity of 5,200 cfs. Because of their combined export capacity and geographic location, the SWP and CVP have a substantial effect on how freshwater routes through the Delta, and specifically, can draw water south through Old and Middle rivers which are distributaries of the San Joaquin River (Monsen et al. 2007). The magnitude of southward Old and Middle river flows combined with the seasonal occurrence of Longfin Smelt was shown to be a predictor of entrainment, which is defined as the involuntary movement of fish into the water diversions at the SWP and CVP (Grimaldo et al. 2009).

To both understand and minimize loss of fish to water diversions, the SWP and CVP constructed fish salvage facilities to collect fish which would have otherwise been entrained into the diversion pumps (Morinaka 2013a; Reyes et al. 2018). These facilities are located a short distance upstream of each project's pumping plants. The fish salvage facilities utilize a series of behavioral barriers, which are referred to as louvers, to guide fish into a bypass system, rather than a positive barrier which is meant to exclude fish, such as a fish screen (Brown et al. 1996). Upon collection, fish are counted and identified before being transported by truck back to the Estuary and released. This process is referred to as "salvage" (Morinaka 2013b). Data collected from the fish salvage facilities has been important in understanding impacts to native fish species associated with freshwater diversions of the Delta (Kimmerer 2008). However, the salvage process was not designed to salvage larval fishes. This is due to the size bias of louvers and the salvage process itself. The louvers are most effective on fish that can swim against strong currents (Castillo et al. 2012) which means that small,

<sup>1</sup> <https://water.ca.gov/Programs/State-Water-Project>

<sup>2</sup> <https://www.usbr.gov/mp/cvp/>

passively swimming larvae are unlikely to be diverted into the bypass system. Additionally, the louvers are most effective on fish larger than 30 mm fork length (Kimmerer 2008) and fish that are less than 20 mm fork length are not identified or counted within the salvage data (Morinaka 2013b). Because of this, a substantial data gap existed regarding entrainment and loss of Longfin Smelt larvae to SWP and CVP diversions.

As a result of the CESA listing, CDFW issued the California Department of Water Resources (DWR) an Incidental Take Permit (ITP) (CDFG 2009b), which permitted DWR to operate the SWP with some amount of incidental impact to Longfin Smelt. To account for the magnitude of larval Longfin Smelt entrainment at the SWP, CDFW estimated larval entrainment by coupling larval catch information collected from 1991–1994 and 2005 with outputs from a hydrodynamic model (CDFG 2009b). This approach involved some speculation as larval Longfin Smelt distribution data was limited and salvage data was unavailable for this life stage. This method demonstrated that in some years, larval entrainment could be relatively high if distribution of Longfin Smelt extended into the southern portion of the Delta (CDFG 2009b).

Pursuant to CESA, the ITP required DWR to avoid, minimize, and fully mitigate all impacts to Longfin Smelt due to operations of the SWP. Specifically, minimization of Longfin Smelt entrainment at the SWP diversions is achieved, in part, by managing water exports in real-time. This strategy relies on empirical negative relationships between Old and Middle river flows, fish distribution, and salvage (CDFG 2009b; Grimaldo et al. 2009). However, larval Longfin Smelt salvage and distribution data did not exist at the time, and without this information it would be difficult to minimize entrainment of larval Longfin Smelt in the winter and early spring (January–March). To partly address this issue, CDFW developed and implemented a new monitoring program in 2009, the Smelt Larva Survey (SLS), to monitor early-post hatch larval Longfin Smelt distribution between January and March across the Delta, Suisun Bay, and Suisun Marsh.

Unlike most other long-term monitoring programs of the Estuary, the SLS was explicitly designed to provide resource agency managers with real-time distribution information for weekly assessments of larval Longfin Smelt entrainment risk into the SWP. To best manage entrainment impacts on Longfin Smelt larva, resource managers utilized the SLS as a means of detecting uncommon but important distribution events. This required biologists to implement the SLS and then disseminate the data in real-time and determine if actions were needed to minimize the entrainment of Longfin Smelt larva (CDFG 2009b). Data collected by the SLS would affect operations of the SWP through distribution and density criteria identified within the ITP, or through expert opinion (CDFG 2009b). Because of this explicit need, the SLS does not sample the geographic extent of Longfin Smelt spawning within the Estuary, and instead focuses on distribution information east of San Pablo Bay.

Here I take an opportunity to analyze data collected by the SLS since 2009, and ask specific questions related to the distribution and abundance of post-hatch larval Longfin Smelt within the Upper Estuary.

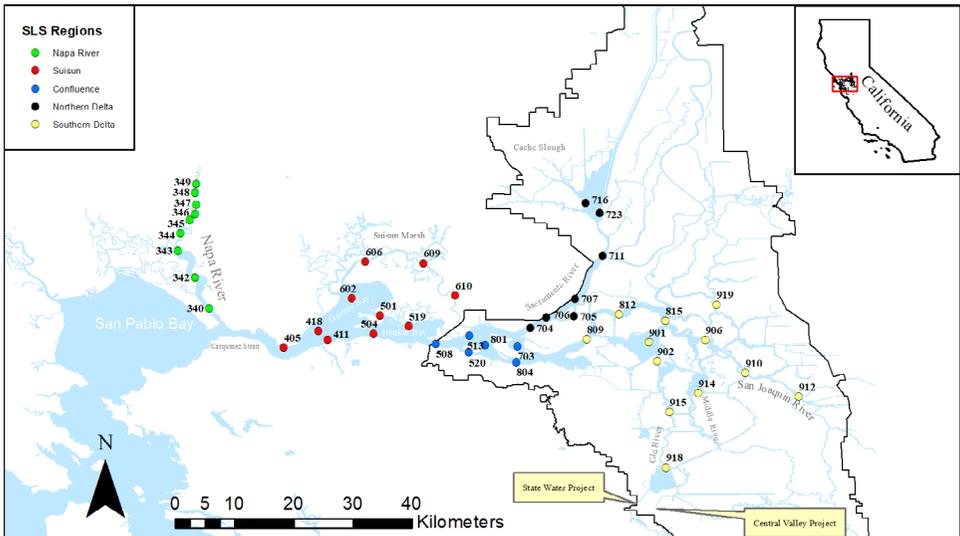
1. Has the abundance of larval Longfin Smelt changed since 2009 within the Upper Estuary?
2. Did previously described regional differences in distribution of Longfin Smelt in the Upper Estuary persist?

## METHODS

### Study Area

The San Francisco Estuary is where freshwater flows from California's Central Valley watersheds meet the landward influence of the Pacific Ocean. The Estuary is one of the largest estuaries on the west coast of the United States and consists of large, tidally influenced waterways which vary in salinity based on freshwater inflows. Here, following the spatial sampling grid of the SLS, I delineate the upper, eastward region of the Estuary as the waters east of, and including, Carquinez Strait (Fig. 1). The Upper Estuary consists of two major regions: (1) the Delta, which is mostly riverine channels lined with armored levees designed to hold back water and manage flood risk, and (2) Suisun Bay, which is the area between the Delta and the Carquinez Strait. Suisun Bay consists of large bays of mostly open water with tidal marshes and managed wetlands. Suisun Bay is comprised of Grizzly and Honker bays as well as Suisun Marsh on its northern side. Low salinity habitat typically occurs within Suisun Bay and is an important feature of nursery habitat for native fish species (Meng and Matern 2001; Hobbs et al. 2006).

The Delta is where the two major drainage basins of California's Central Valley converge to eventually flow into the Pacific Ocean. The Sacramento River enters the Delta from the northeast and provides most of the freshwater inflow, while the San Joaquin River enters from the southeast. The CVP and SWP water export facilities are located on the most southern end of the Delta and export water directly from distributaries of the San Joaquin River.



**Figure 1.** The geographic extent of the SLS sampling grid in San Francisco Estuary, delineated into five sub-regions: Napa River, Suisun, Confluence, Northern Delta, Southern Delta. Colored circles represent the geographic location of 44 survey stations associated with the Smelt Larva Survey. Black solid line represents the Legal Delta boundary

To compare distribution patterns of larval Longfin Smelt across the study area, I delineated the SLS sampling grid into five sub regions. These sub regions were based on a combination of ITP criteria, the legal Delta as defined by the Delta Protection Act, and general geography. (1) the Southern Delta region includes the 12 station entrainment criteria described in the ITP (CDFG 2009b) and geographically encompasses the San Joaquin River and its distributaries within the Delta. (2) the Northern Delta region includes seven SLS stations on the Sacramento River and Cache Slough which are upstream of the geographic confluence with the San Joaquin River. (3) the Confluence region is the geographic location of where the Sacramento and San Joaquin Rivers converge within the Delta and includes the remaining stations within the legal Delta boundary. (4) The Suisun region includes stations within Suisun Marsh, Grizzly Bay, and Honker Bay. (5) the Napa River region includes all stations within the Napa River (Fig. 1).

### Monitoring Data

For this analysis, I utilized data collected from two long-term monitoring programs: The Fall Midwater Trawl (FMWT) and the SLS. The FMWT started in 1967 with the purpose of monitoring the effects of water exports on the relative abundance and distribution of age-0 Striped Bass (Stevens 1977). However, the FMWT also collected data on other, predominantly pelagic, species and has since become important in monitoring long-term trends in relative abundance for some of the native species of the Estuary, including Longfin Smelt (CDFG 2009b; Nobriga and Rosenfield 2016; Eakin et al. 2020). Because Longfin Smelt typically spawns in its second year of life, I use the FMWT<sub>*n-2*</sub> indices of relative abundance calculated from all ages of Longfin Smelt collected in the fall from 2007 through 2017 to provide a reference to relative spawning stock size for each year *n* (Table 1).

The SLS was included as part of the broader Interagency Ecological Program<sup>3</sup> (IEP) – a multi-agency science consortium – and is one of several fish monitoring programs implemented by IEP within the Estuary. The SLS samples bi-weekly between January and March at 44 stations across the Upper Estuary and the Napa River, obliquely towing a fixed frame sled with skis for a single 10-minute tow. The 505 $\mu$ m mesh net has a 0.37 m<sup>2</sup> mouth area opening and is most effective at catching newly hatched Longfin Smelt ( $\leq 10$  mm fork length; Grimaldo et al. 2017). Data used for this analysis were collected by the SLS from 2009–2019. These data are publicly available through CDFW (IEP 2020).

As previously described, the SLS monitoring program was developed and implemented to provide real-time distribution information to agency managers regarding larval Longfin Smelt entrainment risk. Since its implementation in 2009, some changes have occurred to the SLS. First, the inaugural year of the SLS had only five surveys occurring from January–March. By 2010, the SLS was expanded in its temporal range to include a sixth survey and has conducted six surveys per season since. Second, as part of an agreement with SWP stakeholders, the SLS was expanded into the Napa River in 2014. The purpose for this expansion was to test hypotheses related to production of Longfin Smelt in smaller tributaries of the Estuary relative to production of Longfin Smelt within the Delta and Suisun. Napa River sampling ceased at the end of the 2018 sampling season due to a combination of funding and closing of the agreement.

The 2009 ITP concluded that the position of the low salinity zone within the Estuary would relatively predict the extent of adult Longfin Smelt spawning migrations into

<sup>3</sup> <https://iep.ca.gov/>

**Table 1.** Relative abundance of Longfin smelt and water year indices for the Sacramento and San Joaquin Rivers for each year of the Smelt Larva Survey. Fall Midwater Trawl abundance represents the relative size of the adult Longfin Smelt spawning stock in the Estuary each year.

Year	Previous FMWT <sup>a</sup>	Sacramento Valley Index	San Joaquin Valley Index
2009	13	Dry	Below Normal
2010	139	Below Normal	Above Normal
2011	65	Wet	Wet
2012	191	Below Normal	Dry
2013	477	Dry	Dry
2014	61	Critically Dry	Critically Dry
2015	164	Critically Dry	Critically Dry
2016	16	Below Normal	Dry
2017	4	Wet	Wet
2018	7	Below Normal	Below Normal
2019	141	Wet	Wet

<sup>a</sup>The FMWT index occurring from the fall two years prior to the start of the SLS each year.

the Delta during the winter (CDFG 2009b). Essentially, adult Longfin Smelt are going farther into the Delta when its dry and salinity moves further inland compared to when its wet and salinity is further west. To investigate this, I used water year indices developed by DWR which are based on water storage volume in the upstream reservoirs and measured snowpack<sup>4</sup>. Water years are defined as the period between 1 October and the following 30 September to avoid splitting California's wet season in two as use of a calendar year would. Water year classifications do not provide explicit flow information for the winter, but rather, provide a binned evaluation of the hydrologic conditions experienced in each of these years. These water year classifications are derived for both the Sacramento and San Joaquin River basins separately. I relied on the Sacramento Valley Index to represent the water year due to its disproportionate contribution of freshwater to the Estuary. Indices can be one of five classifications: (1) Critically Dry, (2) Dry, (3) Below Normal, (4) Above Normal, and (5) Wet (Table 1).

## Data Analysis

I calculated the Catch Per Unit of Effort (CPUE) for larval Longfin Smelt collected by the SLS, expressed as a relative density, to understand spatial and temporal trends of larval Longfin Smelt across the SLS sampling grid. To do this, I applied the following formula to each tow in the SLS across the entire time series<sup>5</sup>

$$N_t = F_t / V_t * 1000$$

<sup>4</sup> See <https://cdec.water.ca.gov/reportapp/javareports?name=WSIHIST>

<sup>5</sup> Established in the SLS metadata, see <https://wildlife.ca.gov/Conservation/Delta/Smelt-Larva-Survey>

Where  $N_t$  represents the relative density of larvae per 1,000 m<sup>3</sup> of water per tow  $t$ ,  $F_t$  is the number of larvae sampled per tow  $t$ , and  $V_t$  is the amount of water filtered through the net per tow  $t$ . To calculate the amount of water filtering through the net ( $V_t$ ), I applied the following formula

$$V_t = A * K * D_t$$

Where  $A$  is the net mouth opening in m<sup>3</sup>,  $D_t$  is the difference in the flowmeter reading from start to finish of each tow  $t$ , and  $K$  is the calibration factor for the flowmeter used to measure flow in the SLS monitoring program. To understand patterns in density through time, I calculated average CPUE for each station within each year, rounding up to the nearest whole fish. I will refer to this average as the per-station averaged density (PSAD) throughout the rest of this analysis. These PSAD were then plotted for all 44 stations from 2009–2019 simultaneously as a heat map, delineated by region, to visually compare trends through time.

Lastly, to investigate changes in presence over time, I applied an approach described in (Merz et al. 2011; Merz et al. 2013) and calculated a detection frequency for each station across all years to capture variation in larval Longfin Smelt detection by station and region within the SLS. The detection frequency was calculated by applying the following equation:

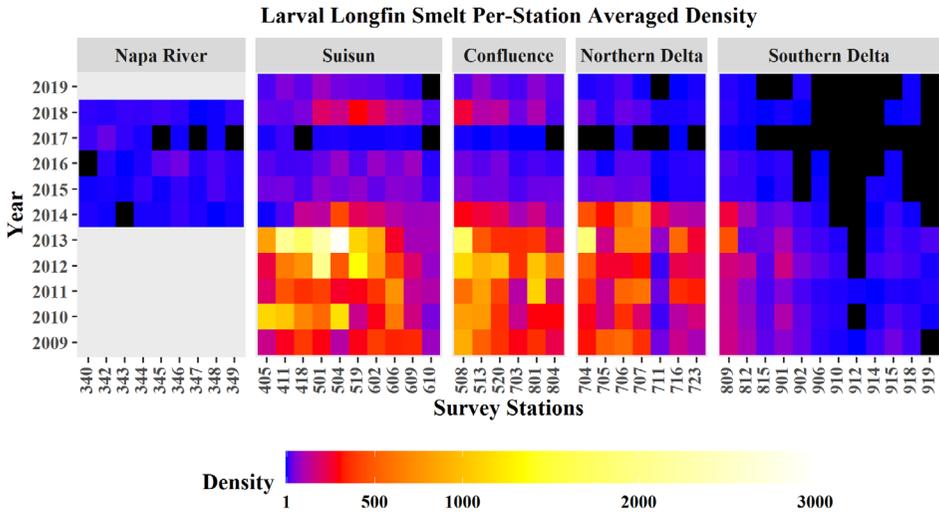
$$P_{sy} = S_{sy} / T_{sy} * 100$$

Where  $P_{sy}$  is the proportion of tows where larval Longfin Smelt were detected by station  $s$  per year  $y$ ,  $S_{sy}$  is the number of tows where larval Longfin Smelt were detected at station  $s$  per year  $y$ , and  $T_{sy}$  is the total number of tows conducted at station  $s$  per year  $y$ . To understand the relationship between CPUE and frequency of detection within the SLS, I used the R statistical software to calculate a Spearman's correlation coefficient between the two variables.

## RESULTS

### Per-Station Averaged Density

There were 2,463 SLS tows across the 44 monitoring stations in the Estuary, with a median tow volume of 185.452 m<sup>3</sup>. Longfin Smelt were collected in 60% (1,484) of SLS tows, resulting in a total of 78,955 Longfin Smelt. Of those fish, 246 (<1%) were collected in the Napa River region, 37,540 (48%) in the Suisun region, 21,818 (28%) in the Confluence region, 16,011 (20%) in the Northern Delta region, and 3,340 (4.3%) in the Southern Delta region. Relative densities of larval Longfin Smelt varied through time across all five regions, declining towards the end of the time series. PSAD were lowest in the Napa River and Southern Delta regions, with median PSAD of 4 fish per 1,000 m<sup>3</sup> and 3 fish per 1,000 m<sup>3</sup> respectively. Of the five regions, PSAD were highest in the Confluence region, with a median PSAD of 152 fish per 1,000 m<sup>3</sup>. Peak densities occurred in 2013, with a median PSAD of 274 fish per 1,000 m<sup>3</sup> across all regions. In contrast, 2017 had the lowest densities across the time series with a median PSAD of 1 fish per 1,000 m<sup>3</sup> across all regions. PSAD sharply declined starting in 2014, with median PSAD ranging from 154 to 274 fish per 1,000 m<sup>3</sup> between 2009 to 2013 to median PSAD of 1 to 65 fish per 1,000 m<sup>3</sup> between 2014 and 2019 (Fig. 2). The Spearman's correlation coefficient between PSAD and detection frequency showed a strong positive correlation, with a  $r_s = 0.8962383$  (Fig. 4).



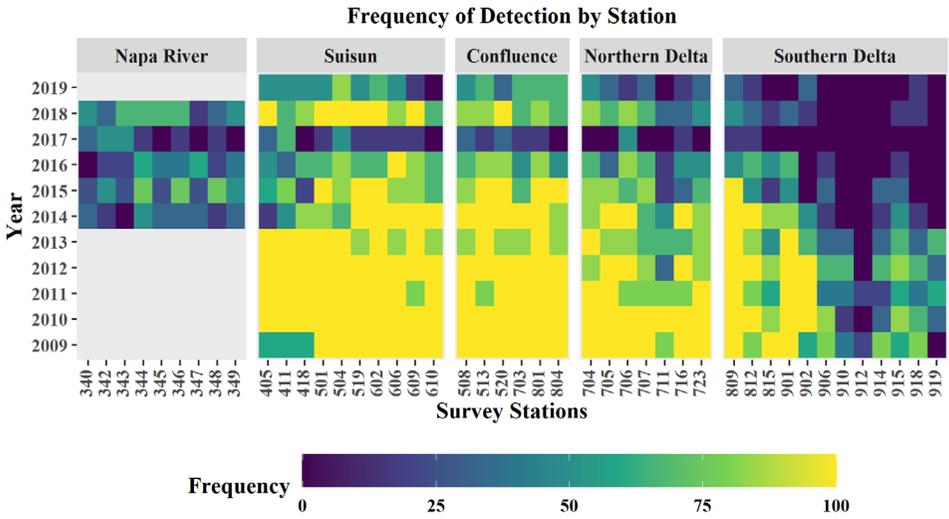
**Figure 2.** Per-station averaged densities for larval Longfin Smelt across four regions of the Upper Estuary. Densities were averaged at each station for each year of the Smelt Larva Survey. Legend presents average densities per 1,000 m<sup>3</sup>. Black boxes represent sampling stations which did not detect Longfin Smelt across all tows for that year.

### Frequency of Detection

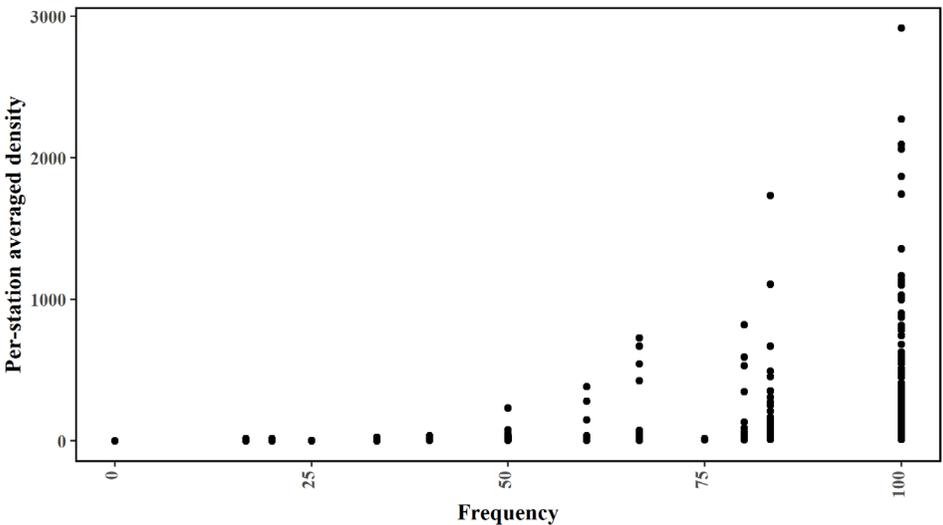
Longfin Smelt larva were detected in all 5 regions, however the frequency of these detections decreased through time (Fig. 3). In the Suisun region, detection frequency was highest in the early part of the time series, when fish were detected throughout the entire sampling period. Median detection frequencies were 100% from 2009 through 2014, but became more variable in recent years. Detection frequencies in the Confluence region followed a similar pattern to the Suisun region, where median detection frequencies ranged from 83.33% to 100% from 2009 through 2015 but declined in recent years. In contrast to the Suisun and Confluence regions, detection frequency in the Northern and Southern Delta regions was more variable throughout the time series, but still showed a similar decline through time. Median detection frequencies in the Northern Delta were 100% in 2009 and 2010 and reached a low of 0% in 2017. In the Southern Delta region, detection frequencies were highest in 2009 and 2010 with median frequencies of 80% and 83.33% respectively, however, detection frequencies in the latter part of the time series are the lowest of all regions, with median detection frequencies ranging from 0% to 33.33% from 2014 to 2019. Median detection frequencies in the Napa River region ranged from 16.67% to 50% for all years sampled.

## DISCUSSION

Understanding life stage specific trends in distribution and abundance through time is important for managing a rare species. For Longfin Smelt, understanding the density and distribution of larvae into the the southern portions of the Delta has been paramount for minimizing entrainment impacts in real-time. Because of this, a relatively new long-term monitoring program was created, the SLS, with a specific purpose of being used as tool



**Figure 3.** Frequency of larval Longfin Smelt detection by station across four regions of the Upper Estuary. The number of tows where larval longfin smelt were collected over the total number of tows conducted at a given station per year. Legend represents percent frequencies of detection from 0–100%.



**Figure 4.** Scatterplot of per-station averaged density on the y axis, expressed as fish per 1,000 m<sup>3</sup>. X-axis represents frequencies of detection from 0–100%.

for implementation of the 2009 ITP. However, I’ve shown here that this data provides important information which resource managers can also use to further the comprehensive understanding of the species within the Estuary.

## Estuary-Wide Change

My analysis displayed an abrupt change in larval densities across all stations and regions in the Upper Estuary, except for the Napa River. This abrupt change started in 2014 and persisted through 2019. Densities in the Napa River were already low when sampling began in 2014, and stayed consistently low until sampling ended in 2018. This trend follows a continuing decline of the species and has been observed since monitoring began several decades ago (Rosenfield and Baxter 2007; Thomson et al. 2010; Nobriga and Rosenfield 2016). These changes coincide with a recent drought that started in 2012 and lasted through 2016 (Lund et al. 2018). Given the continued decline combined with the expectation that droughts would negatively impact native species of the Estuary, low densities would be expected across the Estuary until conditions improved. Spawning stock abundance also hit record lows during this time, and a combination of dry conditions and low abundance is the most likely explanation for the patterns observed within the data. However, there are other pieces of evidence that also explain some patterns in the density and distribution of larval Longfin Smelt observed in the SLS. For example, young of the year Longfin Smelt aggregate within and around the low salinity zone (Dege and Brown 2004), which is commonly located in the Suisun and Confluence regions during the winter in most years. The mechanism behind the aggregation of young of the year Longfin Smelt in the low salinity zone has two prevailing hypotheses: (1) Longfin Smelt are transported down stream by freshwater flows and are then retained in the low salinity habitat, or (2) Longfin Smelt are hatching in or near the low salinity zone and are locally dispersed by tidal forces, as demonstrated in a particle tracking simulation by Kimerer et al. (2014). Data collected by the SLS may elucidate these mechanisms by providing data on newly-hatched Longfin Smelt, and one of the next steps in analyzing this data should be the use of quantitative models to investigate the distribution of recently hatched Longfin Smelt and their relationship with salinity to determine if aggregation within the low salinity zone is due to transport, local hatching, or a combination of both. Expansion of the SLS into portions of the Lower Estuary would also provide data that could inform how transport mechanisms affect larval distribution within the Estuary.

Relative abundance of longfin smelt in the fall was variable through this period, with the lowest FMWT index occurring in the fall of 2015. Longfin Smelt abundance is known to be positively influenced by the magnitude of freshwater flows exiting the Delta (Jassby et al. 1995; Kimerer 2002; Kimerer et al. 2009). Therefore, prolonged dry periods are expected to negatively impact relative abundance, while wetter years are expected to positively impact relative abundance. The high flows observed in 2017, one of the wettest years on record, contributed to a moderate increase in relative abundance from the previous year. However, increases in larval densities which were observed in the Suisun and Confluence areas in 2019 were still lower than those observed prior to the 2014 decline. In particular, larval densities in the Northern Delta region decreased by up to two orders of magnitude, and 2019 data from this region showed relatively little change in density in response to the moderate increase in spawning stock abundance from 2017.

## Regional Distribution

My findings show that prior to 2014, larval Longfin Smelt were consistently detected at nearly every station across all regions except the Southern Delta. Adding to previous descriptions of Longfin Smelt distribution, this data suggests that spawning within the

Southern Delta may be more consistent and widespread than previously described in 2009 (CDFG 2009a). However, densities observed in this region remain relatively low and are largely driven by catches at stations along the eastern portion of the San Joaquin River.

The Southern Delta region is an important area for California's water conveyance and infrastructure system as this is where freshwater is exported from the Delta. Longfin Smelt larvae can occur in the Southern Delta as a result of multiple processes: 1) voluntary movement to spawning habitat by adults resulting in hatching in the region, 2) entrainment into the region via hydrodynamic processes, or 3) a combination of both (CDFG 2009b). The data presented here demonstrates that Longfin Smelt are spawning in the Southern Delta. This is consistent with historical descriptions of adult Longfin Smelt presence in portions of the lower San Joaquin River (CDFG 2009a; Merz et al. 2013; Rosenfield 2010). However, my findings show that detection of Longfin Smelt larva in the SLS is positively correlated to relative larval densities at a given sampling location. Due to this correlation, resource managers may face challenges when attempting to discern a Southern Delta distribution of Longfin Smelt when abundance is low. This may result in a higher reliance on expert opinion to adequately minimize entrainment impacts to larvae under such circumstances.

### **Napa River Production**

Recently, the Napa River has been shown to be a potentially important spawning area for Longfin Smelt in some years (Lewis et al. 2019). Contributions of larval Longfin Smelt from San Francisco Bay tributaries were hypothesized to be substantially higher in wetter years (CDFW 2014) which was posited as an explanation for increases in Longfin Smelt abundance following a wet winter and spring period. SLS data collected from the Napa River shows that detection of Longfin Smelt is inconsistent, and when detected, Longfin Smelt densities were typically low when compared to areas east of the Napa River. Importantly, when densities in the Upper Estuary increased following the drought (2014–2016), catches in the Napa River stayed consistently low. This data indicates that the importance of the Napa River regarding Longfin Smelt production is relatively low when compared to portions of the Delta and Suisun, even under wet conditions such as those experienced in 2017. However, 2017 is the only wet year where the SLS sampled the Napa River, and this data may not represent a more typical wet year. For example, Lewis et al. (2019) were able to collect larvae on the Napa River for 2019 and although they used a different net, they did show substantially higher densities of larvae in the Napa River in 2019, a wet year not sampled by the SLS.

### **Conclusion**

Newly developed monitoring programs can provide important information regarding data gaps for a rare species. Here I evaluated how a long-term monitoring program, which was launched in response to the listing of a species under CESA, has provided some useful insight into larval Longfin Smelt densities over time, but remains consistent with previous descriptions of Longfin Smelt spawning within the Estuary. Data presented in this analysis is helpful in understanding the current distribution and abundance of young of the year Longfin Smelt within the Upper Estuary, but requires more sampling in the Lower Estuary as well as regular sampling of the Napa River to better understand the relationship that larval Longfin Smelt have with fresh water flows. The SLS data also demonstrates that distribution in the

Upper Estuary can be extensive during wet years, as was observed in 2011. Next steps should focus on investigating changes in habitat conditions in these regional areas, specifically changes in spawning and rearing habitat for Longfin Smelt eggs and larvae and how those affect detection within the SLS. Additionally, there is a need to further investigate the interaction between larval Longfin Smelt distribution and hydrodynamic processes of the Estuary, which include entrainment into and within the Southern Delta region. Lastly, there is a need to sample a broader area to capture the full extent of larval Longfin Smelt distribution within the Estuary, specifically expanding sampling into the Lower Estuary, such as San Pablo Bay and San Francisco Bay. A broader sampling area will provide a more complete understanding of the importance the Upper Estuary has in the production of Longfin Smelt each year.

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# Amphibians



# Amphibians

Photos, previous page:

Photo captions and credits from left to right, top to bottom

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1. Santa Cruz long-toed salamander (*Ambystoma macrodactylum croceum*).  
Photo Credit: Mark Allaback, Biosearch Environmental Consulting
2. Black toad (*Anaxyrus exsul*). Photo Credit: Scott Trageser.
3. California tiger salamander (*Ambystoma californiense*).  
Photo Credit: Mike van Hattem, CDFW
4. Foothill yellow-legged frog (*Rana boylei*).  
Photo Credit: Mark Allaback, Biosearch Environmental Consulting
5. Cascades frog (*Rana cascadae*).  
Photo Credit: Oregon Caves, National Park Service, CC BY 2.0
6. Limestone salamander (*Hydromantes brunus*).  
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FULL RESEARCH ARTICLE

## **Foothill yellow-legged frog breeding biology in a semi-regulated river, Humboldt County, CA**

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River-breeding foothill yellow-legged frogs (*Rana boylei*) are endemic to California and Oregon. Across this wide geographic range, many populations have declined due habitat loss, non-native competitors and predators (e.g., American bullfrogs [*Lithobates catesbeianus*], Centrarchid fish), and disrupted water flow due to dams. Even when flow conditions are not extensively regulated, managers still require basic and region-specific information about the breeding biology of this species to prevent further decline. To document spatiotemporal dynamics of reproductive output during drought and high flow years, we surveyed a 13.5 km reach of the lower Mad River, Humboldt County, CA approximately 70 km downstream of Matthews Dam. We found relatively high densities of egg masses (39 to 59 masses / km). Egg masses were generally laid on small cobbles (mean  $\pm$ SE diameter =  $11 \pm 0.24$  cm) at depths between 0 and 20 cm, and 95% of egg masses were laid within 6 m of the wetted edge. Egg masses were disproportionately found in the tailouts of fast runs and glides, and found less often than expected in side arms, runs, and riffles than would be expected by chance. Breeding timing appeared to be more related to rapid decreases in stream flow variance than air temperature. Taken with previous information about the species, our results suggest that *R. boylei* rely on multiple cues to initiate breeding. Our results can be used to help inform breeding timing and habitat use by *R. boylei* breeding under natural flow regimes in Northern California. Our recommendations for future research include further investigating upland habitat use by post-metamorphic life stages factors that influence breeding site selection.

**Key words:** amphibian, flow rate, land use, oviposition site selection, *Rana boylei*, river-breeding frog, tributaries

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River ecosystems can be highly vulnerable to human disturbances, which often affect the survival and spatial distribution of aquatic species (Bondi et al. 2013). Anthropogenic activities, such as water diversion, hydroelectric power generation, flow regulation, and other habitat modifications alter the regular flow regimes of rivers and streams, which can cause changes in species assemblages and biodiversity loss (Kupferberg 1996; Femmer 2002; Tonkin et al. 2018; Best 2019). Dam construction and operation can disrupt natural water flow cycles and pose a threat to river-breeding and flood-plain-breeding frogs (Eskew 2011; Kupferberg et al. 2012; Peek et al. 2020). In particular, dam operations to generate electricity can affect the reproductive success of amphibian populations, as egg masses are subject to scouring and stranding from irregular flow timing (Wheeler and Welsh 2008). Flow rates from dams can fluctuate seasonally, monthly, daily, sporadically, or remain static depending on the type of dam and management plan (Kupferberg et al. 2011). Changes in flow regime from historical norms are dependent on multiple factors, including the purpose of damming (i.e. hydroelectric, reservoir, and flood control), size of and distance to the dam, and number of downstream tributaries (Bunn and Arthington 2002). Here, we define “semi-regulated” to describe rivers that are dammed but generally mimic historical seasonal patterns of flow timing and magnitude. Understanding the breeding ecology of sensitive species in semi-regulated rivers can offer valuable insight into beneficial flow regimes in regulated systems.

Foothill yellow-legged frogs (*Rana boylei*) are obligate stream breeding ranid frogs that are particularly vulnerable to perturbations from river and stream flow regulation. Through much of their native range in California and Oregon, *R. boylei* are experiencing population declines (CDFW 2019). Several regionally distinct genetic clades were recently listed under the California Endangered Species Act, including the lineages in the Southwest/South Coast, West/Central Coast, and East/Southern Sierra clades (endangered) and the Northeast/Northern Sierra and Feather River clades (threatened; CFGC 2020). While the California Fish and Game Commission determined that listing of the Northwest/North Coast clade was not warranted, this clade is still designated as a Species of Special Concern in the state. *Rana boylei* population declines have been attributed to anthropogenic disturbances such as river modifications from damming and introduction of invasive species including the American bullfrog (*Lithobates catesbeianus*), signal crayfish (*Pacifastacus leniusculus*) and largemouth bass (*Micropterus salmoides*) (Wiseman et al. 2005; CFGC 2020).

*Rana boylei* relies on key habitat features for reproduction in lotic environments, including low flow velocity sites and cobble-sized substrate for oviposition sites (Rombough and Hayes 2005; Lind et al. 2016). Eggs and tadpoles are most successful under natural seasonal flow patterns wherein rivers flow quickly during wet winters and slowly during dry summers (Kupferberg 1996; Lind et al. 1996). *Rana boylei* typically initiates breeding in late spring, when temperatures increase, and river flow rates have declined and stabilized (Wheeler et al. 2018). Breeding season duration is site specific and variable by population and year, spanning from 14 to 31 days (Storer 1925; Zweifel 1955; and Van Wagner 1996). Nonetheless, *R. boylei* breeding appears to be relatively synchronous within a population compared to other ranids such as lentic breeding northern red-legged frogs (*R. aurora*; Wheeler et al. 2018). *Rana boylei* can delay oviposition in response to increased rain during the breeding season, which may allow for higher reproductive success by avoidance

of egg mass scouring during late-season flooding events (Kupferberg 1996; Ashton et al. 1998; Bondi et al. 2013). Additionally, oviposition has been correlated with increasing air temperatures, a proxy for warmer water temperatures that likely facilitate successful reproduction (Wheeler et al. 2018) and presumably correspond with lower summer flows. While tadpoles survive higher flow rates than egg masses (Lind et al. 1996), larval growth and survival may be negatively impacted by flow rates that are exceptionally high or that occur late in the breeding season (Kupferberg et al. 2011). Metamorphosis typically occurs three to four months after hatching, although the timing of metamorphosis is dependent on water temperature and food availability (Ashton et al. 1998). A central concern for conservation of *R. boylei* depends largely on understanding the impacts of regulated flow regimes on instream habitats, particularly during the species' reproductive and growing seasons.

The terrestrial environment surrounding rivers may also negatively affect *R. boylei* populations, and spatial patterns of extirpation are consistent with a habitat destruction hypothesis (Davidson et al. 2002). Previous work has found that preserving a riparian buffer around water sources is important for amphibian conservation (Semlitsch and Bodie 2003; Marczak et al. 2010). Urbanization and human encroachment pose a threat to over one-third of the world's amphibian species as a result of habitat loss, fragmentation, and degradation of available habitat (Hamer and McDonnell 2008). However, riparian habitats are often overlooked in amphibian conservation even though they are vital for the completion of life stages for many species (Semlitsch and Bodie 2003). With the rate of urbanization rising on a global level it is necessary to consider the implications of land use changes on neighboring riparian and aquatic habitats (Alig et al. 2004).

The Mad River and its watershed, in Humboldt County, California, have been altered by agriculture, urbanization, and the installation of the Matthews Dam, which captures approximately 25% of the total upstream watershed runoff. Releases have been designed to maintain aquatic habitat for listed salmonids during low-flow months, typically in late summer and early fall (HBMWD 2004). Despite having some hydroelectric capacity, the flows from this dam are mostly regulated by overflow, creating relatively natural seasonal flow patterns downstream. In addition, much of the lower Mad River is surrounded by agriculture and urban development.

Given that the Matthews Dam provides a relatively natural flow regime for oviposition sites, investigating suitable breeding habitat along the Mad River may be useful for informing future management strategies to conserve *R. boylei* in more regulated systems. In addition, uncertainty in future water availability caused in part by recent variation in precipitation, including a historic multi-year drought, is a major driver of environmental politics and management in California (e.g., Doremus and Tarlock 2008). In this study, we aimed to incorporate the differences of water availability during years with normal flow (2011), drought (2015), and high flow during El Niño Southern Oscillation (2016) in an effort to provide guidance on river conditions that support reproduction of *R. boylei*. Additionally, this study aimed to describe the spatial and temporal patterns of oviposition in relation to adjacent terrestrial habitat and seasonal temperature patterns to offer insight into the role that riparian buffers play in supporting the adult life stage of *R. boylei*.

To address these issues, we explored three complementary hypotheses regarding oviposition sites on the lower Mad River. First, we assessed the impacts of flow rates and air temperature on oviposition timing within years by identifying the stability of flow rate during the breeding period as well as the number of days since peak flow. We expected to find a pattern of early oviposition during low flow rates (drought year), and later oviposition

during years with high flow rates. Second, we compared land-use types and river tributaries' spatial relationship to oviposition sites, predicting that oviposition may be associated with riparian areas, closer to tributaries, and further from urban areas. Finally, we identified spatial patterns of oviposition sites within the Mad River, describing distance to wetted edge, depth of egg mass or larvae, and river features used for oviposition, and we predicted these physical parameters would be similar to those used by frogs in other regions (Bondi et al. 2013).

## METHODS

### Study Area

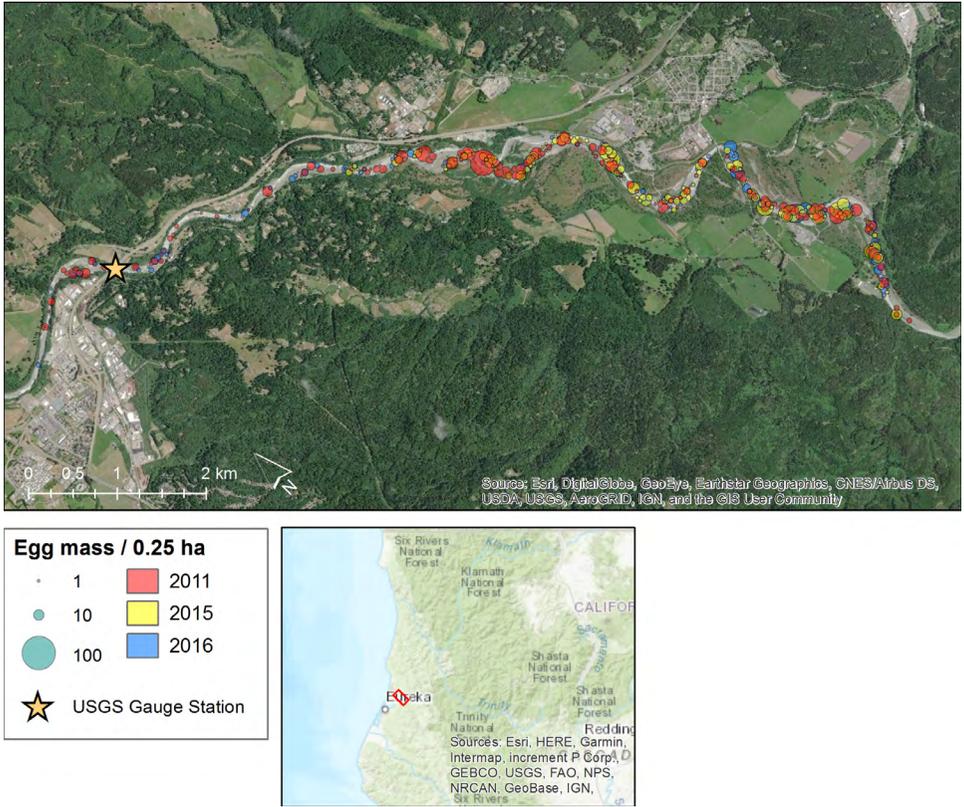
We conducted fieldwork in a 13.5-km reach of the Mad River (between 40.91, -124.01 and 40.84, -123.98, NAD83) approximately 70 km downstream from Matthews Dam in Humboldt County, California (Fig. 1). Two major tributaries join the Mad River below the Dam, Maple Creek and the North Fork Mad River. We completed surveys during the breeding season in three years (2011, 2015, 2016). We classified 2011 and 2016 as high flow years, whereas 2015 was classified as a year of peak drought. Annual mean discharge rates (in cubic feet per second; cfs) during study years varied from 770–1945 cfs (USGS NWIS 2017). Annual discharge rates were available for 2008–2016 and ranged from 410–1945 cfs, indicating that our study years spanned the range. Land use of the floodplain near the river consisted primarily of small-scale agriculture, rural residential use, and urban development.

### Field Surveys

We conducted field surveys in 2011, 2015, and 2016. We timed surveys based on the estimated peak egg mass deposition, which was variable between years. Therefore, to determine the timing of peak *R. boylei* breeding activity, and initiation of survey, we relied on spot checks at reliably occupied upstream sites as the breeding season approached (Bourque and Bettaso 2011). We surveyed the entire study area once per year. To complete visual encounter surveys, two or more biologists walked the wetted edge, either upstream or downstream, depending on access points. We counted all egg masses, assigned an approximate embryonic development stage (i.e., Gosner stage, Gosner 1960) and recorded egg mass location using a global positioning system (GPS) unit. We grouped Gosner stages into four approximate categories: round (1–14), bean (15–18), tail (19–21), and hatching (i.e., when larva were observed on/at the egg mass). Additionally, in 2015, we recorded microhabitat variables for each egg mass, including depth of egg mass, substrate size (median diameter), and distance to wetted edge.

### Oviposition Timing

To study the effect of flow rate on *R. boylei* breeding in the lower Mad River, we estimated breeding season start and end dates, and compared these date ranges with discharge rates during years of high (2011 and 2016), and drought (2015) flows. We estimated oviposition start date (beginning of breeding season) as 21 days prior to the first observation of a 'hatching' (latest stage) egg mass. Estimated breeding season end date was estimated as 21 days after the last observation of a 'round' (earliest stage) egg mass. To quantify the relationship between flow rate and oviposition date, we estimated standard deviation of daily discharge rate, as measured at the USGS Mad River Station near Arcata (USGS NWIS



**Figure 1.** Study site along the lower Mad River, Humboldt County, California with foothill yellow-legged frog egg mass locations from three survey years.

2017), using a moving time window of 21 days. While precise estimation of breeding onset is difficult using this backdating approach, we selected a window based on development rates in a lab setting with mean air temperatures comparable to the conditions during the breeding season in our study area (Kupferberg et al. 2011). We then used piecewise regression to identify the breakpoint in the standard deviation of flow rate (Toms and Lesperance 2003), after which we compared breakpoints to the estimated oviposition start date. We also assessed whether pulse flows that may have resulted in decreased survival occurred during the breeding season time period. We compared the log ratio of daily maximum and minimum discharge to survival rates measured in Kupferberg et al. (2012).

To estimate the effects of temperature on breeding initiation, we compared air temperature within and between years because water temperature was not available. Relationships between air and water temperature are generally correlated, although not necessarily 1:1 (e.g., Morrill et al. 2005). We acquired daily maximum, minimum, and mean temperatures from the National Weather Service's weather station on Woodley Island, Eureka, California (USW00024213). We used ANOVA to compare minimum and maximum temperatures during estimated breeding season, one week prior and post estimated breeding start date, and mean temperatures between 15 March and 15 April during each study year and pooled across years.

## Oviposition Site Selection

To investigate the influence of adjacent terrestrial land use on oviposition site selection, we digitized the area around the study reach using NAIP imagery (US Department of Agriculture 7 June 2014, 1 m resolution), dividing it into six land types. These types included the following: agriculture, developed, open areas, ponds, trees, and the river (Table 1). We digitized these areas by relying on our collective knowledge of land use in the study area. We defined river habitat as anywhere within the bankful margin (i.e., the area where water is contained within the channel under most flow conditions, and where flows do not connect with the floodplain). Previous research has found that amphibians are most successful when there is a 159 to 290 m riparian buffer around aquatic habitat (Semlitsch and Bodie 2003). With this in mind, we digitized all land within 1 km of the survey area. Egg masses tended to be concentrated within particular sections of the river. To control for spatial autocorrelation among points, we calculated Ripley's K to identify a distance at which egg mass density was no longer correlated (Bivand et al. 2008). We then created a raster of egg mass density using a cell size (50 m) equivalent to the minimum distance that resulted in no significant spatial autocorrelation.

Next, we used generalized linear models with a Poisson link to examine the relationship between egg mass density and percent of each land use type at different distances from the egg masses. For each survey year, we created multiple models based on different buffer distances (from 100 m to 1,000 m, in intervals of 50 m) around each cell to identify the distance from egg masses that best explained variation in egg density. For example, for the 100 m buffer, we calculated percent of each of the six land use types within 100 m of each 50m cell and created a generalized linear model to predict the relationship between the two. We created separate models for each buffer distance. These distances represented different "hypotheses" related to the most important spatial scale to explain difference in egg mass density. Models were ranked using Akaike's Information Criterion corrected for small sample sizes ("AICc") (Burnham and Anderson 2002). We then assessed the relative contribution of different land use types to explain the spatial variation in egg mass density. We expected that egg mass density would be positively correlated with trees and undeveloped open space and negatively related to agriculture and developed areas.

Finally, we examined oviposition microhabitat selection within the river in two ways. First, in 2015, we measured three variables at every egg mass: water depth, distance to wet-

**Table 1.** Land cover/land use categories used to digitize terrestrial areas surrounding the lower Mad River.

Type	Description
Riparian	Low lying vegetation and broadleaved trees near bodies of water
Trees	Mainly conifers. Other trees indistinguishable between conifers and riparian
Agriculture	Actively or recently farmed
Open	Meadows, lawns, pastures, and areas that may have been previously farmed but had no observable signs of agriculture
Ponds	Standing water
Developed	Pavement, houses, and other signs of human habitation or development
River	Bankful margin of the Mad River

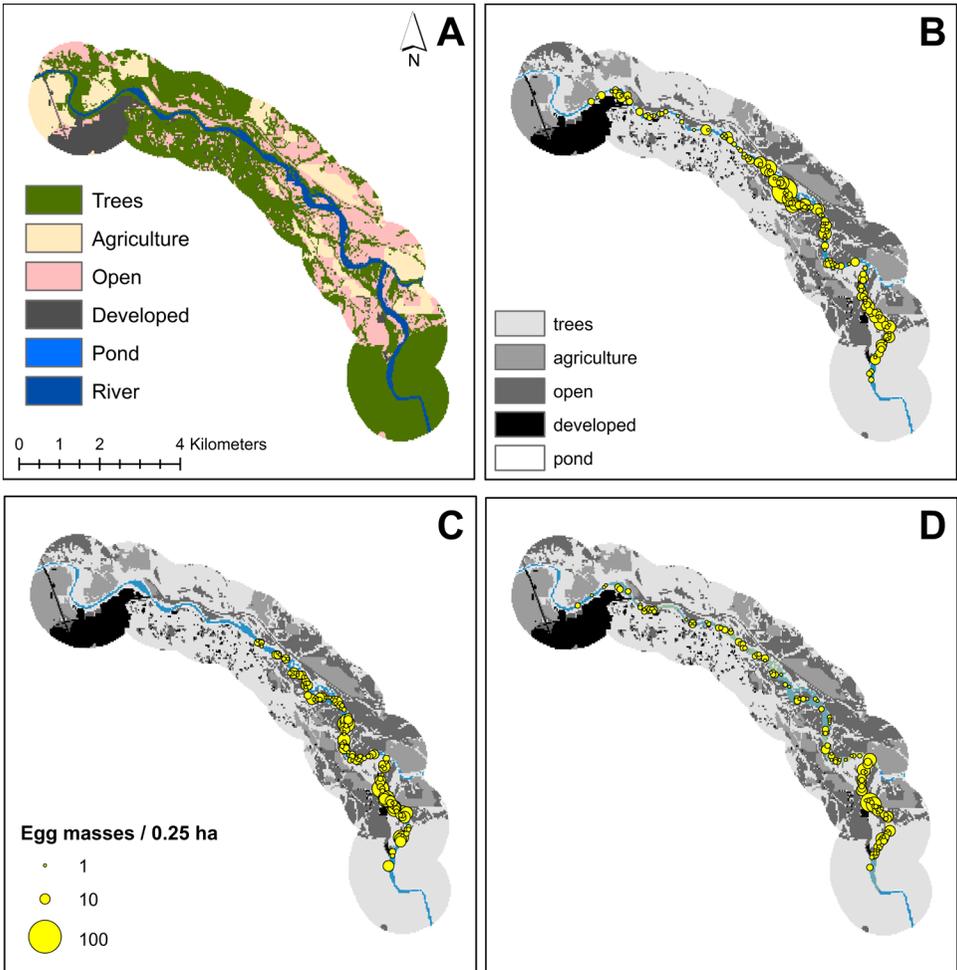
ted edge, and substrate size. We summarized these data in relation to growth stage. Next, we digitized the river within the survey area using standard classifications for river features believed to influence *R. boylii* oviposition sites: backwater, disconnected pool, fast run, fast run tailout, glide, rapid, riffle, riffle tailout, run, run tailout, and side arm (Hauer et al. 2009). We acquired georectified aerial imagery generated by National Marine Fisheries Service from flights flown in May or June of each year of the study. Resolution and precision were  $<0.5$  m (i.e., the imagery was more precise than the GPS locations of the egg masses themselves). Each mapped egg mass was assigned the river feature within which it fell, or was closest to, if the egg mass fell outside of the mapped area. We then calculated total percent area of each river feature represented within the survey area and compared these percentages with the percent of total egg masses found in each. Following standard habitat selection theory, we assumed that river features with a higher percentage of egg masses than was available in the area represented features “selected” by the frogs, with areas under-represented by egg masses were “avoided.” We expected egg masses to be concentrated in “slower” parts of the river (disconnected pools, backwaters, and tailouts), and that these concentrations would be consistent across years.

## RESULTS

We observed 2,308 egg masses total during our three years of sampling in the study area (Fig. 2; Table 2). The spatial distribution of these egg masses varied considerably across the study reach and egg mass density ranged from  $0.004/\text{m}^2$  to  $0.024/\text{m}^2$ . We detected particularly high egg mass densities in a few locations (e.g., around the confluence of the north and south forks of the Mad River (Fig. 2), which remained consistent across the three study years. Egg mass density was lower toward the downstream end of the study area during the drought year (2015) compared to high flow (2011 and 2016) years.

### Oviposition Timing

Mean daily discharge rates (in cfs) in the lower Mad River peaked many times throughout the winter season, while the onset of spring brought a final peak and subsequent decline (Fig. 3). In 2011 and 2016 (high flow years), the estimated oviposition start date occurred 26 and 31 days after a sharp reduction in the standard deviation of flow rate (Fig. 3). However, in 2015 (drought year), flow rate stabilized much earlier in the year and the estimated oviposition start date occurred approximately seven weeks after this breakpoint. The estimated breeding season started and ended earlier during the drought year (April 17 to June 9) compared to the high flow years of 2011 (May 25 to July 9) and 2016 (May 3 to June 24). Flow on the estimated breeding start date was 844, 437 and 551 cfs in 2011, 2015 and 2016, respectively, and it declined to 182, 78, and 102 cfs by the estimated breeding end. During the estimated breeding seasons, the min:max discharge ratio did not exceed one, indicating that there were no post-breeding pulses that would negatively impact survival. Across all three years, the onset of the breeding season was marked by significantly warmer ambient air temperatures compared to the week prior to onset and between March 15 and April 15. Across all three years, there was a particularly significant increase in the daily minimum temperature, from  $7.4^\circ\text{C}$  one week prior to breeding onset to  $8.7^\circ\text{C}$  one week after the estimated onset (Appendix Table 1).



**Figure 2.** Land use / land cover types along the lower Mad River, Humboldt County, California (A); foothill yellow-legged frog egg mass densities in 2011 (B), 2015 (C), and 2016 (D).

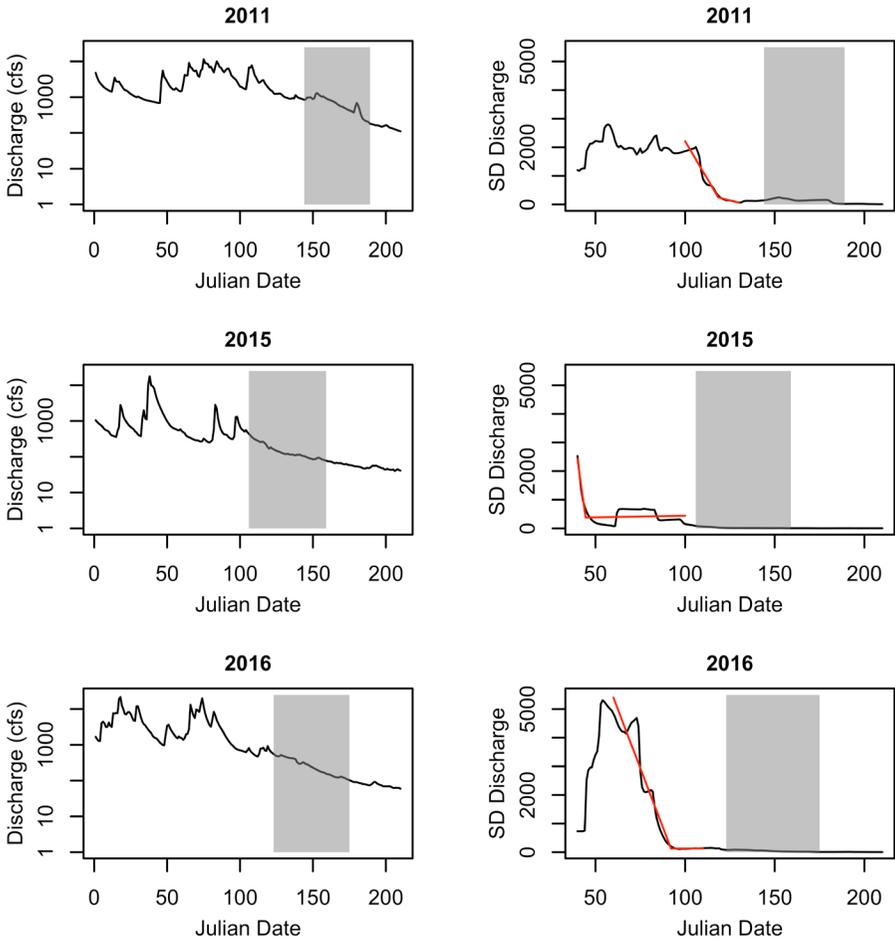
### Oviposition Site Selection

The buffer distance that best explained variation in egg mass density compared to surrounding land use types varied by year (Appendix Table 2-4). In 2016, the best model included a buffer distance of 400 m; in 2011, 450 m; and in 2015, 900 m (Table 3). Selection of land use types did not neatly fit with our original expectations. In all three years, the percent cover of trees within the buffered area was negatively related to egg mass density. By contrast, the percentage of agriculture, open areas, and ponds were all positively related to egg mass density. In 2011, developed area was positively correlated with egg mass density, but in 2015 and 2016 it was negatively related.

Egg masses were almost exclusively found between 1 and 10 m from the wetted edge and between 0 and 20 cm in depth (Fig. 4), with 95% found within approximately 6 m of the wetted edge. Mean substrate diameter was 11 cm (min = 2 cm, max = 120 cm, 25% =

**Table 2.** Results of egg mass surveys in lower Mad River, Humboldt County, CA, during peak breeding season across three years.

Year	Survey Dates	Number of Egg Masses Observed	Egg Mass Density per Kilometer
2011	June 14 - June 17	795	59
2015	May 7 - May 22	986	73
2016	May 23 - June 3	526	39

**Figure 3.** Flow rate (mean, left and standard deviation, right) at the Mad River Gauge Station, Arcata, California from January 1 to July 31 during years of foothill yellow-legged frog survey. Gray rectangles indicate estimated breeding season, defined as a 21-day hatching period on either side of survey dates. Red line indicates the piecewise linear regression and breakpoint. Standard deviation was calculated within a 21-day moving window.

**Table 3.** Coefficient estimates for the top model from each year relating to foothill yellow-legged frog egg mass density to percent cover of surrounding land use types in the lower Mad River, California (standard error given in parentheses) using buffer distances that best explained spatial variation in egg mass density for that year.

Year	Best supported buffer distance	Intercept	Trees	Agriculture	Open	Developed	Pond
2011	50 m	-5.15 (0.33)	-1.57 (4.75)	-4.74 (1.13)	8.67 (0.56)	NA	7.45 (0.47)
2015	900 m	-4.98 (0.33)	-16.45 (1.39)	9.16 (1.87)	4.82 (0.63)	-920.93 (128.6)	61.96 (5.27)
2016	950 m	-2.76 (0.32)	-7.34 (1.14)	4.29 (1.34)	0.89 (0.80)	-1,975.72 (226.65)	37.47 (6.36)

7 cm, 75% = 14 cm). Overall, egg masses were significantly, disproportionately found in the tailouts of fast runs and glides, but under-represented in side arms, runs, and riffles ( $\chi^2 = 392.03$ ,  $df = 10$ ,  $p < 0.001$ ) (Fig. 5).

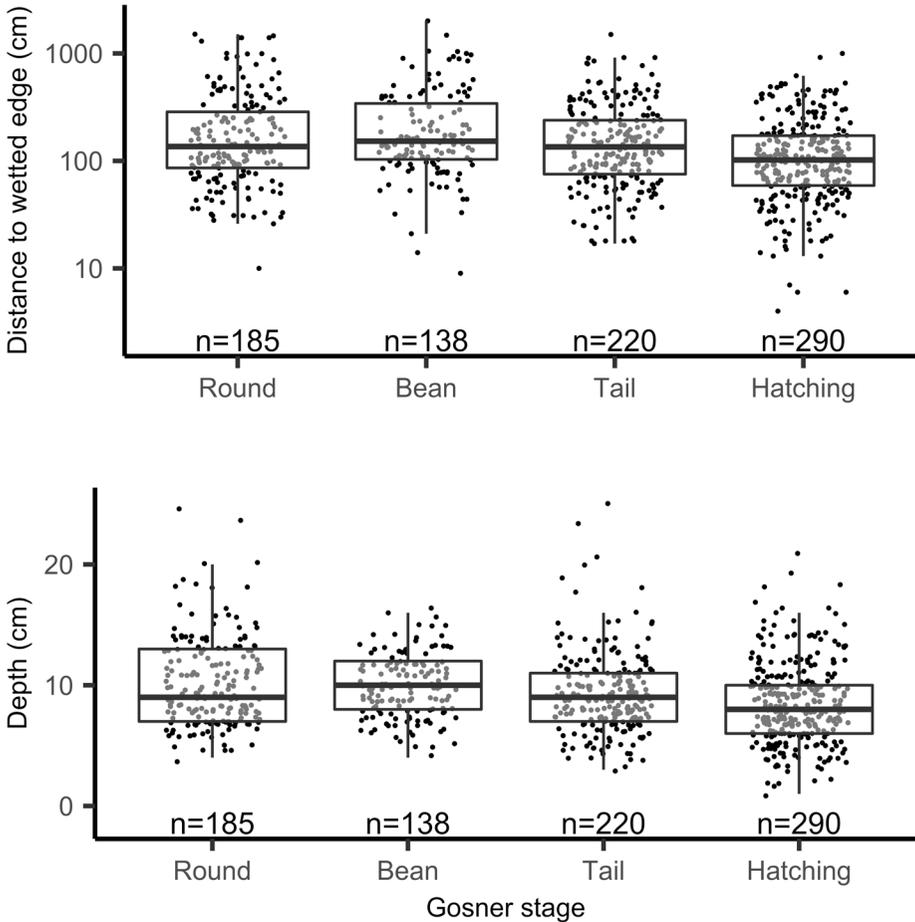
## DISCUSSION

In this study, we examined spatial and temporal distribution of *R. boylii* egg masses across two high flow (2011, 2016) and one drought year (2015). In each of the three years, onset of breeding occurred more than a month after the final major pulse of rainfall, and minimum air temperatures one week after estimated breeding onset were significantly warmer than previous weeks. High egg mass densities occurred closer to agriculture, open areas, and ponds, and further from trees and developed areas.

Egg mass density has been used as a metric of population size in *R. boylii* and unregulated rivers maintain a more consistent level of egg mass density (Kupferberg 2012). Egg mass densities calculated over the total length of our study reach (39 to 59 egg masses/km, Table 2) were comparable to density estimates from the South Fork Trinity River (35 to 47 egg masses/km) and Hurdygurdy Creek (18 to 30 egg masses/km) in northern California (Lind et al. 2016). However, there was substantial variation along the reach, with egg mass densities approaching 200 / km in certain 1 km stretches of the river (Fig. 2).

Average substrate size at observed egg mass in our study (11 cm) were comparable to a study in the South Fork Trinity River and Hurdygurdy Creek, which found the majority of egg masses on cobbles between 6.4 and 25.6 cm in diameter (Lind et al. 2016). However, our results differ from a study in the Sierra Nevada, where egg masses were observed fairly equally on cobble and boulder substrate (Yarnell et al. 2011). This could be a result of the difference in stream habitat characteristics between higher elevation mountain and coastal drainages, but it may also reflect differences in traits between *R. boylii* clades.

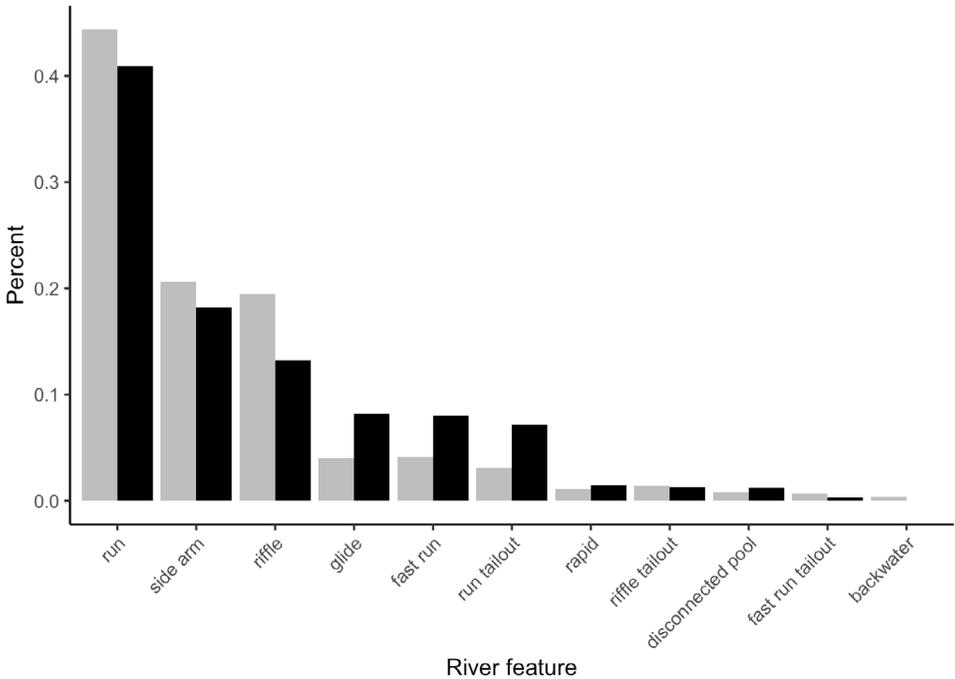
Results from the broader analysis of habitat selection were inconsistent with previous findings on *R. boylii* breeding habitat. Unexpectedly, we found that egg mass densities were higher with greater percent cover of agricultural areas. We suspect this is most likely



**Figure 4.** Box plots representing distance of foothill yellow-legged frog egg masses from wetted edge (top) and river depth at egg masses (bottom) on the lower Mad River, Humboldt County, California at time of survey in 2016.

a coincidence – riparian vegetation may have much better explanatory power, but we did not have reliable spatial data to measure riparian vegetation within the study area. There may have been other, more localized factors that contributed to high egg mass density in areas that happened to fall closer to agricultural areas – for example, flatter, wider reaches preferred by *R. boylii* for egg mass deposition (Lind et al. 2016) may have been more likely further downstream, where the majority of agricultural land use is found.

Our results on the microhabitat conditions for oviposition match with previous literature. Lind et al. (2016) found a narrow range of oviposition microhabitat characteristics, with *R. boylii* selecting wide, shallow segments of the stream with low water velocity and cobble-sized substrates for oviposition sites. Virtually all the egg masses we found were in depths between 0 and 20 cm, although some of these were up to 10 m from shore – in other



**Figure 5.** Left: proportion used (black) vs. available (gray) for foothill yellow-legged frog oviposition sites among river features on the lower Mad River, Humboldt County, California in 2011, 2015, and 2016 combined. Right: difference in used and available proportion of river features for 2011 (light gray), 2015 (dark gray), and 2016 (black), ordered from most to least used; positive percent suggests selection of that feature for oviposition, negative percent suggests avoidance, order from most to least selected.

words, wide, shallow segments of the river. We noted a general lack of stranding in this study, consistent with the beneficial stream morphology and limited anthropogenic water development in the lower Mad River.

Oviposition occurred earlier in years of low base flow (2015) compared to years of higher discharge (2011, 2016), consistent with *R. boylei* on the South Fork Eel River (Kupferberg 1996). Onset of breeding season in relation to flow patterns suggests *R. boylei* use periods of steady or decreasing flow rates as a cue to initiate oviposition (Kupferberg et al. 2012). As demonstrated in this study, the start of breeding approximately two weeks after flow stabilization suggests that durations of low variation in flow signal adequate oviposition conditions. However, during the drought year of 2015, flows stabilized more than seven weeks prior to initiation of oviposition – breeding onset is therefore likely triggered by multiple environmental cues. For example, in dry water years, when discharge is limited and flows decrease earlier than during more average water years, *R. boylei* may also use day length or water temperature cues to initiate breeding.

Similar to Kupferberg (1996), air temperature during the first week of oviposition were significantly warmer than previous weeks, with a notable increase in average minimum temperature one week after our estimated breeding onset compared to the week prior. However, 2015 was a warm year and air temperatures in mid-February were similar to temperatures at 2011 and 2016's breeding onset in April. The early increase in temperature and steady flows during this extreme drought may have exceeded the species' plasticity in breeding timing, or *R. boylei* may rely on multiple cues to initiate breeding. Further, our estimate of breeding onset is based on backdating from peak egg mass surveys. It is likely that some frogs began breeding earlier than our breeding start estimate because development times are temperature dependent, thus backdating from Gosner stages is only an estimate of oviposition dates. Finally, estimates of water temperature rather than ambient air temperature would have been more useful to estimate breeding onset and development time, as well as potentially more informative to explain breeding timing. Surveys to determine first date of oviposition may provide further insight into the relationship of flow, temperature, and breeding.

Increased flows due to dam release during late spring and summer could have a negative effect on the density and survival of *R. boylei*, as these flows do not coincide with natural patterns and do not provide environmental cues (Kupferberg 2012). Late-season releases from the Matthews Dam, however, seem to be buffered at our study site by distance from the dam and incoming tributaries below the dam, as summer peaks were not visible in flow data from the USGS Mad River gauge station near Arcata (USGS NWIS 2017). We found that there were no late season pulses that would impact egg or tadpole survival in any of the three years (Lind et al. 1996; Kupferberg et al. 2012), suggesting Matthews Dam did not negatively impact frog breeding in our study area during these three years.

### ACKNOWLEDGMENTS

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## RESEARCH NOTE

**Notes on reproduction of black toads from California**

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The black toad, *Anaxyrus exsul* (Myers 1942), is a mainly diurnal species known only from marshes and springs feeding Deep Springs Lake, Deep Springs Valley (37.333°N, 118.01759°W, 1,525 m elevation), Inyo County, California. The valley is enclosed between the White and Inyo Mountains (Dodd 2013). The entire natural range of *A. exsul* is limited to approximately 15 ha, one of the smallest ranges for any North American amphibian (Green et al. 2013). Because of this small distribution there is concern that an environmental catastrophe such as introduction of a disease or predator could result in the extinction of *A. exsul* (Green et al. 2013).

Information on *A. exsul* reproduction is limited. *Anaxyrus exsul* reproduction occurs in freshwater marshes and sloughs associated with Deep Springs Lake (Dodd 2013). Breeding of *A. exsul* occurs in the early spring, from late March to late April (Schuierer 1962; Kagarise Sherman 1980). Stebbins and McGinnis (2018) reported *A. exsul* breeding occurred from mid-March to May, perhaps as late as June; they are active in the fall until November (Dodd 2013). *Anaxyrus exsul* is rated critically imperiled (G1) by NatureServe Explorer (2019), state threatened in the California Natural Diversity Database (CNDDB 2019) and vulnerable by Hammerson (2004). In this paper, I provide additional information on the reproductive cycle of *A. exsul* from an examination of museum specimens. The values for minimum sizes for males and females at maturity, based on histological examination, may be valuable in efforts to reestablish or maintain populations of *A. exsul*. The use of museum collections for obtaining reproductive data avoids euthanizing specimens and avoids the need for collecting permits from state and federal authorities.

I examined a sample of 22 *A. exsul* from Inyo County, California collected 1954 to 1967 consisting of 15 adult males (mean snout-vent length, SVL = 46.5 mm ± 4.0 SD, range = 38–51 mm) and seven adult females (mean SVL = 50.7 mm ± 2.3 SD, range = 48–53 mm) from the herpetology collection of the Natural History Museum of Los Angeles County (LACM), Los Angeles, CA, USA.

I made a small incision in the lower part of the abdomen, removed the left testis from males and a piece of the left ovary from females, embedded them in paraffin, cut sections at 5 µm and stained them with Harris hematoxylin followed by eosin counterstain (Presnell and Schreibman 1997). I deposited histology slides at LACM and tested for differences

between adult male and female SVLs using an unpaired  $t$ -test (Instat, vers. 3.0b, Graphpad Software, San Diego, CA).

The testicular morphology of *A. exsul* is similar to that of other anurans as detailed in Ogielska and Bartmanska (2009a). Within the seminiferous tubules, spermiogenesis occurs in cysts which are closed until the late spermatid stage is reached; cysts then open and differentiating sperm reach the lumina of the seminiferous tubules (Ogielska and Bartmanska 2009a). All fifteen adult males in my sample exhibited spermiogenesis. By month these were: April (N = 2), June (N = 4), November (N = 9). One June male (LACM 36676, SVL = 48 mm) contained only small clusters of sperm in the seminiferous tubules, in comparison to the abundant sperm in the testes of the other males. Because of the presence of sperm, I considered this male to be an adult, although it is not known if it would have successfully mated. The smallest mature *A. exsul* males exhibiting full spermiogenesis measured 38 mm SVL (LACM 26128, 26144) and were from November. Wright and Wright (1933) measured body sizes (body length) from the tip of the snout to the rear end of the body back of the vent, (equivalent to SVL), and reported adult males of *A. exsul* ranged from 44 to 59 mm in body length. The smaller value for male maturity of *A. exsul* reported herein may be the result of my histological examination of the testes which was not done by Wright and Wright (1933).

The mean SVL of *A. exsul* females was significantly larger than that of males ( $t = 2.6$ ,  $df = 20$ ,  $P = 0.017$ ). The ovaries of *A. exsul* are similar to those of other anurans in being paired organs lying on the ventral sides of the kidneys; in adults the ovaries are filled with diplotene oocytes in various stages of development (Ogielska and Bartmanska 2009b). Mature oocytes are filled with yolk droplets; the layer of surrounding follicular cells is thinly stretched. My female sample consisted of seven: two from April, three from June and two from November. All were in spawning condition in which mature oocytes predominated. Adult females of *A. exsul* ranged from 46 to 61.5 mm in body length (Wright and Wright 1933). The smallest mature *A. exsul* females (in spawning condition in my LACM sample both measured 48 mm SVL (LACM 26097, 26098) which is within the size range for adults of *A. exsul* in Wright and Wright (1933).

Atresia is a widespread process occurring in the ovaries of all vertebrates (Uribe Aranzábal, 2009). It is common in the amphibian ovary (Saidapur 1978) and is the spontaneous digestion of a diplotene oocyte by its own hypertrophied and phagocytic granulosa cells which invade the follicle and eventually degenerate after accumulating dark pigment (Ogielska and Bartmanska 2009b). See Saidapur and Nadkarni (1973) and Ogielska et al. (2010) for a detailed description of stages of atresia in the frog ovary. Atretic follicles were observed in (100%, 7/7) of my *A. exsul* mature female sample. Atresia plays an important role in fecundity by influencing numbers of ovulated oocytes (Uribe Aranzábal 2011). Incidences of atresia increase late in the reproductive cycle when follicles that did not ovulate are resorbed (Saidapur 1978). Saved energy is presumably utilized in the next activity season. The causes of follicular atresia in nonmammalian vertebrates are not fully understood although it has been associated with captivity, food availability, crowding and irradiation (Saidapur 1978). In amphibians adverse environmental conditions such as starvation and suboptimal lighting may cause atresia of vitellogenic oocytes (Jørgensen 1992).

It is likely the two gravid *A. exsul* females from November would have kept their eggs until spring before spawning. This appears to be the case for *Rana boylei* (Goldberg 2019; Zweifel 1955) and *Rana cascadae* (Goldberg 2020), both from California, in which females from autumn with mature oocytes apparently delay spawning until spring. Jørgensen

et al. (1979) reported that by the time of hibernation, ovaries are close to breeding size in frogs from the temperate zone. Therefore, it may be advantageous for *A. exsul* to be capable of spawning soon after emergence from hibernation rather than delaying reproduction to undergo a period of yolk deposition.

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## APPENDIX

Twenty-two *A. exsul* from Inyo County, California examined from the herpetology collection of the Natural History Museum of Los Angeles County (LACM), Los Angeles, California. LACM 26091, 26092, 26094, 26097, 26098, 26105, 26114, 26118, 26128, 26135, 26137, 26140, 26142, 26144, 26147, 26148, 26154, 26156, 26158, 36673, 36676, 87716.

RESEARCH NOTE

## Salvage and translocation of endangered Santa Cruz long-toed salamander larvae

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**Key words:** *Ambystoma macrodactylum croceum*, connectivity, habitat fragmentation, larvae, metamorphosis, oak woodland, Santa Cruz long-toed salamander, translocation

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Like many biphasic amphibians that migrate between non-breeding upland and aquatic breeding locations, the endangered Santa Cruz long-toed salamander (*Ambystoma macrodactylum croceum*) is susceptible to the effects of habitat loss, alteration, and fragmentation (Bury and Ruth 1972; USFWS 1978; Stebbins and McGinnis 2012). The subspecies is particularly imperiled due to its restricted range within a heavily populated landscape in southwest Santa Cruz County and extreme northwest Monterey County (Bury and Ruth 1972; Bury et al. 1980; USFWS 1999; Stebbins and McGinnis 2012). Threats to survival and recovery include ongoing modification or loss of upland habitat and increased barriers to terrestrial movements during migration and dispersal (Bury and Ruth 1972; Allaback and Laabs 2003). Typical barriers include houses and related infrastructure including retaining walls, busy roads, vertical curbs, and solid fences that extend below grade. In many instances, these barriers prevent or severely reduce access between breeding ponds and surrounding upland habitat, while also forcing individuals to navigate converted grasslands and/or other unsuitable terrain (Bury and Ruth 1972).

The range of the subspecies in Santa Cruz County was reported to be approximately 7,580 ha (USFWS 1999), but many areas do not provide suitable habitat and *A. m. croceum*

likely inhabits less than half of the remaining available area, much of which is under private ownership subdivided into several hundred parcels (T. Kasteen, personal observation). This remaining habitat is mostly rural-residential embedded in hilly topography (Bury and Ruth 1972), and access to homes often requires long driveways and substantial vegetation clearing for fire safety, which negatively affects *A. m. croceum* especially during migration and dispersal. A small number of breeding sites are protected and managed for the subspecies, while most are on private property and inaccessible to determine species presence (C. Mitcham, personal observation).

*Ambystoma m. croceum* was originally listed as endangered by the federal government in 1967, and although a recovery plan was subsequently approved and critical habitat proposed, it was based on only two known locations (USFWS 1977, 1978). A revised draft recovery plan adopted in 1999 put forth a more comprehensive management strategy (USFWS 1999), but a revision is necessary based on data collected over the last 20 years. Since the mid-2000s, both the California Department of Fish and Wildlife, which considers the subspecies fully protected and endangered, and the United States Fish and Wildlife Service have conserved and enhanced acreage to support recovery actions. The subspecies *A. m. croceum* is isolated from other subspecies by more than 240 km and genetic analysis suggests that full species status may be warranted (USFWS 1978; Savage 2008). Efforts to identify and conserve adequate movement corridors between existing protected lands and undeveloped uplands are ongoing, since the best available genetic data indicates that the groups of populations in Santa Cruz County may be described as two or three subpopulations (W. Savage, University of Massachusetts Lowell, unpublished data). *A. m. croceum* breeding populations in Santa Cruz County were likely fragmented by the construction and subsequent expansion of State Route 1 and areas disturbed by human development in the coastal zone (USFWS 1999, 2019). Extensive agricultural and residential development for many decades has isolated the Santa Cruz County population from the few scattered locations that still support the subspecies in north coastal Monterey County (C. Mitcham, personal observation). In Monterey County, *A. m. croceum* faces extirpation/extinction (Bury and Ruth 1972; USFWS 1978), and active management (USFWS 2019) is likely necessary, such as captive rearing to restore genetic variability if inbreeding is prevalent, and/or translocation of eggs or larvae to suitable breeding sites in the remaining oak woodlands east of Elkhorn Slough (C. Mitcham and K. Camara, personal observation).

Conservation biologists have understandably debated concerns that translocation may stress individuals or negatively affect a recipient population by affecting the genetic composition or spreading disease (Griffith et al. 1989; Germano and Bishop 2009). We used the best available data to consider these issues and, after identifying an appropriate study area, balanced our decision to test translocation based on the myriad of threats throughout the entire range of the subspecies, especially habitat fragmentation. Here, we use the operational definition of translocation as moving larval individuals within a fragmented, genetically similar subpopulation, to a pond constructed with adequate surrounding upland habitat known to have supported adults as recently as 2002 (M. Allaback and D. Laabs, unpublished data). The intent was to allow larvae to undergo metamorphosis at the new pond and establish site fidelity, so that individuals would colonize the surrounding protected lands and create a self-sustaining breeding population.

The 26-ha Willow Canyon Unit of the Santa Cruz Long-toed Salamander Ecological Reserve (Reserve) was established in 2009 and is owned and managed by the California

Department of Fish and Wildlife (Fig. 1). Topography is hilly with two seasonal springs dominated by oak woodlands and patches of coastal scrub, willow riparian and non-native *Acacia*. In 2012, the Resource Conservation District of Santa Cruz County constructed a pond on the Reserve (36.964045, -121.875488, elevation 65 m; Figs. 1 and 2). The pond appeared to provide suitable breeding habitat based on a sufficient hydro-period and supported colonization by Pacific (= Sierran) treefrog (*Hyla* {= *Pseudacris*} *regilla*) and macro-invertebrates. The Seascape Uplands Pond is a productive breeding site within the known dispersal distance of *A. m. croceum* located 730 m offsite to the southeast (Fig. 1). Approximately 1,100 m to the north is the Valencia Lagoon Unit of the Santa Cruz Long-toed Salamander Ecological Reserve, where the subspecies was discovered in 1954 (Russell and Anderson 1956). However, there is limited or no upland habitat connectivity between Valencia Lagoon and Willow Canyon because of residential development.

The Valencia Lagoon Unit was established in 1973 and today consists of a 2.4-ha strip of land situated between Bonita Road and State Highway 1, as well as a patchwork of undeveloped residential lots in the nearby area (Fig. 1). Following various modifications of the original marsh that was compromised by the construction and expansion of State Route 1, a 1.5 ha freshwater impoundment was completed in 1978 that has provided a consistent breeding site referred to as Valencia Lagoon (36.972423, -121.879883, elevation 34 m). Maximum depth reaches nearly 2 m and it generally dries by late summer (M. Allaback and T. Kasteen, personal observation). The upland at the Valencia Lagoon Unit is densely vegetated with a mixture of native and non-native plants, primarily willow riparian and oak woodland with some nonnative green wattle (*Acacia decurrens*), black cottonwood (*Populus balsamifera*), and Monterey pine (*Pinus radiata*). The portion of the Valencia Lagoon Unit supporting upland habitat situated immediately adjacent to the intersection of Bonita Drive and Encino Drive is considered critical, because it is unlikely that adult *A. m. croceum* are able to complete their breeding migration beyond approximately 300 m from Valencia Lagoon, mainly due to residential housing that creates barriers to terrestrial movements (Fig. 1; Allaback and Laabs 2003). Since the Valencia Lagoon Unit is constrained by State Route 1 and habitat conversion to the south, it is effectively isolated from all other potential *A. m. croceum* habitat. However, the best available genetic information indicates common ancestry between Valencia Lagoon and Seascape animals, which places the Willow Canyon Unit within the same subpopulation (W. Savage, University of Massachusetts Lowell, unpublished data).

Between State Route 1 and the Valencia Lagoon Unit is a drainage channel that supports a narrow band of mostly willow riparian habitat extending approximately 700 m along the south edge of the highway between Freedom and Rio Del Mar Boulevards. The California Department of Transportation (Caltrans) periodically removes emergent marsh vegetation from the dry channel in the late summer prior to the onset of winter rains. Approximately 75 m east of Valencia Lagoon, a 0.1-ha seasonal pool forms in the drainage channel during the winter that typically dries in May or June. It is the only pool along the entire drainage channel with a sufficient hydro-period to provide breeding habitat for *A. m. croceum* (M. Allaback and T. Kasteen, personal observation). However, the channel receives erratic pulse flows during rain events and dries rapidly each spring even during years of above average rainfall, regularly stranding larvae prior to transformation (M. Allaback and T. Kasteen, personal observation). Furthermore, late spring rains frequently wash a portion of the larval cohort up to 350 m or more out of the deep pool and down the channel into a series of shallow pools that dry rapidly such that no larvae survive without intervention.



**Figure 1.** Aerial image of CDFW Santa Cruz Long-toed Salamander Ecological Reserve - Valencia Lagoon Unit (northern grouping of red circles) and Willow Canyon Unit, Santa Cruz County, CA, USA.



**Figure 2.** Coverboards placed at highwater line around Willow Canyon Pond (facing north), Santa Cruz County, CA, USA, 28 June 2016.

Beginning in 2013, we surveyed the constructed pond at Willow Canyon annually using long-handled dipnets and seines and did not detect evidence of *A. m. croceum* breeding. From 2016–2018, after aquatic sampling at the Willow Canyon Pond was negative, we translocated larvae from the Valencia drainage channel to the Willow Canyon Pond as both a salvage and recovery effort. Two or three sampling visits were required each year beginning in May through early June, with at least six days between visits. Within 90 minutes of initial capture, individuals were transported to the Willow Canyon Pond; allowed to acclimate in shallow water for 30–60 minutes; and released at 2–6 separate locations along the perimeter within patches of emergent vegetation dominated by spikerush (*Eleocharis* sp.).

A total of 947 larvae were translocated (300 in 2016, 144 in 2017, and 503 in 2018). On 21 June 2016, 18 days after that year's final translocation of 136 larvae (average length 69.2 mm TL,  $n = 15$ ), we began a coverboard study to determine if translocated larvae reached metamorphosis. We placed 26 small, 1.3 cm thick plywood coverboards (20 boards = 30.5 cm x 30.5 cm; six boards = 30.5 cm x 61 cm) around the perimeter of the pond at the high-water line (Fig. 2). From 21 June through 11 August 2016, the coverboards were inspected every morning and periodically adjusted perpendicularly to near the edge of high water as the pond dried. When encountered, post-metamorphic juveniles were measured, weighed, and moved approximately 10–30 m to an area of moist leaf litter in nearby uplands under mature willow (*Salix* sp.) and coast live oak (*Quercus agrifolia*) canopy.

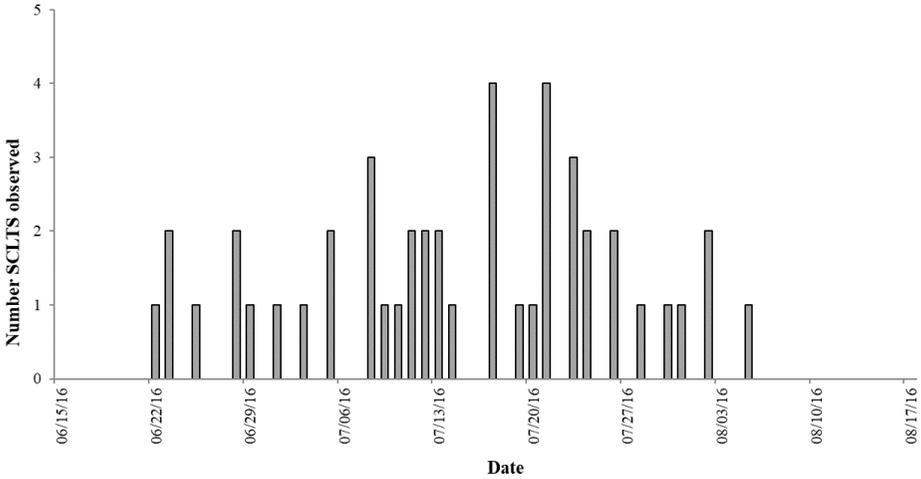
Forty-six post-metamorphic juveniles (i.e., metamorphs) were detected under coverboards from 22 June through 5 August 2016 (Figs. 3 and 4). Metamorphs were observed during 27 of the 52 days coverboards were monitored, with the highest number ( $n = 4$ ) on 17 and 21 July 2016, near the midpoint of the field study (Fig. 4). Metamorphs were detected under 22 of the 26 cover board locations, and in all cardinal directions, but were most common on the southeastern (i.e., downstream) locations (Fig. 5). Individual metamorphs averaged 66.6 mm total length (range 47–86;  $n = 34$ ), 36.4 mm snout-vent length (range 22–46;  $n = 34$ ), and 1.3 g (range 0.4–2.6;  $n = 32$ ) (Fig. 6). Coverboards were removed on 11 August 2016, following six days without any observations of metamorphs and while the water depth was approximately 35 cm.

During subsequent years, translocations were conducted from 11 May 2017 through 8 June 2017 and 8–16 May 2018. To monitor larval development at the recipient (=Willow Canyon) pond, aquatic sampling was conducted after the final transfer each year. Aquatic sampling on 23 April 2019 yielded an abundance of larvae, indicating that unassisted breeding occurred at Willow Canyon Pond. On 27 September 2019, 20 metamorphs were observed under woody debris (Fig. 7). This juvenile aggregation was consistent with behavior reported by Alvarado (1967) and Anderson (1967). We believe this indicates that resident (previously translocated) *A. m. croceum* successfully bred and metamorphosed at the Willow Canyon Unit for the first time in 2019. On 21 May 2020, larvae were readily detected for the second consecutive year, and we assumed that a self-sustaining breeding population was established at the recipient pond that could be periodically monitored in the future.

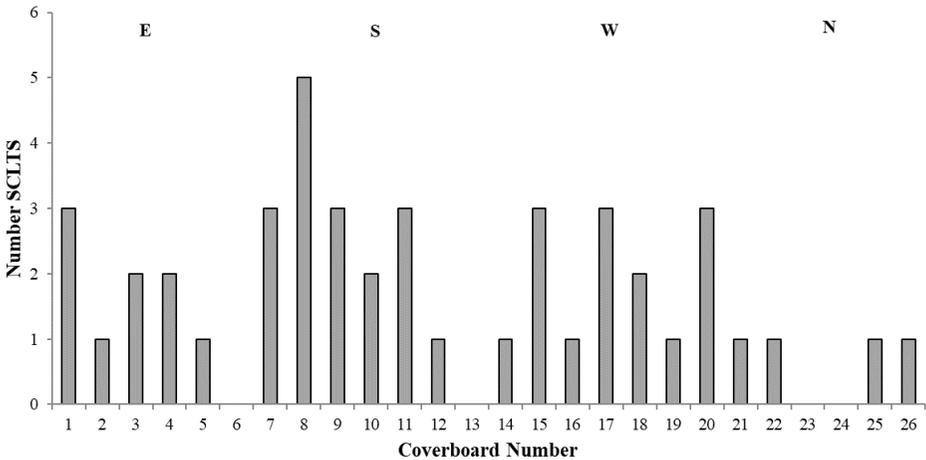
We used minimally invasive field methods to translocate *A. m. croceum* larvae from an atypical, at-risk breeding location to a constructed pond on a Reserve near the northern limit of the subspecies' range. Results of the coverboard study revealed useful data regarding the timing of metamorphosis, including body mass at transformation. Data suggest that metamorphs dispersed in all directions, and distribution was uneven and may be influenced



**Figure 3.** Newly metamorphosed Santa Cruz long-toed salamander, Willow Canyon Pond, Santa Cruz County, CA, USA, 30 July 2016.

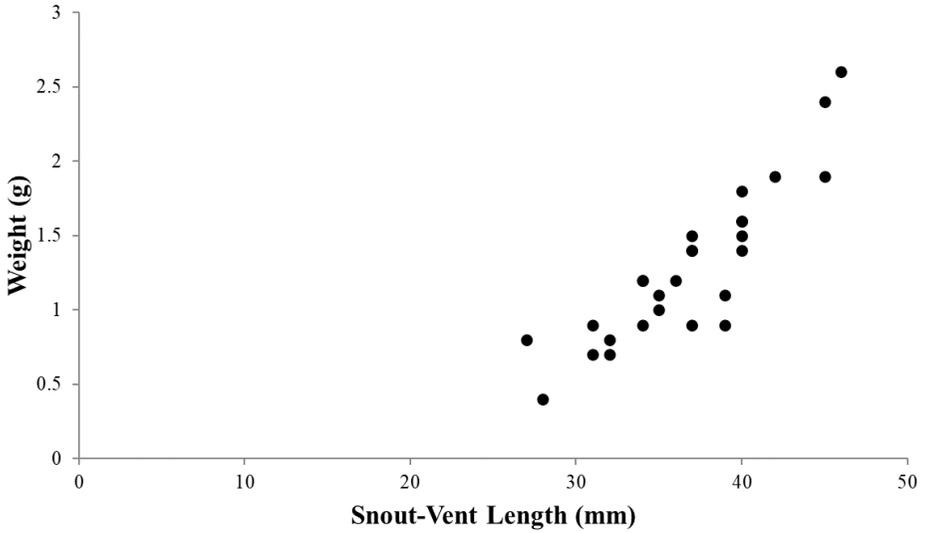


**Figure 4.** Number of Santa Cruz long-toed salamander (SCLTS) metamorphs observed by date under coverboards at Willow Canyon Pond, Santa Cruz County, CA, USA, 2016.



**Figure 5.** Frequency and distribution of Santa Cruz long-toed salamander (SCLTS) metamorphs by coverboard at the Willow Canyon Pond, Santa Cruz County, CA, USA, 2016 (approximate cardinal directions indicated).

by perimeter cover near the high-water line (see: Petranka 1998) or the topography around the pond basin. Given the consistent number of detections under small coverboards, we speculated that emerging metamorphs circle portions of the high-water line seeking cover or perhaps gentle topography, a behavior that may influence the direction of dispersal and may also have important management implications. The Willow Canyon Pond was not naturally colonized within five years after construction, despite being situated in optimal uplands with limited habitat connectivity to a known breeding pond. A variety of site-specific factors likely affect the time period and dispersal distance required for *A. m. croceum* to colonize new locations, especially in a fragmented landscape.



**Figure 6.** Weight and length of Santa Cruz long-toed salamander metamorphs, Willow Canyon Pond, Santa Cruz County, CA, USA, 2016.



**Figure 7.** Santa Cruz long-toed salamander metamorphs found under a log adjacent to Willow Canyon Pond, Santa Cruz County, CA, USA, 27 September 2019.

We demonstrated that translocating an average of 300+ *A. m. croceum* larvae from consecutive breeding seasons appeared to establish a self-sustaining population within three years, thereby satisfying the success criteria put forth in Germano and Bishop (2009). This is in contrast with Semlitsch (2002) who recommended 10,000–50,000 eggs be moved over several years. In some cases, this large a collection effort may be infeasible, and curtail or eliminate efforts to reestablish populations. If future translocation studies are conducted, the

recipient location should be protected in perpetuity and not only contain a seasonal pond with a six to eight-month hydro-period, but sufficient upland habitat that provides connectivity to other breeding locations (Anderson 1967; USFWS 1978; Griffith et al. 1989; Dodd and Seigel 1991). Although we do not know if one season of translocation is sufficient to establish a self-sustaining population for *A. m. croceum*, we recommend that future efforts also translocate for consecutive years, since multiple generations of different aged adults would more quickly establish a robust breeding population and may also increase genetic diversity. However, our results suggest that a single round of larval translocations may be sufficient to initiate breeding of *A. m. croceum*, since individuals may reach sexual maturity within two years after metamorphosis (Anderson 1967). It is therefore possible that individuals from the first- and/or second-year translocations successfully recruited and returned to breed. While pond construction is typically expensive and requires periodic maintenance, the traditional methods used to implement and monitor this translocation effort did not require significant time or expense, especially with multiple volunteers. Given the paucity of breeding sites throughout its range, particularly on protected lands, pond construction linked with salvage and translocation may be a viable tool for future *A. m. croceum* recovery efforts.

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## RESEARCH NOTE

## Use of atypical aquatic breeding habitat by the California tiger salamander

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**Key words:** aquatic, atypical, breeding, *Ambystoma californiense*, eggs, habitat, plasticity, sites, threatened

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The California tiger salamander (*Ambystoma californiense*) has been a species of focused study due to habitat loss and population declines. The species is state and federally listed as either threatened or endangered throughout its entire range in California (USFWS 2000, 2002, 2004). *Ambystoma californiense* has historically been closely associated with its putative breeding habitat in lentic waters, in particular, vernal pools—typically shallow, rain-filled water bodies with hydroperiods that last up to several months (Storer 1925; Fitzpatrick and Shaffer 2004; Lannoo 2005). Several investigators have suggested that the decline of the species is related to declines in vernal pools throughout the species' range (Stebbins and Cohen 1995; Loredó and Van Vuren 1996; Fitzpatrick and Shaffer 2004). However, there is an increased understanding of the ability of *A. californiense* to frequently use perennial waterbodies and seasonal cattle stock ponds as aquatic breeding habitat, which may ultimately contribute to the conservation of the species (Alvarez 2004a,b; Wang et al. 2009; Wilcox et al. 2015). Here we report on observations of additional breeding site plasticity in *A. californiense* which were found breeding in atypical habitats, such as perennial creeks and anthropogenic structures.

We conducted opportunistic visual encounter surveys, often while conducting other watershed management activities, and observed *A. californiense* using perennial or nearly perennial sections of three creeks in eastern Contra Costa County, CA. During two reproductive seasons separated by 12 years (i.e., 1996 and 2008), approximately 30–40 *A. californiense* adults and thousands of eggs were observed in Brushy Creek (a fishless creek approximately 3.5 m wide and 1 m deep; Fig. 1a). This site included a seasonally flowing, intermittent creek with a silt bottom that slowly flowed through level ground within open

annual grasslands. Vegetation consisted of live and decadent non-native annual grasses and scattered clumps of *Juncus balticus*. The nearest known breeding location of *A. californiense* to this location was a seasonal stock pond approximately 300 m west of Brushy Creek. In 2010, approximately 10 *A. californiense* eggs were detected in Kellogg Creek, an adjacent watershed (approximately 5 m wide and 2 m deep; Fig. 1b). This site included a perennial creek with a silt bottom that was bordered by valley oaks (*Quercus lobata*), Fremont cottonwood (*Populus fremontii*), and willow (*Salix* spp.), and included emergent patches of *Typha latifolia*. It flowed very slowly, meandering through level ground among low hills covered by annual grasslands. Kellogg Creek is hydrologically connected to the Sacramento-San Joaquin River Delta and supports numerous species of fishes from the families: Cottidae, Cyprinidae, and Centrarchidae, as well as Sacramento sucker (*Catostomus occidentalis*). The nearest known breeding location of *A. californiense* to this location was a perennial stock pond, approximately 710 m northwest of Kellogg Creek. At a third site, approximately 25–100 *A. californiense* larvae (ranged in number depending upon year) ( $\leq 75$  mm TL) were observed from 2001–2012 inhabiting a nearly perennial (i.e., wet through mid to late-summer), fishless pool within an unnamed tributary of Kellogg Creek (Fig. 1c). This site consisted of a seasonal, rain-filled pool (35 m x 3 m and 0.5 m deep) with a firm clay bottom that was bordered by live and decadent non-native annual grasses and scattered clumps of *Juncus balticus*. Generally, there was no flow within this pool, but it was hydrologically connected to Kellogg Creek during peak inundation (i.e., during rain events). The nearest known breeding location of *A. californiense* to this site was a perennial stock pond, approximately 630 m northwest of Kellogg Creek.

In 2000, also in eastern Contra Costa County, we had single season observations of adult *A. californiense* breeding in two long-lived rain-filled depressions (1 m x 3 m, 1 m deep) created from soil excavation at a construction site. This steep-sided excavation supported standing-water for approximately 2 months and was located 330 m north of a known breeding location for *A. californiense*. Similarly, in 2010, *A. californiense* were observed breeding in a rain-filled pool created by soil compaction surrounding a horse trough. This pool was approximately 5 m x 5 m x 0.3 m deep, with a 2 m circular horse trough at its center. The nearest known breeding location of *A. californiense* to this location was an seasonal stock pond, approximately 640 m to the east. Larvae were noted in both pools during subsequent visits to these individual sites, but no attempt was made to determine if metamorphosis occurred.

During the springs of 2008, 2011, 2018 and 2019, in eastern Santa Clara County on the Blue Oak Ranch Reserve,  $> 100$  *A. californiense* eggs were observed during each visit in a partially buried concrete trough (3 m x 1 m, 0.5 m deep; Fig. 1d). The nearest known breeding location of *A. californiense* to this perennial water body was a perennial stock pond, approximately 240 m west. During three of the four years, we observed *A. californiense* larvae among dense algal growth and debris within the trough. *A. californiense* were able to access the trough directly due to the trough being buried to soil level on three sides, however it is unknown whether these larvae metamorphosed and/or were able to leave the trough. A small piece of hardware cloth was placed in the trough in 2012 to facilitate self-escape by newly metamorphosed individuals.

Although it is likely that some of these rain-filled seasonal aquatic breeding sites (e.g., inundated excavation, horse trough depression) dried prior to the metamorphosis of *A. californiense* larvae, clearly such features attract adults, which use them as ovipositing sites. Some of these observations occurred in habitat that was reported to be perennial, or





**Figure 1.** Atypical breeding habitat used by *Ambystoma californiense* in Contra Costa and Santa Clara Counties, California.  
a. Brushy Creek. Photo by J. Alvarez;  
b. Kellogg Creek. Photo by J. Alvarez;  
c. Unnamed tributary of Kellogg Creek. Photo by J. Alvarez;  
d. Buried concrete cattle trough. Red arrows indicate examples of eggs of *Ambystoma californiense*. Photo by J.T. Wilcox.

nearly so (i.e., Brushy Creek, Kellogg Creek, and an unnamed tributary), which may have facilitated successful metamorphosis in some years.

Storer (1925) reported collecting eggs of *A. californiense* from pools that formed in “ephemeral drainages” in the foothills of the Sierra Nevada range. Storer’s report of the use of lenite sections of a lotic system would be consistent with the general types of observations we made. The observations reported here, including *A. californiense* breeding in newly created sites, suggests that some *A. californiense* breed in pools that are encountered opportunistically. The reported use of a large variety of aquatic breeding habitat (i.e., vernal pools, stock ponds, intermittent creeks, fishless perennial creeks, and temporary pools) supports our contention. Opportunistic use of available aquatic breeding habitat could facilitate colonization of new sites, making it possible for long-term persistence in areas with few or no vernal pools, or where large-scale habitat changes have occurred. Alternatively, *A. californiense* utilizing these atypical breeding sites, in some years, may experience a reproductive sink—reproduction occurs, larvae hatch, but no metamorphosis occurs due to limited hydroperiod, inability to self-escape (i.e., concrete trough or step-sided excavations), or direct impact (i.e., trampling by horses using horse trough).

*Ambystoma californiense* is a species in decline and continues to be adversely affected by modification and/or loss of aquatic breeding habitat. Although we do not suggest that fishless intermittent or perennial creeks could be a reasonable long-term aquatic breeding site surrogate, we believe that a landscape-scale understanding of all potential *A. californiense* habitat use, including atypical breeding habitats, is critical for conducting complete site assessments for this species. More specifically, within upland habitat that includes a mix of vernal pools, stock ponds, creeks, and other potential aquatic breeding sites, long-term persistence of *A. californiense* may rely on the availability of all of these habitat types, including the use of sites previously characterized as atypical. Reproductive site plasticity may play a role in population persistence through climactic changes, stochastic events, and adjacent habitat alterations. Therefore, use of these atypical sites as potential breeding habitat for *A. californiense* should be considered during site assessments and other regulatory actions.

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# Reptiles



# Reptiles

Photos, previous page:

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1. Alameda whip snake (*Masticophis lateralis euryxanthus*).  
Photo Credit: Jeff Alvarez, The Wildlife Project
2. Desert tortoise (*Gopherus agassizii*).  
Photo Credit: Public Domain
3. Coachella Valley fringe-toed lizard (*Uma inornate*).  
Photo Credit: Cameron Barrows, University of California, Riverside
4. Barefoot gecko (*Coleonyx switaki*).  
Photo Credit: Gary Nafis, Californiaherps.com, CC BY-NC-ND 3.0
5. Giant garter snake (*Thamnophis gigas*).  
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6. Pacific leatherback sea turtle (*Dermochelys coriacea*).  
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## FULL RESEARCH ARTICLE

## Forty years later: monitoring and status of the endangered Coachella Valley fringe-toed lizard

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The Coachella Valley fringe-toed lizard, *Uma inornata*, was listed as endangered under the California Endangered Species Act in 1980. By that time, the lizard's habitat was already reduced by 90%, fragmented into isolated habitat islands on private property among hundreds of land-owners. Ecosystem processes that are essential for delivering sand and maintaining the lizard's sand dune habitat were already compromised. As challenging as it was to protect its habitat under these conditions, populations of this lizard still occur across much of the area where it was found forty years ago. Annual monitoring was designed to assess the ongoing viability of these populations by quantifying the effects of potential threats and stressors and focusing adaptive management actions where they are most needed. Here we demonstrate how hypothesis-based monitoring identified specific locations where invasive plant control and sand corridor management were needed to maintain the lizard's populations. By monitoring lizard densities within the context of environmental variables that either drive or inhibit population growth, this monitoring approach informs if, when, and where management actions are needed.

**Key words:** aeolian sand, California, hypothesis-based monitoring, management intervention, natural versus anthropogenic-driven population fluctuations, nested-scale monitoring, reptile, stressors, *Uma inornata*

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The Coachella Valley fringe-toed lizard, *Uma inornata*, (the lizard) (Fig. 1) was listed in 1980 as endangered under the California Endangered Species Act (CESA) and threatened under the federal Endangered Species Act (ESA). Listing a species as endangered requires evidence that habitat loss and/or impacts from other stressors have put that species on a trajectory to extinction. However, the act of listing a species does not alone ensure its protection. The habitat loss and associated stressors that warranted listing need to be managed to halt or reverse population declines. Monitoring informs and assesses the success of ongoing critical management tasks. Here we present a case study underlining the importance of monitoring and management for the protection of the lizard. Now, forty years after those listings, we assess this species' status and the successes and failures of efforts to protect it.



**Figure 1.** An adult male Coachella Valley fringe-toed lizard, *Uma inornata*. Fringes along the trailing edges of their toes, countersunk lower jaw, overlapping eyelids, and valvular nostrils that keep sand that protect their respiratory tract from breathing in sand particles all provide adaptations for living in an aeolian sand habitat.

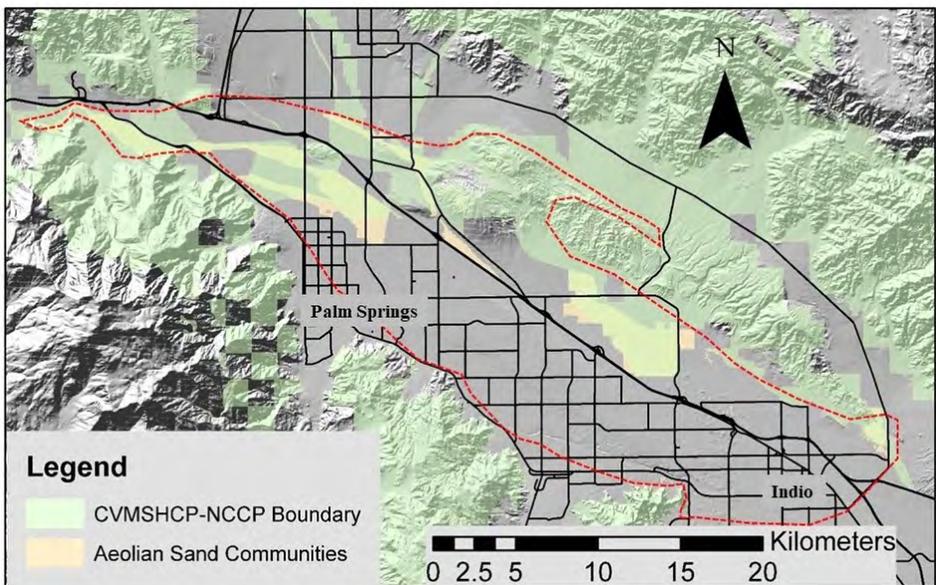
The conservation planning and implementation steps for the protection of the lizard have been detailed elsewhere (Barrows 2019). In short, the federal ESA initially took precedence as it offered flexibility under 1982 amendments that allowed the creation of Habitat Conservation Plans (HCPs). HCPs facilitate regional landscape scale conservation planning, not just project by project regulatory requirements for mitigation in response to proposed development impacting endangered species' habitat. Regional planning was an essential and critical task to protect ecosystem processes that transport sand to the lizard's habitat. Since the lizard did not occupy key sand transport corridors, those corridors would not necessarily receive protection under traditional regulatory approaches. With the creation of the Natural Community Conservation Planning Act (NCCP) in 1991, protection efforts for CESA-listed species were given an analogous regional conservation planning approach. The initial single-species HCP for the lizard was signed in 1986 with the fanfare of being the first-ever approved after the 1982 amendments to the ESA. The Coachella Valley Fringe-toed Lizard HCP included multiple municipalities and hundreds of landowners. Being first also meant that there was no template outlining how to proceed and no criteria for defining success or failure.

The lizard's habitat was once a continuous landscape of 33,500 ha of aeolian-sand; however, prior to the 1980 listing and the onset of conservation planning and implementation for this species, the sand dunes had already been reduced by close to 90%, with remaining habitat fragments isolated by roads, freeways, rail corridors, golf courses, agriculture, and suburban developments (Barrows et al. 2008; Fig. 2). A critical concern was that the sand transport corridors were all compromised to one degree or another. A decade after the original lizard HCP was signed it became increasingly clear that the sand corridors were not being adequately protected. Planning began in 1996 to create a federal multiple species HCP (MSHCP) and state NCCP with an explicit ecosystem focus. This effort recognized

the need to correct the shortcomings in the original lizard HCP and to extend protection for 27 plant and animal species (including the fringe-toed lizard) and 27 natural communities. Four of the natural communities together encompass the range of aeolian-sand habitats occupied by the lizard: 1) active dunes, 2) stabilized sand fields, 3) ephemeral sand fields, and 4) honey mesquite hummocks and dunes. The state and federal permits for the joint MSHCP/NCCP were signed in 2008.

A monitoring program to assess the degree to which the plan was successful in protecting the lizard and other covered species was developed concurrent with conservation planning efforts. Historically, biological monitoring has focused on periodic counts of a species. Results were limited to determining presence or absence and occupancy trends. However, even healthy populations increase and decrease over time in response to natural fluctuations of limiting resources, predator densities, and other factors. Such natural fluctuations do not necessarily warrant management intervention. Occupancy or abundance data alone do not provide insights as to why changes are happening or what, if any, management prescription might enhance population persistence.

Precipitation is the primary driver of population growth in arid environments (Noy-Meir 1973; Kearney *et al.* 2018). However, the relationship between the lizard's population growth and rainfall is not linear; the seasonality, intensity, and amount of rainfall all have differential effects (Barrows *et al.* 2009). Monitoring in arid habitats must be able to partition the complex effects of rainfall from other anthropogenic effects to identify if management actions are warranted to reverse population declines. A novel monitoring approach was developed as the MSHCP/NCCP was being negotiated (Barrows *et al.* 2005; Barrows and Allen 2007a,b). That approach considered monitoring as a series of hypothesis-driven



**Figure 2.** The entire historical range of the Coachella Valley fringe-toed lizard (red-dashed line), as well as remaining aeolian sand habitat, land designated for protection (CVMSHCP-NCCP boundary), and the level of existing fragmentation of those remaining habitats. The aeolian sand habitats shown here are based on US Geologic Survey soil maps but are not precise equivalents to habitat occupied by the lizard. Smaller, isolated habitat fragments and peripheral areas within larger mapped habitat polygons no longer support lizard populations.

experiments using the varying intensity of drivers and stressors over time and space as independent variables, and changes in the lizard's abundance as the dependent, or response variable. Here we present results of monitoring data, employing this hypothesis-driven approach for Coachella Valley fringe-toed lizards covering a 19-year period from 2002–2020.

## METHODS

### Study Area

The Coachella Valley is located at the northwestern corner of the Colorado Desert, a drier subset of the Sonoran Desert with less influence from summer monsoonal precipitation, broadly stretching west from the Colorado River. This valley is bounded to the west by the Santa Rosa and San Jacinto Mountains, and to the east by the Little San Bernardino Mountains (Fig. 2). The northern boundary of the Coachella Valley is delineated by the southeastern terminus of the San Bernardino Mountains, and the valley extends south to the Salton Sea. The Coachella Valley includes nine incorporated municipalities with a year-round resident population of roughly 400,000 people, from Palm Springs and Desert Hot Springs in the west to Indio and Coachella in the east. However, the number of residents can more than triple during the cooler winter and spring months when seasonal “snowbirds” swell the human population. The regional economy is focused on tourism, second homes, and agriculture.

Habitat conservation efforts are coordinated by the Coachella Valley Conservation Commission (CVCC), a Joint Powers Authority whose members are elected representatives of Coachella Valley cities, indigenous tribes, water districts, and Riverside County. While the lizard's habitat was initially a patchwork of hundreds of privately-owned parcels, current conservation landownership of that habitat includes the U.S. Fish and Wildlife Service National Wildlife Refuges, California Department of Fish and Wildlife Ecological Reserves, U.S. Bureau of Land Management, Coachella Valley Water District, Coachella Valley Association of Governments (CVAG), Coachella Valley Mountains Conservancy (a State of California conservancy), and Friends of the Desert Mountains (a private, non-profit organization). Individual conservation landowners are responsible for land management, while biological monitoring is funded and coordinated by the CVCC. Monitoring protocols are therefore applied evenly across the remaining lizard habitat, independent of land ownership.

Coachella Valley fringe-toed lizards are among six species of the genus *Uma* occupying the Mojave and Colorado Deserts in California, Arizona, and northwestern Mexico (Gottscho et al. 2017; Derycke et al. 2020). Two additional *Uma* species occur in the Chihuahu Desert in north-central Mexico. All species of *Uma* are restricted to or are found at their highest densities on fine, well-sorted, aeolian sand landscapes, with many confined to discrete sand dune systems. Among those eight *Uma* species, two are especially impacted by expanding human development (*U. inornata* and *U. exsul*; García-De La Peña et al. 2015), with the degree of habitat loss and fragmentation most severe for *U. inornata*, the Coachella Valley fringe-toed lizard (Barrows et al. 2008).

### Survey Protocol and Dependent Variables

The lizard's sand dune habitat is extremely dynamic. Aeolian sand habitats are continuously shifting down wind, while new upwind sand additions are dependent on stochastic flood events bringing sediments out of the surrounding mountains (Barrows 1996). The aeolian sand habitat includes four different natural community types that comprise the

remnants of the original aeolian sand landscape; they are defined by unique wind, sand, and vegetation characteristics (Table 1). Protection goals included maintaining sustaining populations of the lizard within each of these community types. Monitoring goals focused on quantifying lizard densities in response to precipitation, the variation in habitat quality due to aeolian and fluvial sand dynamics, and anthropogenic stressors (Table 2) across each of the four natural communities. We tested and rejected multiple approaches for visual counts of the lizards. Fisher et al. (2020) monitored this species via a mark/recapture approach on a single 2.25 ha plot for +31 years, marking each resident lizard with a unique combination of three colored beads attached to the base of their tails (Fisher and Muth 1989). They were able to acquire both accurate annual population estimates and delineation of home ranges for resident lizards. However, their method was time and effort intensive, typically requiring dozens of surveys per year, and so was impractical to apply to more than one or two plots.

Our solution was to not count the lizards directly, but to quantify lizard densities using their tracks left in the fine aeolian sand. By using tracks, we eliminated the problem of the lizard's variable, inconsistent activity patterns—if any individual was active on a plot during or prior to the survey we could detect it by the diagnostic tracks it left behind. However, determining which species had left tracks, and how many individuals were present introduced challenges. To determine how many lizards were represented by the tracks observed on each transect we used four criteria. First, we only surveyed on mornings after a night with strong enough winds to clear all tracks from the previous day. Second, we followed each set of tracks to determine if it connected with the tracks of a previously counted lizard. Third, we looked for interactions between lizards to determine if we were looking at one or multiple individuals. Fourth, there are considerable size differences between male and female lizards and between juveniles and adults (Barrows and Fisher 2009) and those differences are mirrored in the track widths. Ensuring that the species-track identification was accurate was resolved with adequate training, and when in doubt following the tracks to the lizard that created them. Much like learning to count birds by their calls and songs, accurately identifying tracks is a learnable skill.

A benefit of this method was that we could detect many more lizards, and so could reduce plot size to just 0.1 ha and still have adequate numbers of lizard sightings for robust statistical analyses. With smaller plots and smaller time and effort per plot, we were able to survey 68 core plots (plots resurveyed every year) across the entire range of the lizard, with 4–6 repeated surveys per plot within a six-week survey window. We configured the 0.1 ha plots as 10-m × 100-m rectangles. Those plots were then clustered (3–7 plots) within separate dunes or habitats within the same natural community type, with plot clusters > 500 m apart, (with the exception two clusters that were < 500 m apart as a result of a random placement) from an adjacent plot cluster. Placement of the initial plot within a cluster was random. Thereafter additional plots were either placed randomly or regularly to answer specific questions (such as edge effects). Non-random plot placements occurred within three clusters where we wanted to measure the effect of distance from a road/powerline that formed a habitat edge. Within a cluster we placed plots  $\geq 50$  m apart to avoid individual lizards overlapping adjacent plots. Fisher et al. (2020) identified home range sizes for females ( $\bar{x} = 505 \text{ m}^2$ ) and males ( $\bar{x} = 662 \text{ m}^2$ ), which, assuming roughly circular home ranges, equate to home range diameters of 25–29 m, well below the 50-m separation between plots.

Population densities can vary as habitat characteristics vary, and responses to those shifting habitat qualities can become apparent at different scales (Morris 1987; Smith and

**Table 1.** Characteristics that distinguish the four aeolian sand natural communities found in the Coachella Valley that provide habitat for the Coachella Valley fringe-toed lizard.

Aeolian Community Characteristics	Active Dunes	Stabilized Sand Fields	Ephemeral Sand Fields	Honey Mesquite Dunes
Habitat Area / Number of Habitat Fragments	1370 ha / 5	400 ha / 1	1700 ha / 4	200 ha / 1
Sand	Deep, continuous, well-sorted fine sand with low silt or finer particle content	Well-sorted fine sands form discontinuous shallow layers over compacted layers with higher silt content.	Discontinuous patches of well-sorted fine sands, coarse sands, gravel, rocks, and boulders	Deep, well-sorted fine sand with low silt or finer particle content
Sand Movement	High mobility shifting dunes	Low mobility	Extremely high mobility	Low mobility
Perennial and annual Plant Composition	Sparse perennial and annual cover: <i>Larrea</i> sp. and <i>Atriplex</i> sp.	Moderate cover of perennials, seasonally high cover of annuals <i>Larrea</i> sp. and <i>Atriplex</i> sp.	Moderate cover of perennials, sparse annual cover: <i>Larrea</i> sp., <i>Psoralea</i> sp., <i>Croton</i> sp., and <i>Petalonyx</i> sp.	High cover of mesquite, low to moderate cover of other shrubs: <i>Prosopis</i> sp., <i>Larrea</i> sp., <i>Atriplex</i> , and <i>Isocoma</i> sp.
Invasive Plant Species	Low to moderate cover of <i>Brassica</i> sp.	Moderate to high cover of <i>Brassica</i> sp. and <i>Schismus</i> sp.	Low to zero cover of invasive species	Moderate cover of <i>Brassica</i> sp. and <i>Schismus</i> sp.

Ballinger 2001). Collecting lizard densities at a plot scale (0.1 ha) that can be combined and analyzed as plot clusters provides analytic flexibility at multiple scales. Plot clusters can be combined at the natural community or landscape scale. Our 68 core plots included replicates within the four natural communities as follows (plot clusters/total # of plots): active dunes (4/18); mesquite dunes (1/11); ephemeral sand fields (3/18); and stabilized sand fields (3/21).

Two to three people surveyed each plot: a professional biologist plus 1–2 volunteer community scientists. Surveyors slowly walked equidistant from each other along the length of the plot, noting and identifying all vertebrate tracks, which were then verified and recorded by the biologist. The addition of the community scientists significantly increased detection rates for lizards and their tracks (Barrows et al. 2016).

While population density is a useful metric, it is dependent on long-term habitat conditions. It can take multiple years for a population to substantially increase density due to the finite number of breeding adults. Similarly, it can take years for densities to decline due to multiple-year lifespans. Population growth rate ( $\gamma$ ) can prove to be a more sensitive response variable to shorter term changes in independent variables. Here population growth was calculated as  $\gamma = \ln(N_{i+1}/N_i)$ , where  $N_i$  is the population density in year  $i$ , and  $N_{i+1}$  is the population density the following year.

**Table 2.** Primary stressors impacting the Coachella Valley fringe-toed lizard, their effects, and management responses for reducing those impacts.

Stressor	Scale	Effect	Management Response
Climate Change	Broad, but most severe at the eastern, hotter/drier conserved habitats	Reduced surface activity for the lizards, more severe droughts, reduced vegetation cover. Higher mortality and lower recruitment rates	Reduce impacts from other stressors
Invasive Plant Species	Localized, varies between sites, and between species. Most severe where there are lower sand transport rates	Sand stabilization, outcompetes native annuals, reducing both plant and insect food resources for the lizards. Notably, insect abundance and diversity are reduced as Sahara mustard increases	Hand removal is the safest, but the scale of the infestations easily overwhelms staff or volunteers for large scale removal efforts. Removal efforts then need to be strategically targeted to the habitats with the greatest benefits
Edge Effects	Localized	Increased predation from greater roadrunners, American kestrels, and common ravens	Remove anthropogenic nesting sites and power lines used as perches by predators
Loss of Genetic Heterogeneity	Broad, but most severe on the smallest habitat patches	Potential reduced adaptability to climate change and other stressors. Otherwise unexplained population declines	Translocation of gravid females and/or hatchlings to increase heterogeneity. Adults do not appear to translocate as successfully.
Loss of Ecosystem Processes	Localized	Increased sand stabilization, reduced active, loose sand habitats	Keep sand corridors open. Recycle fugitive sand (sand on roads or otherwise unwanted areas) to sand corridors
Off-road Vehicle Trespass	Localized	Reduced perennial vegetation cover. Increased debris dumping	Maintain fencing, increased law enforcement patrols

## Independent Variables

Although this region receives occasional isolated summer rain that can result in localized flooding, primary productivity and breeding success of the lizards is usually catalyzed by cool season rains (Noy-Meir 1973; Kearney *et al.* 2018). To illustrate the relationship between rainfall and the lizards' population dynamics we compared annual November-April rainfall totals from the eastern-most protected habitat, the Coachella Valley National Wildlife Refuge and California State Ecological Reserve. Rainfall data were collected on site and were found to be nearly identical to a nearby, internet accessible weather station in the city of Indio (<https://wrcc.dri.edu/cgi-bin/cliMAIN.pl?ca4259>). Rainfall levels do vary across the Coachella Valley, with an increase toward the western edge of the valley at the western limits of the lizards' remaining habitat; however, the relative trajectories (drought, average rainfall, or relatively wet conditions) are consistent throughout the region. Using this rainfall

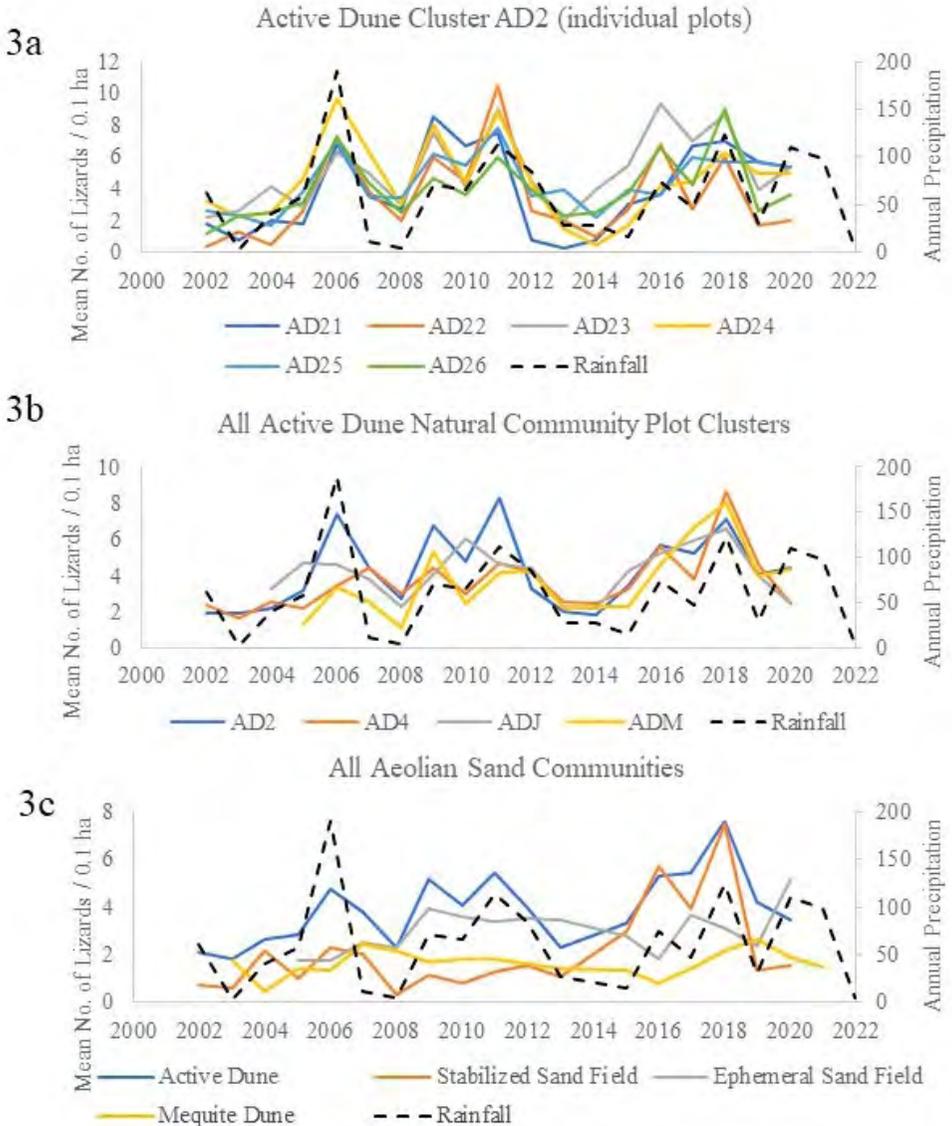
metric to illustrate relationships between rainfall and lizard population dynamics throughout the lizards' range, while not precise for specific locations, provides the opportunity to assess how drought or wetter conditions influence the lizards' population densities. Rainfall levels provide a coarse-scale expectation of population growth rate trajectories.

Additional independent data that we collected annually on each 0.1 ha plot included: 1) spring annual and perennial plant abundance and density by species, including both native and non-native species; 2) arthropod abundance and species diversity, 3) sand compaction, and 4) associated vertebrates, using track counts collected at the same time that the lizards were surveyed. These metrics provided fine-scale, plot-specific indicators of habitat characteristics. For annual vegetation cover we measured both density and percent cover by species, on 12, 1-m<sup>2</sup> sub-plots, four at each end and one in the center of each 0.1 ha plot. We measured arthropods using three pitfall traps placed overnight, one at each end and one in the center of each 0.1 ha plot. One of those arthropods, the beetle *Asbolus* (previously *Cryptoglossa*) *laevis*, (Tenebrionidae) proved to be a useful indicator of sand compaction, only occurring on the less compacted sands of active dunes (Barrows 2000). Sand compaction was measured using a Pocket Penetrometer (AMS Inc.). Twenty-five compaction measurements, each separated by roughly 4 m, were made along the mid-line of each plot. We measured associated vertebrates using the same track protocol used to measure the lizard densities. Some of the associated vertebrates are predators and so could influence fringe-toed lizard abundance. Potential predators include leopard lizards (*Gambelia wislizenii*), sidewinders (*Crotalus cerastes*), coachwhips (*Masticophis flagellum*), glossy snakes (*Arizona elegans*), greater roadrunners (*Geococcyx californianus*), loggerheaded shrikes (*Lanius ludovicianus*), common ravens (*Corvus corax*), American kestrels (*Falco sparverius*), coyotes (*Canis latrans*), and potentially some species of rodents (Timberlake and Washburne 1989). Others are possible competitors such as zebra-tailed lizards (*Callisaurus draconoides*) and flat-tailed horned lizards (*Phrynosoma mcallii*), but none are as habitat specific to active aeolian sand as are fringe-toed lizards.

## RESULTS

Figure 3 illustrates the nested-scale character of the fringe-toed lizard monitoring data. At the finest scale (Fig. 3a) are individual plots clustered within a single active dune (AD2). Means for the combined plots within each of the four individual active dune plot clusters (replicates within the active dune natural community) are shown in Figure 3b (middle scale). Finally, at the coarsest scale (Fig. 3c) are the combined means for each of the four natural communities across the lizards' entire range. At each of these scales the data can reveal patterns that provide insights regarding the status of the lizard. At both the fine-scale plot level for the AD2/active dune cluster (Fig. 3a) and the combined active dune natural community (Fig. 3b) scale, precipitation levels positively correlate with lizard densities (Pearson's Correlation: AD2 plot cluster:  $df = 17$ ,  $r = 0.717$ ,  $P = 0.0008$ ; all active dune communities:  $df = 17$ ,  $r = 0.581$ ,  $P = 0.011$ ). At the coarsest natural community scale (Fig. 3c), the correlation ( $r$ ) between lizard density and precipitation was uneven. The strongest correlation was with active dunes. Next was the mesquite dunes ( $df = 17$ ,  $r = 0.514$ ,  $P = 0.029$ ), followed by non-significant rainfall-lizard density correlations for stabilized sand fields ( $df = 17$ ,  $r = 0.317$ ,  $P = 0.199$ ), and ephemeral sand fields ( $df = 14$ ,  $r = 0.077$ ,  $P = 0.785$ ).

Since the plots are replicate surveys within each dune, and the dunes are replicates within the natural community, the general within year synchrony provides validation for



**Figure 3.** Annual changes in lizard density at multiple scales within the context of precipitation to show how the lizards’ population fluctuations are often synchronized with rainfall patterns. Since lizard density is in part a reflection of the previous year’s reproductive recruitment, precipitation is shifted back by one year so that lizard density aligns with the precipitation effects.

the ability of the plot size and survey methodology to detect real change when it happens. Large population swings are a regular occurrence and should not influence management responses if they are synchronized in direction and amplitude with shifting rainfall levels. The question then is when does asynchronous, or non-significant correlations between precipitation and lizard densities indicate a need for management intervention?

A list of potential stressors that could warrant management responses is shown in Table 2. Of those that have localized impacts, off-road vehicles could be discounted as no recent vehicle trespasses were observed. Invasive species impacts and losses of ecosystem processes (reduced sand delivery) can be interrelated and so are difficult to partition. However, looking at that middle scale graph, in 2020 there were opposite population trajectories for the AD2 and ADM plot clusters (increasing) versus the AD4 and ADJ clusters (decreasing). Those divergent trajectories warranted further analyses. The AD2 and ADM plot clusters did have significantly less Sahara mustard, *Brassica tournefortii*, than the AD4 and ADJ sites (Means 13.23 versus 24.75 plants/m<sup>2</sup>; ANOVA df = 1, F = 4.5313, P = 0.049), and had a significantly higher (and positive) population growth rate (means  $\gamma$  = 0.103 versus -0.644; ANOVA df = 1, F = 18.9855, P = 0.00049). While densities AD2 and ADM were less than that for the Ephemeral Sand Field natural community (Fig. 1), a habitat that lacked Sahara mustard, their respective population growth rates were not significantly different (means  $\gamma$  = 0.103 versus 0.57; ANOVA df = 1, F = 4.0887, P = 0.0561). The mustard densities on AD4 and ADJ appear to have exceeded a tipping point for negatively impacting the lizards. An illustration of the varying Sahara mustard densities that can occur across the active dunes and stabilized sand fields are shown in Figure 4.

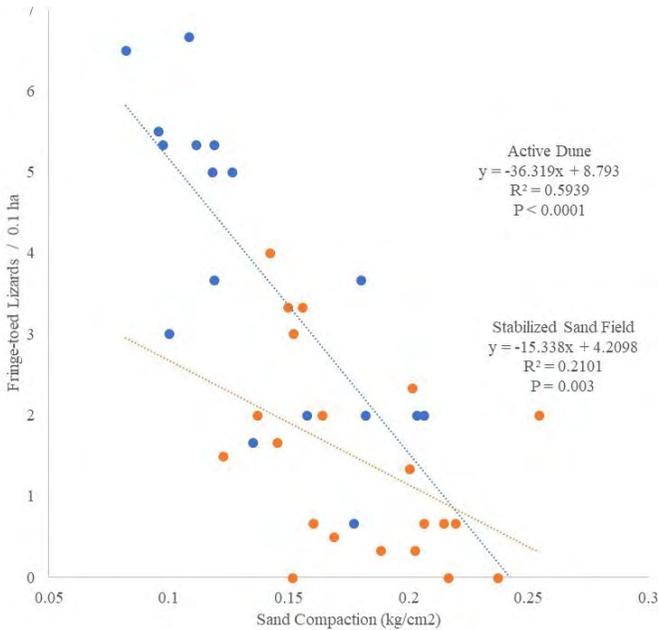
The regression of 2020 lizard density versus sand compaction was significant for both active dunes ( $R^2 = 0.5939$ ;  $P < 0.00001$ ) and stabilized sand fields ( $R^2 = 0.2101$ ;  $P < 0.003$ ); less compacted sand in correlated with higher densities of fringe-toed lizards (Fig. 5). There appears to be a sand compaction level of approximately 0.125 kg / cm<sup>2</sup> that distinguishes most active dunes from stabilized sand fields. Of the AD2 and ADM plots designated *a priori* as active dunes, 75% had sand compaction levels fitting to that natural community. However, for the AD4 and ADJ active dune plots, just 30% had sand compaction levels  $\leq$  0.125 kg / cm<sup>2</sup>. The occurrence of plots previously identified as active dunes, but now with sand compaction and lizard densities well within the stabilized sand field range, identified a need to initiate remedial management. Although roadrunner, kestrel, and raven densities increased with proximity to human development, we did not find any support for other additional explanations, such as edge effects which are manifested by increases in potentially anthropogenically augmented predator densities (i.e., roadrunners, ravens, or kestrels). However, both the roadrunner (except on the mesquite dune natural community) and kestrel were dependent on planted non-native trees and shrubs for nesting sites. Our data identified that management intervention to remove mustard as well as remove any other barriers to aeolian sand movement was warranted on the AD4 and ADJ dunes. The lack of synchrony between lizard density and coarse scale precipitation data identified that a potential problem existed; finer scale invasive species densities and sand compaction data identified the cause and management solutions.

## DISCUSSION

Wild populations fluctuate naturally in size from year to year. The challenge for managing endangered species that are facing multiple stressors is distinguishing natural population



**Figure 4.** The top image shows the infestation of Sahara mustard (the dense, straw colored plants) on an active dune (AD2) during the wet spring of 2005. The lower image shows the density of mustard on an adjacent stabilized sand field that same year.



**Figure 5.** Patterns of Fringe-toed lizard densities in relationship to sand compaction in 2020. Active dunes (each plot indicated by a blue circle) generally have less compacted sand and higher lizard densities, whereas stabilized sand field plots (orange circles) have more compacted sand and fewer lizards. The regression of lizard density versus sand compaction for each habitat type show statistically significant correlations. The plots identified as active dunes, but that have values that are well within those for stabilized sand fields are not receiving new sand and are being invaded by Sahara mustard.

oscillations from population shifts that are anthropogenic driven that, if not managed, could result in population declines leading to extinction. Here we provided examples of how the hypothesis-driven monitoring approach employed for the Coachella Valley fringe-toed lizard has clarified those distinctions and identified site-specific management recommendations. Using two abiotic metrics, precipitation (coarse scale) and sand compaction (fine scale), plus a biotic metric (invasive plant densities), we identified site-specific priorities for managing an invasive weed, Sahara mustard, to promote more sustainable lizard populations. Without management intervention, some active dune communities, habitats that where the lizard populations are consistently the densest throughout its range, appear to be transitioning to stabilized sand fields, a natural community with consistently the lowest lizard densities.

We continue to find that increasing mustard density decreases native plant abundance (Barrows et al. 2009), decreases arthropod abundance (Hulton et al. 2013), and increases sand compaction. As Sahara mustard density increased, lizards became increasingly scarce, and ultimately absent. Our findings indicate that the mustard continues to be a significant threat to the sustainability of the lizard populations, especially on stabilized sand fields and active dunes. This is in contrast to our findings that another invasive weed, Russian thistle, *Salsola tragus*, had a benign to positive impact on the lizards (Barrows 1997).

The density of mustard is tied to both the amount of rainfall and sand transport rates - the more rainfall and the more stable the sand, the denser the mustard. Mustard density is influenced by both the amount of rainfall and the timing of rainfall. Heavy early December rains guarantee a dense growth of mustard, but if the rains do not start until late February

or March, little mustard germinates (Barrows et al. 2009). If there is a sequence of storms beginning in December and continuing through February, a new cohort of mustard germinates after each storm. These patterns complicate control efforts. Herbicides that kill mustard will also kill native annual plant species, and mustard will still germinate following an herbicide treatment if more storms occur. Accordingly, “surgical” hand pulling, focusing on areas where mustard removal will yield the greatest benefits, is the preferred control method. Unless a safe, species-specific biological control for the mustard is identified, hand pulling will be an ongoing management task.

Stabilized sand fields did not have significant correlations with precipitation. Stabilized sand fields have the highest levels of Sahara mustard infestation as well as the highest sand compaction levels of any of the aeolian sand communities. *Asbolis laevis* beetles were not detected in this dune type, and fringe-toed lizards only rarely exceeded a mean of 2 lizards/plot (Figs. 3, 5).

Ephemeral sand fields also did not have significant correlations with precipitation; this community occurs in a region of the Coachella Valley where wind and sand transport are so strong as to continue to blow deposited sand downwind and scour rocks into ventifacts (Table 1). Within the ephemeral sand fields, due to these strong winds, sand residence time is relatively short compared to the other aeolian sand-based natural communities. These scouring winds also inhibit annual plant growth (including non-native invasive species), so higher annual rainfall that supports annual plant growth and arthropod prey for the lizards elsewhere has less of an impact on the lizard’s population dynamics here, and a close correlation between annual precipitation and the lizard’s population growth is not expected. Rather, when sand delivery is sufficient to build sand hummocks, and when that coincides with sequential years of average or greater rainfall to maintain high soil moisture to support leaf and flower production of perennial shrubs, the lizard population grows, as it did in 2020. Understanding site-specific interactions between abiotic inputs and biotic responses is critical for developing models from which the need for management interventions can be determined. For this natural community there are up-wind sand corridor challenges, such as sand and gravel mining, channelization for aquifer re-charging, and conflicts associated with roadways that cross the sand corridor. Each of these could restrict sand delivery to this habitat, and each needs to be monitored to ensure sand delivery is not constrained.

We have previously addressed questions that included whether the high degree of habitat fragmentation had resulted in a loss of genetic diversity in the lizards. Based on tissue samples collected in the mid-1990s, Hedtke et al. (2007) found no genetic structure associated with the lizard populations occupying the different fragments; their genetic profile reflected the pre-fragmentation, panmictic condition. A follow-up study analyzing tissues collected in 2008, (Vandergast et al. 2016) found a different result; lizard populations occupying each habitat fragment had a unique genetic signature, and each population had lost genetic diversity relative to that 1990s baseline. Climate change also looms as a threat to the lizards. Barrows et al. (2010) modeled the response of the fringe-toed lizards to expected levels of climate change if no significant reductions in anthropogenic greenhouse gases occur and found that only the westernmost habitat areas will likely continue to provide the climate envelope currently preferred by the lizards. For the present, we found lizards are sustaining populations as expected with respect to annual rainfall and Sahara mustard densities in all the remaining protected habitats. Given that land managers do not have the capacity to alter the course of climate change, it is imperative that they address those threats that they can affect. These include controlling invasive plants and keeping sand corridors

unobstructed, and reducing other stressors that might, together with climate change, result in local extirpations.

Forty years after the listing of the Coachella Valley fringe-toed lizard as endangered, this species continues to occupy much of the same landscape they occupied in 1980. Land protection efforts, purchasing essential private parcels and so taking them out of a trajectory toward future development, has been extremely successful. However, long-term success, defined as maintaining sustaining fringe-toed lizard populations across those protected lands, will depend on effective management informed by hypothesis-based monitoring.

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## RESEARCH NOTE

## Observations on the phenology of the threatened Alameda whipsnake

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**Key words:** activity, Alameda whipsnake, annual, management, observations, phenology, predict, temperature, threatened, winter

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The Alameda whipsnake (*Masticophis lateralis euryxanthus*) is a federally and State threatened subspecies of the California whipsnake (*M. lateralis*) and is restricted to a specific geographic range (Jennings 1983, USFWS 1997, USFWS 2002, Stebbins and McGinnis 2012). Until recently, the subspecies was believed to occur in Alameda and Contra Costa Counties, and in small portions of northwest San Joaquin County and northern Santa Clara County. Recent work suggests that the range of the subspecies may include eastern Santa Clara County and northern San Benito County, making the subspecies active in a wider area than previously believed (Richmond et al. 2016). Within its accepted range, the Alameda whipsnake has been the subject of study for nearly four decades, yet there remains a paucity of published work on this subspecies. After its initial description (Riemer 1954), Hammerson (1978, 1979) was the first to study the subspecies, and described its thermal ecology and reproduction; Larsen et al. (1991) and Shafer and Hein (2005) reported on the snake's feeding ecology with little detail about its general natural history; and Swaim and McGinnis (1992), Swaim (1994), Alvarez et al. (2005), Alvarez (2006), and Alvarez et al. (in press) looked at the subspecies' use of habitat, with conflicting conclusions. These conflicts included a disagreement on the types of habitats, and slope aspects used. Currently, the natural history of the Alameda whipsnake remains mostly conjecture and assumption, with inferences made from work by Swaim (1994) or stemming largely from grey literature (i.e., unpublished technical reports). The draft recovery plan for the Alameda whipsnake, which summarizes the natural history, also uses limited published literature related to the Alameda whipsnake's biology. Here we examine and analyze a large data set on Alameda whipsnake activity and add to the published literature by reporting on its annual activity period.

We reviewed and compiled 610 records in Alameda and Contra Costa Counties, and small portions of San Joaquin and Santa Clara Counties, *a posteriori*. In previous compilations we looked at habitat use; here we recompiled and updated our data to look at phenology of the subspecies (Alvarez et al. 2005, Alvarez 2006). Our analysis included both pure Alameda whipsnakes and any intercross specimens (between *M. l. euryxanthus* and *M. l. lateralis*) delineated within the “zone of intergradation” by Jennings (1983). Due to the ambiguity of the phenotypic and genetic boundary, we elected to limit our analyses to specimens throughout the zone of intergradation, as far south as extreme northern Santa Clara County. Our methodology closely incorporates the guidelines proposed by the USFWS (1996), which suggest that any listed species, subspecies, and possible intercross specimens be managed as if all were listed species or subspecies. Here we will refer to both Alameda whipsnakes and the intercross specimens as Alameda whipsnakes.

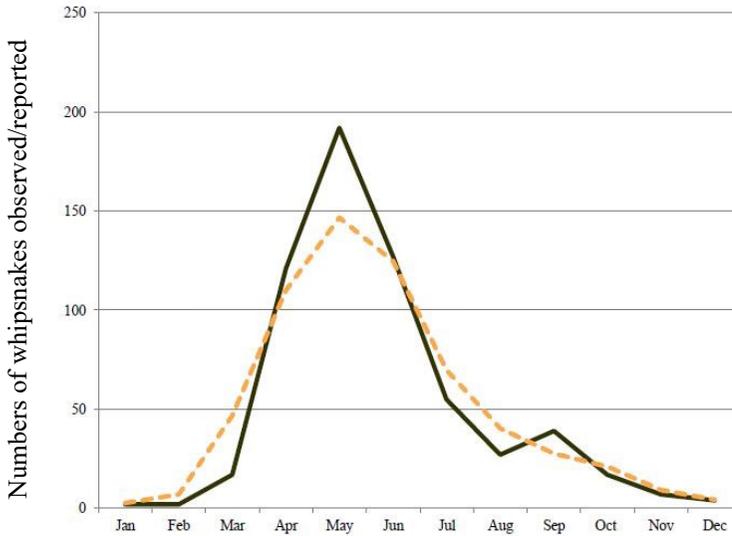
We analyzed the following aggregate data: our own observations; all known published accounts; reports from the California Natural Diversity Data Base (CNDDDB 2020); records and specimens from museums and universities; publicly accessible consulting reports from survey efforts; and anecdotal observations (i.e., personal communications) from demonstrably knowledgeable individuals. Data collected, analyzed, and used in this study included the reported date and location of each observation. If the date or location was ambiguous in any manner, the record was discarded.

Twenty-one percent of the observations used here were also used in Alvarez et al. (2005). As in that study, we acknowledge the shortcomings of using this aggregate data in our analysis, including the potential for misidentification of snakes by the various observers. Verifications of observations followed that of Alvarez et al. (2005) whenever possible. Questionable observational reports were discarded. We also acknowledge the inherent sampling biases of randomly reported sightings, including, but not limited to: the highly variable expertise of biologists working seasonally in the field; omissions in reporting when the species was observed and identified; our inclusion of focused field efforts to detect the snake (i.e., trapping surveys); and the inaccessibility of some habitats (i.e., closed/muddy roads) during specific times of year (see: Oliver 1947). Despite the shortcomings of this data set, we believe the methodology reasonably supports the conclusions drawn.

We also attempted to collect high and mean temperatures for dates where snakes were active between November and February (subjectively categorized as winter months due to increased rain potential and the mean temperature dropping below the reported emergence temperature for the subspecies). Historic weather data were collected from the nearest weather station ( $\leq 4.0$  km [2.5 miles] in all cases) to a snake observation. The subset of data points from which we were able to collect location and temperature data reflect a significantly narrower window (1953–2006) than the total range of our reported observations (i.e., 1940–2020). We therefore only analyzed temperature data for those observations that occurred between November and February 1953–2006.

The 610 reported observations we reviewed included data collected or reported from 10 November 1940 to 1 August 2020, in all months of the year. Our data set indicated an obvious peak in observations (31.5%) in the month of May for all observations combined, followed by a minor, secondary peak (6.4%) in September (Fig. 1). More than 72% ( $n = 440$ ) of all observations were reported April–June, with only 2.5% ( $n = 15$ ) of the observations reported during the coldest months of the year (i.e., November–February; Table 1).

We found that the Alameda whipsnake appears to follow the typical seasonal pattern of many snake species in North America—a reduced period of activity during the winter



**Figure 1.** The frequency of reported observations of Alameda whipsnakes (solid line) over the course of 12-month year, collected from observations between 1940 and 2020 in Alameda, Contra Costa, and portions of San Joaquin and Santa Clara Counties. Dashed line is a 3-point moving average, which was used as a smoothing function.

**Table 1.** High and mean daily temperatures associated with the dates of Alameda whipsnake observations during the months of November through February 1952–2015<sup>a</sup>. Weather station location was the nearest recording station to the observed location (measured in km to the nearest 0.5 km). Data source for weather from: Weather Underground (<http://www.wunderground.com>). Three data points discarded due to imprecise location (e.g., location = “Contra Costa County”).

Season	Date	Daily High	Daily Mean	Weather Station Location
Fall	2 Nov 1990	20.5° C (69° F)	15.5° C (60° F)	Berkeley (3.5 km)
	18 Nov 1990	16.1° C (61° F)	13.3° C (56° F)	Berkeley (3.5 km)
	12 Nov 1952	18.8° C (66° F)	12.8° C (55° F)	Berkeley (1.5 km)
	12 Nov 2013	21.6° C (71° F)	16.1° C (61° F)	Walnut Creek (4.0 km)
	22 Nov 1989	16.7° C (62° F)	10.6° C (51° F)	Livermore (2.5 km)
	2 Dec 2006	16.1° C (61° F)	10.6° C (51° F)	Mt Diablo (4.0 km)
	12 Dec 2015	13.9° C (57° F)	10.0° C (50° F)	Pittsburg (3.0 km)
Winter	26 Jan 1953	13.9° C (57° F)	10.0° C (50° F)	Oakland (1.5 km)
	25 Feb 1991	17.7° C (64° F)	11.7° C (53° F)	Oakland (4.0 km)
	28 Feb 1992	18.8° C (66° F)	15.5° C (60° F)	Berkeley (3.0 km)

<sup>a</sup> The subset of data points from which we were able to collect precise location and temperature data reflect a significantly narrower window (1952–2015) than the total range of our reported observations (i.e., 1940–2020).

months (Conant 1938; Cowles 1941; Oliver 1947; Seigel et al. 1987). Work conducted by Swaim (1994) showed a similar pattern of activity to that provided here, but our robust sample size eliminates the site-specific and time-limited potential biases that may result from a short-term project. Both studies suggest that the activity pattern of the Alameda whipsnake is annually bimodal, however in our work, a peak in fall observations was significantly less well defined (Fig. 1) than that reported by Swaim (1994), whose data were comprised primarily of hatchling and juvenile specimens during the fall. Larsen et al. (1991) reported a relative abundance of juvenile Alameda whipsnakes peaking in mid-October and declining through November and December (estimated from their Fig. 5, absent specific reported data)—representing a decline at least one month later than that found in our data set. This may suggest inter-annual variability in the peak timing of activity in this species. Nevertheless, the bimodal activity pattern we found in Alameda whipsnakes follows very closely with that reported for the ring-necked snake (*Diadophis punctatus*), common kingsnake (*Lampropeltis getula*), and other North American species (Conant 1938; Oliver 1947; Gibbons and Semlitsch 1982; see also: Seigel et al. 1987).

Heliothermic animals, including many snake species, require solar exposure in order to attain an activity level sufficient for foraging (Brattstrom 1965) and reproduction (Hammerson 1978, 1979). During tests in semi-natural outdoor enclosures from May through July, Hammerson (1979) found that Alameda whipsnakes emerged from refugia to bask and later to forage when soil surface temperatures were as low as 19° C (66° F; inferred from his Fig. 1.). Our data show that the vast majority (72.1%) of the 610 reported observations we analyzed occurred within the mating, gravid, and egg-laying period, which is generally April–late June (Hammerson 1978; personal observation). This three-month period also coincided with typical average daily air temperatures ranging above the lowest temperature for emergence reported by Hammerson (1979).

The acceptance of whipsnakes as endogenously inactive during the winter months has been treated as putative, particularly in the grey literature (i.e., technical reports; pers. obs.). Although Swaim (1994) reported that this subspecies is in brumation during this period, we note that Alameda whipsnakes were reported active on the surface on 10 days when daily high temperatures were as low as 13.9° C (Table 1). The USFWS (2002) suggested that “short, above-ground movements may occur in the winter” with no reference to the origin of their contention. The data analyzed here show that these above-ground movements occur at temperatures that are lower than previously reported and may occur more frequently than indicated by the data set we analyzed.

Siegel et al. (1987) reported that, “...evidence suggests that movement by snakes is highly deterministic and the potential exists to predict the conditions under which individuals will be active.” We contend that predicting the phenology of Alameda whipsnake activity will require substantially more research. When compared to Larsen et al. (1991), our data indicate the possibility of inter-annual variability in peak activity timing. We strongly suggest that land managers and regulators consider management conditions that are climate-focused, rather than focused on the months of the year. In this way, adjustments for climate change would be automatically incorporated into regulatory compliance and land-management policy. Because the Alameda whipsnake can be at the surface and potentially active at any time of year, albeit differentially, habitat-altering activities (i.e., ground disturbing and vegetation clearing) within areas suspected to be occupied by Alameda whipsnake should carefully consider this species and, more specifically, assume that active snakes may be pres-

ent above ground when daily high temperatures are as low as 13.9° C. More specifically, we recommend conducting vegetation clearing activities in winter months, when snakes are less active. Care should be taken within this subspecies' range if vegetation is stacked to be burned, as the use of brush piles by Alameda whipsnakes remains undetermined. Ground disturbing activity (grading, trenching excavating, etc.) may pose a very significant risk to snakes that are inactive and underground during winter months (Cowles 1941). We would recommend that ground disturbing activity take place after the majority of snakes have emerged from winter hibernacula (i.e., March), so that active snakes may be able to move out of harms-way as opposed to being unearthed when they are inactive. We would further add that a biological monitor should be in place during any ground disturbing activity in order to prevent loss of snakes, and also to record behaviors that then should be reported.

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REVIEW PAPER

## **A tale of two valleys: endangered species policy and the fate of the giant gartersnake**

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By the mid-20th Century, giant gartersnakes (*Thamnophis gigas*) had lost more than 90% of their Central Valley marsh habitat and were extirpated from more than two-thirds of their range. This massive habitat loss led to their inclusion in the inaugural list of rare species under the California Endangered Species Act (CESA). Listing under the CESA provided giant gartersnakes legal protection and mechanisms for recovery, and subsequent listing under the U.S. Endangered Species Act (federal ESA) further fortified these protections. But how effective has listing under these endangered species acts (ESAs) been at achieving their goal of giant gartersnake recovery? Herein, we review relevant aspects of giant gartersnake ecology, illustrate how listing has benefited giant gartersnakes and what challenges have been faced in slowing declines and recovering populations, and chart a course toward improved conservation, management, and recovery of giant gartersnakes. Although listing as threatened under both state and federal ESAs has not yet achieved recovery of giant gartersnakes, the increased knowledge gained and mechanisms for protecting giant gartersnake habitat on private and public lands developed over the past 50 years has improved conservation of this endemic California snake.

**Key words:** Central Valley, conservation, garter snake, private land, rice, *Thamnophis gigas*, water management, wetlands

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Giant gartersnakes (*Thamnophis gigas*) are semi-aquatic snakes endemic to marshes, sloughs and other stagnant or slow-moving aquatic habitats of California's Central Valley. Although they once ranged through all but the most northern portions of the Central Valley,

the loss of wetlands has extirpated them from about two-thirds of their historical range. Because of this loss of habitat, giant gartersnakes were among the inaugural list of rare species under the California Endangered Species Act (CESA; California Department of Fish and Game Commission 1971). Herein, we review giant gartersnake biology and the conditions leading to their listing as rare, discuss how listing has helped conserve giant gartersnakes and what challenges have been encountered, and chart a path toward giant gartersnake recovery.

## GIANT GARTERSNAKE BIOLOGY AND LISTING HISTORY

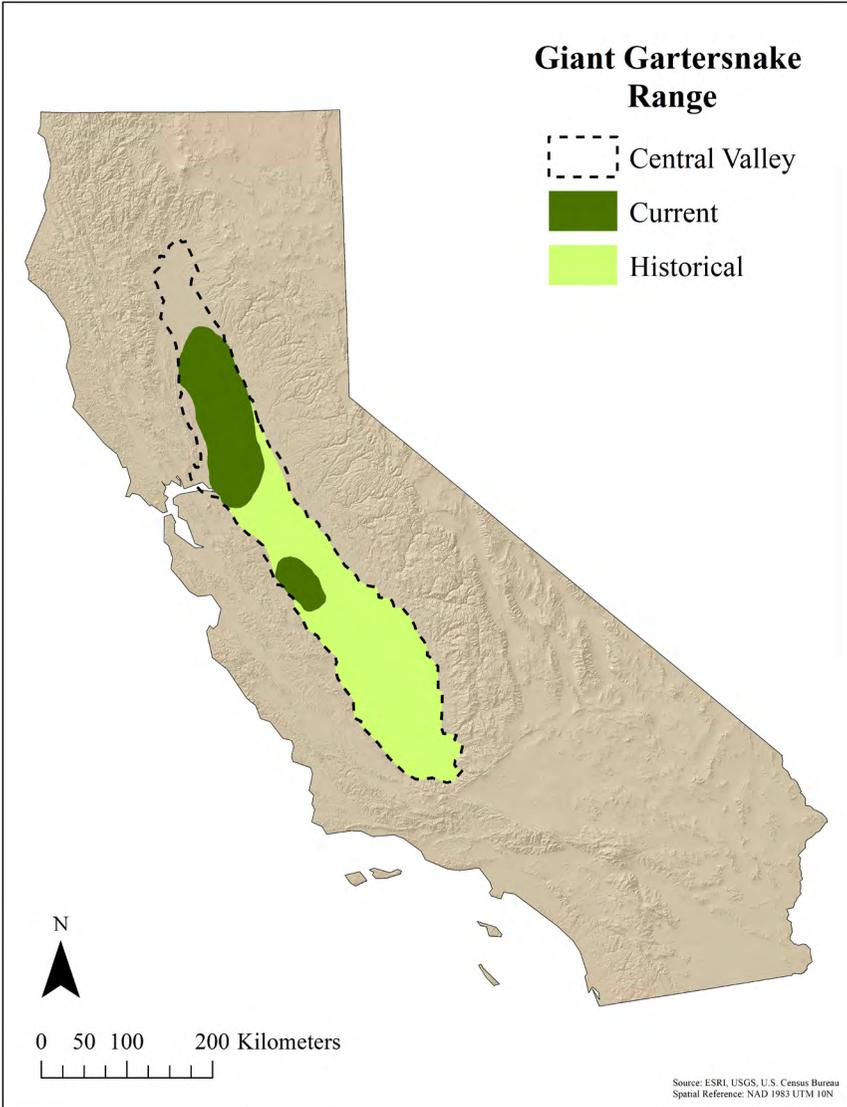
### Description

Giant gartersnakes currently range from Butte County to Fresno County, although few populations remain in the southern portion of their range (Fig. 1; Hansen and Brode 1980; Ernst and Ernst 2003; Wylie and Amarello 2006). Body coloration in giant gartersnakes is typically brown or olive with a yellow dorsal stripe and a light-yellow lateral stripe on either side of the body (Fig. 2). Between the dorsal and lateral stripes are paired rows of dark blotches. The ventral surface is generally pale olive yellow in color, though giant gartersnakes exhibit variation in color from overall darker specimens with dark olive dorsal and lateral stripes to snakes with vivid orange dorsal and lateral stripes and peach-colored ventral surfaces. Historically, giant gartersnakes reached a maximum total body length of 162.6 cm, making them the largest species of gartersnake (Ernst and Ernst 2003). From 1995 to 2020, the largest giant gartersnake captured and measured by the U.S. Geological Survey (USGS) was 142.0 cm total length in 2019 (U.S. Geological Survey, unpublished data). Large giant gartersnakes are thick-bodied snakes, reaching weights of up to 930 g. Female giant gartersnakes reach larger sizes than males (Wylie et al. 2010), though males reach their maximum size quicker with a faster growth rate (Rose et al. 2018b).

### Population and Community Ecology

Because of their secretive nature, estimating abundance of giant gartersnake populations and understanding giant gartersnake population ecology requires intensive field study and sophisticated analytical methods. Long-term capture-mark-recapture (CMR) studies of giant gartersnakes have provided valuable information on demographic vital rates and their contribution to population growth. Giant gartersnake annual survival rates increase with higher emergent vegetation cover at a site and higher precipitation the previous year (Rose et al. 2018c), and with individual size. Hansen et al. (2015) found a linear increase in annual survival with snout-vent length (SVL), whereas Rose et al. (2018c) found that survival increased with SVL up to a peak for individuals near 800 mm SVL, followed by a plateau or slight decrease for even larger individuals. Female fecundity also increases with size (Rose et al. 2018a), and the greater reproductive value of large adult females means that the survival of this life stage has the greatest influence on population growth rates (Rose et al. 2019). The growth and survival rates of juvenile (1 year old) giant gartersnakes also have an important influence on population growth, especially when the probability of recruitment from neonate to 1 year old is higher (Rose et al. 2019). The value of large females and growth and survival of juvenile snakes suggests the importance of prey availability to the life history of giant gartersnakes.

Historically, giant gartersnakes likely preyed on soft-rayed fishes and amphibians, likely including some locally extinct species (Rossman et al. 1996). Around the turn of the



**Figure 1.** Estimated giant gartersnake (*Thamnophis gigas*) historical and current ranges based on Rossman et al. (1996), California Department of Fish and Wildlife (2016), and California Natural Diversity Database (CNDDDB; 2020).

century, many generalist fish species and American bullfrogs (*Lithobates catesbeianus*; hereinafter bullfrogs) were introduced from the eastern United States and have dramatically changed the prey composition available to giant gartersnakes. Despite the dominance of nonnative prey, giant gartersnakes retain a preference for native anurans and generally select anurans (native and introduced) over introduced fishes (Ersan et al. 2020a, 2020b). Naïve neonate giant gartersnakes in the laboratory respond most strongly to extracts of and most readily consume larval and adult Sierran treefrogs (*Pseudacris sierra*; Ersan et al.



**Figure 2.** An adult giant gartersnake (*Thamnophis gigas*) next to a canal in habitat typical of the rice agricultural landscape in which most extant populations occur. U.S. Geological Survey photograph taken by Elliot Schoenig.

2020a), and wild giant gartersnakes also strongly select native treefrogs (Ersan et al. 2020b). Although bullfrog tadpoles and juveniles also are positively selected as prey (Ersan et al. 2020a, 2020b), adult bullfrogs are predators of small giant gartersnakes (Wylie et al. 2003).

Giant gartersnakes are prey to a variety of native and nonnative vertebrates. Mesopredators, especially North American river otters (*Lontra canadensis*); wading birds; and raptors eat giant gartersnakes, and smaller predators like American mink (*Neovison vison*) likely take small giant gartersnakes. Introduced predators, particularly large centrarchid fishes, also prey on giant gartersnakes. Although giant gartersnakes can eat the young of these introduced fishes, they are less important as prey than bullfrogs, and because they deplete native frog populations (Adams et al. 2003), their presence is unlikely to benefit giant gartersnakes in any meaningful way. The effects of bullfrogs, however, are less clear, because they serve both as important prey and predators. Ongoing research seeks to elucidate the intraguild predation relationship between giant gartersnakes and bullfrogs to develop bullfrog management strategies that result in the greatest benefit to giant gartersnakes. Regardless of the predator, however, the influence of predation at the population level might be somewhat ameliorated by habitat structure.

### **Habitat, Past and Present**

The Central Valley historically comprised a network of rivers and floodplains supporting expansive permanent and seasonally flooded freshwater marshes and shallow lakes

across several drainage basins (Frayer et al. 1989; Garone 2011). Marshes were primarily characterized by emergent vegetation, predominantly hardstem bulrushes (*Schoenoplectus acutus*; colloquially and hereinafter referred to as “tules”) and cattails (*Typha* spp.). In the contemporary landscape, giant gartersnakes spend > 50% of their time in the active season on land basking or using cover such as rodent burrows, with most of this terrestrial time spent within 10 m of water (Halstead et al. 2015). During brumation, nearly all snakes are found underground in the terrestrial environment (Halstead et al. 2015).

Today, the Central Valley has lost > 90% of its historical marshes due to agricultural conversion and urban development (Frayer et al. 1989; Garone 2011). Floodplains have been permanently altered by levees built to protect development and agriculture, and artificial water delivery canals have replaced the network of rivers and sloughs that once supported natural marshes and lakes (Frayer et al. 1989; Garone 2011). Most extant giant gartersnake populations are associated with rice agriculture in the Sacramento Valley, where the network of canals, drains, and rice fields themselves provide marsh-like habitat during the giant gartersnake active season (Halstead et al. 2010). Although rice agriculture supports giant gartersnake populations, simplification of habitat structure and mismatches in the phenology of rice agriculture and giant gartersnake ecology limit its suitability relative to marshes (Halstead et al. 2010, 2012, 2019; Wylie et al. 2010). Nonetheless, giant gartersnakes with less rice in and near their home ranges have lower survival; therefore, giant gartersnakes are considered reliant on rice agriculture in the Sacramento Valley (Halstead et al. 2019).

### **Conservation Status**

The California Endangered Species Act (CESA) was enacted in 1970 to conserve and protect native plant and animal species at risk of extinction in the state of California. When the CESA was first enacted, it included take restrictions on killing, trapping, collecting, and harming individuals; however, harm is no longer included in the definition of take (Dwyer and Murphy 1995). The giant gartersnake was declared rare in 1971, as one of the 42 animals inaugurally protected under state law. The primary reason for initial listing was habitat loss caused by land development and conversion from wetlands to agriculture in the Sacramento and San Joaquin valleys (Hansen and Brode 1980). This classification was later changed to threatened in the 1984 amendment of the CESA. The take, possession, purchase, or sale of threatened species is prohibited under state law (California Fish and Game Code, section 2080 *et seq.*). The California Department of Fish and Wildlife (CDFW) may authorize take incidental to lawful activity if the effects of the take are minimized and fully mitigated and taking would not jeopardize the species' continued existence (California Fish and Game Code, section 2080 *et seq.*). Habitat mitigation is often a requirement to fully mitigate take of CESA species.

Giant gartersnakes were listed as threatened under the U.S. Endangered Species Act (federal ESA) in 1993 (USFWS 1993), and the finalized recovery plan was completed in 2017 (USFWS 2017). Giant gartersnakes benefit from further protection under the federal ESA because the federal regulatory definition of “harm” (50 CFR §17.3) more clearly includes significant modification or degradation of habitat of imperiled species where such activity kills or injures wildlife by impairing essential breeding or feeding behavioral patterns (Dwyer and Murphy 1995). Although the federal ESA provides for the designation of critical habitat for threatened and endangered species, no critical habitat has been designated for giant gartersnakes. This dual listing under both the federal ESA and the CESA allows for

more complete protection of giant gartersnakes and their habitat throughout their current range such as when new projects like dams and diversions arise.

The federal Recovery Plan for the Giant Gartersnake highlights six key recovery criteria (USFWS 2017). The first and second stipulate that sufficient habitat is classified under protected status such that populations are supported, and that these populations are connected with corridors of suitable habitat. The third criterion states management plans and best management practices oriented to giant gartersnake conservation are developed and implemented using adaptive management. The fourth and fifth require protected habitat to be supplied with a reliable source of clean water during the active months of the giant gartersnake, and that threats like disease are reduced or removed. The sixth and final recovery criterion requires that monitoring of recovery units demonstrates stable or increasing populations over a 20-year period, including at least one 3-year drought (USFWS 2017). Although these recovery criteria are related to the species' status under the federal ESA, by meeting these criteria the conditions of habitat loss and urbanization under which giant gartersnakes were first listed under the CESA will no longer pose an extinction risk. These criteria provide a roadmap for the recovery of the giant gartersnake, and the eventual delisting under both the U.S. and California ESAs.

Giant gartersnakes have been protected under the CESA for more than 50 years, and under the federal ESA for more than 25 years, and it is appropriate to ask how listing as threatened under both ESAs has promoted the persistence of giant gartersnakes, and what challenges remain unaddressed by listing. In the remainder of this paper, we examine these questions and suggest a path forward to further benefit giant gartersnake conservation.

## **INFLUENCE OF LISTING ON GIANT GARTERSNAKE CONSERVATION**

### **How has listing helped giant gartersnakes?**

The listing of giant gartersnakes under the California and federal ESAs has benefited giant gartersnake conservation in several ways, including (1) providing a mechanism to offset habitat loss by restoring and preserving marshes and funding research to guide recovery; (2) encouraging the development of programs to protect private landowners conducting habitat improvement in good faith or maintaining appropriate conditions for listed species on their lands; (3) changing construction practices to account for giant gartersnakes; (4) influencing water management, particularly water transfers across large distances within California; and (5) increasing knowledge about and concern for the species.

Conservation and restoration of marsh habitat in the Central Valley has accelerated over the past several decades. The increased focus on wetland restoration and management has benefited giant gartersnakes in two ways: (1) increased conservation and restoration of marshes through incentive programs and mitigation requirements and (2) improved wetland management for the benefit of giant gartersnakes. Private landowners, non-governmental organizations, and state and federal refuges have conserved or constructed nearly 90,000 ha of marsh habitat in the Central Valley as of 2015 (Central Valley Joint Venture 2020). Although giant gartersnake conservation has not been the impetus for much of this wetland restoration, these efforts aid in the giant gartersnake's persistence across the contemporary fragmented landscape. Publicly conserved lands within the current range of the giant gartersnake include eight national wildlife refuges, 23 state wildlife or ecological areas, and preserves under partnership management such as Cosumnes River Preserve. These lands

are often publicly accessible and have multiple land management goals that balance public use and wildlife habitat management. Many of these public lands have focused habitat management to include objectives for giant gartersnakes and some, such as Colusa National Wildlife Refuge and the Cosumnes River Preserve (among others), have enhanced and restored habitats that benefit giant gartersnakes. Lands managed by public entities can provide opportunities for partnerships to restore or enhance lands with multi-benefit objectives. The California Department of Water Resources has engaged with multiple partners to restore habitat for both winter waterfowl and giant gartersnakes on lands that may be managed for subsidence reversal or for other purposes. These multi-benefit projects include the western Sacramento-San Joaquin Delta, including Sherman and Twitchell islands where giant gartersnakes have been documented following restoration (CNDDDB 2020).

Compensatory mitigation that can contribute to species conservation is driven by public and private actions as a result of requirements from California and federal ESA permits for effects to giant gartersnakes or their habitat. Projects that require permits can be small scale ditch or culvert repair, wetland restoration, or large-scale projects such as the state or federal water projects for water transfers. The type of conservation land is categorized by the permit and mitigation route and generally falls into three categories: conservation banks, permittee-responsible mitigation, and reserve systems in Habitat Conservation Plans (HCPs) or Natural Community Conservation Plans (NCCPs). These lands typically are not publicly accessible and management goals are focused on species and their habitats. To become approved as banks or other mitigation, restoration and enhancement approved by the U.S. Fish and Wildlife Service (USFWS), CDFW, or both must also include long-term management and not just preservation of habitats (Bunn et al. 2013). Although typically not accessible to the general public, these lands offer research opportunities similar to public conservation lands, and conservation banks such as Gilsizer Slough Giant Garter Snake Conservation Complex and Natomas Basin Conservancy reserves have provided long-term access to research and monitoring (e.g., Halstead et al. 2012, 2015, 2016; Rose et al. 2018c, 2018b, 2019).

The overall footprint of remaining giant gartersnake habitat is certainly enhanced through a variety of means under the influence of both California and federal ESAs. The mitigation lands in the Natomas Basin associated with the HCP alone account for 1600 ha of giant gartersnake habitat that otherwise would not be created or protected. The presence of giant gartersnakes at Colusa National Wildlife Refuge (NWR) was a primary consideration in the purchase of additional inholdings (formerly rice fields) that are now managed for summer water for giant gartersnakes within Tract 24. A major source of the acquisition funding for this purchase came from compensatory mitigation for the Central Valley Project (CVP) through the Central Valley Project Improvement Act (CVPIA) Habitat Restoration Program (HRP). These are just a few examples of habitat being created or conserved because of the giant gartersnake's threatened status under the CESA and the federal ESA.

Safe Harbor Agreements (SHAs) are another mechanism used by USFWS and CDFW to enable conservation and restoration actions on private land, which provides the most habitat for giant gartersnakes. When implemented, SHAs allow for continued commercial, agricultural, or industrial use of habitats as long as baseline habitat conditions are maintained, and habitat restoration, minimization of negative effects, or mitigation are implemented in good faith. Safe Harbor Agreements usually allow for incidental take of listed species during certain activities, including implementing beneficial activities, conducting routine ranching and farming activities, and returning properties to baseline habitat conditions. Two safe

harbor agreements have been implemented for giant gartersnakes under USFWS: A 2013 SHA with the Sacramento River Conservation Area Forum and a 2007 Yolo County SHA with Audubon California. Combined, these SHAs cover 81,000 ha within the range of giant gartersnakes, and the agreements have a duration of 30 years. Although SHAs were codified into state law in 2009, both SHAs covering giant gartersnakes have been implemented solely under federal law.

Another group of programs developed to encourage conservation on private lands is Habitat Exchange Programs (HEPs). Habitat exchange programs allow private landowners to sell mitigation credits for listed species habitat on their lands with the agreement to maintain or improve habitat. Habitat quality and quantity are assessed by an independent entity with oversight from CDFW and USFWS, and better-quality habitat sells at a higher price per acre than poorer quality habitat. Thus, habitat preservation and improvement are incentivized by market forces. Two farms in the Sacramento Valley are currently selling credits for giant gartersnakes under the Central Valley HEP, with a total of nearly 1,600 ha enrolled as of 2020. Habitat Exchange Programs and SHAs are not mutually exclusive; indeed, habitat enhancements to benefit a landowner enrolled in an HEP are often covered by an SHA.

In addition to habitat protection and restoration, the listing of giant gartersnakes has changed some construction practices for the benefit of giant gartersnakes. Seasonal restrictions on groundwork between October and May for permitted projects near wetlands and canals within the range of giant gartersnakes prevents disturbance to overwintering snakes, when most snakes are underground and vulnerable to disturbance (Halstead et al. 2015). This and similar avoidance and minimization measures to limit negative effects to giant gartersnakes would not be in place without listing under the CESA and the federal ESA.

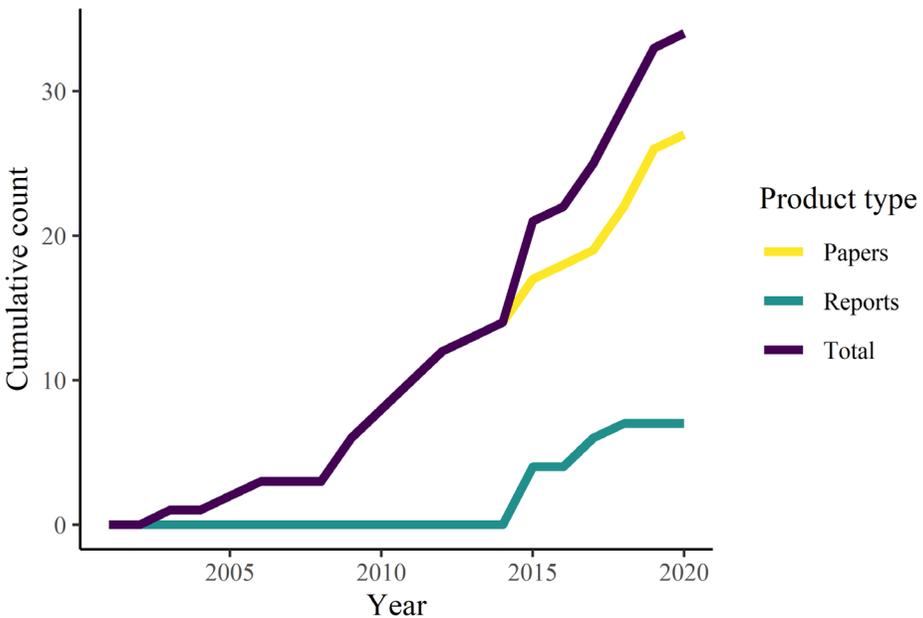
Listing has further resulted in a focus on the effects of water management practices on the species. Recognition of the detrimental effects of wetland loss on wetland-dependent wildlife and the role of the CVP in conversion of wetlands to other uses resulted in the passage of the CVPIA in 1992. The CVPIA mandates changes in CVP management, primarily to benefit fish and wildlife and their habitats through habitat protection, restoration, and management and research to support these efforts. In addition to the CVPIA, the transfer of water through state or federal facilities requires review under the federal ESA, the CESA, or both. Water transfers are perhaps the largest scale and most frequent action that affects giant gartersnakes and requires environmental review. Indeed, water transfers have stimulated and funded the most substantive research on giant gartersnakes both to understand the effects of and mitigate for water transfers from the Sacramento Valley to other parts of California.

One of the greatest benefits listing has conferred to giant gartersnakes is promoting an understanding of the species. Indeed, much of what was known about giant gartersnakes prior to listing was based on historical accounts from sites long since drained for agriculture (e.g., Van Denburgh and Slevin 1918; Fitch 1940; Wright and Wright 1957). From listing under the CESA in 1971 until listing under the federal ESA in 1993, interest in giant gartersnakes from the scientific and conservation communities increased, and an important thesis on the ecology of giant gartersnakes by Hansen (1980) and several important reports documenting the status of giant gartersnakes (e.g., Hansen and Brode 1980; Hansen 1986, 1987) were published. Additional research continued in the late 1990s and early 2000s, but it was not until the provision of larger amounts of longer-term funding for research, largely driven by water transfers, that peer-reviewed research on giant gartersnake ecology and conservation rapidly increased (Fig. 3).

### What challenges remain for giant gartersnake protection and recovery, despite listing?

Despite the numerous ways listing has benefited giant gartersnakes, many challenges to giant gartersnake conservation remain. The limited definition of take under the CESA, which does not include “harm,” means that indirect effects to giant gartersnake habitat by projects without federal involvement are often not consulted on or mitigated for. The lack of coverage under the CESA for loss of habitat and focus directly on killing, trapping, and collecting is especially problematic for secretive and cryptic species like giant gartersnakes. For these species, direct effects are rarely observed, and more insidious habitat conversion, particularly on agricultural lands, is problematic.

Both the CESA and the federal ESA have allowances for normal agricultural practices to continue in the presence of listed species except in the instance of take or when there is a major land conversion (agriculture to other uses, such as commercial or residential development). Crop rotation or permanent conversion, pest control, infrastructure maintenance, or other routine agricultural activities often do not trigger either ESA. Such activities, including conversion of rice to row crops or orchards, pesticide applications, and dredging of canals or grading of canal banks, can, however, negatively affect giant gartersnake populations. When a species like the giant gartersnake largely occurs in an agricultural landscape, most populations are unprotected and reliant on the continuation of conditions that allow them to persist or voluntary conservation actions by private landowners. Although programs like SHAs and HEPs exist to encourage conservation on private lands, these have been underutilized for giant gartersnake conservation.



**Figure 3.** Cumulative number of peer-reviewed journal articles and publicly-available peer-reviewed reports (U.S. Geological Survey Open-File Reports) from a Web of Science™ search from 1985–2020 using the search terms Topic = “giant garter\*” OR Topic = “Thamnophis gigas.” Six peer-reviewed papers in the results were removed because they were not about giant gartersnakes, but compared results for other species to giant gartersnakes.

Agricultural practices do trigger environmental review including ESA processes when state or federal facilities for water transfers are used. In the case of rice agriculture, this primarily occurs when water is sold from water rights holders in the Sacramento Valley to other parts of California and must pass through state or federal water project facilities to reach buyers. Although water transfers have received recent attention because of their potential effects on wetland-dependent wildlife, much remains to be learned about their short-term and cumulative effects on giant gartersnakes and the adequacy of conservation measures for protecting populations.

The attention given to water transfers notwithstanding, the procurement of active season water for giant gartersnakes warrants additional attention. Habitat Conservation Plans and NCCPs often have provisions for emergency water supplies, for example by augmenting surface water with well water. This makes HCPs and NCCPs a valuable resource for giant gartersnake conservation because a reliable supply of water to support marshes from April through October is essential for giant gartersnakes (Halstead et al. 2019). The importance of appropriate water management for giant gartersnakes can be illustrated by comparing the Sacramento Valley, where most extant giant gartersnake populations occur, and the San Joaquin Valley, where few populations remain.

### **A Tale of Two Valleys**

The decision to list giant gartersnakes under the CESA in 1971 was primarily because giant gartersnakes were nearly extirpated from the San Joaquin Valley (Hansen and Brode 1980) with conversion of marsh habitat to cotton and other unsuitable row crop agriculture and orchards (Musoke and Olmstead 1982; Frayer et al. 1989; Garone 2011). The Tulare Basin in particular suffered extensive habitat loss with the 197,000 ha Tulare Lake completely drained by the early 1900s and most of its extensive tule marsh habitat, which represented > 33% of the giant gartersnake's range, now largely eliminated (Fitch 1940; Hansen and Brode 1980; Garone 2011). Only a few isolated snake populations remain in the San Joaquin Valley and Sacramento-San Joaquin Delta today (Hansen and Brode 1980; Hansen 1987; Sloan 2004; Dickert 2005), and surveys south of Fresno in 2006 failed to detect giant gartersnakes despite marsh habitat restoration in some locations (Wylie and Amarello 2006).

In contrast to the conversion of wetlands to orchards and row crops in the San Joaquin Valley, in the Sacramento Valley a mosaic of remnant historical tule marshes (Halstead et al. 2014, 2016), constructed marshes for waterfowl hunting (Frayer et al. 1989; Reyes et al. 2017), and widespread rice agriculture and associated water delivery and drainage canals (Hansen and Brode 1980; Halstead et al. 2010; Reyes et al. 2017) continue to support populations of giant gartersnakes. Rice agriculture acts as surrogate marsh habitat and, in addition to tule marshes, is also used for waterfowl hunting, which may further protect rice from conversion to other types of agriculture unsuitable for giant gartersnakes. Changes in rice irrigation practices (Torbick and Salas 2014; Linqvist et al. 2015), water availability in adjacent canals (Anderson et al. 2017), and ongoing drought leading to rice idling, as well as a rise in conversion to nut tree agriculture (Sleeter et al. 2017) continue to threaten giant gartersnakes in the Sacramento Valley. Despite these remaining challenges, giant gartersnake populations in the Sacramento Valley have unquestionably fared better than those farther south.

The contrast between the two valleys begs the following questions:

1. If rice weren't grown in the Sacramento Valley, would listing have prevented declines like those observed in the San Joaquin Valley?
2. Will its status as a state and federally threatened species recover giant gartersnake populations, or at least maintain existing populations into the future?

The answer to question (1), we suspect, is no. This answer is, in part, because land conversion from wetlands to agriculture had occurred prior to implementation of the CESA. If the land that was converted to rice agriculture had been converted to another crop, the result in the Sacramento Valley likely would have been the same as in the San Joaquin Valley. Giant gartersnakes might have fared slightly better in the Sacramento Valley because of a few remnant wetlands and generally greater water availability than in the San Joaquin Valley, but large-scale extirpation likely would have occurred.

We examine the answer to question (2) below, where we explore ways that the threatened status of the giant gartersnake can be leveraged to improve conservation efforts for the species and the research that will further inform recovery and management of giant gartersnakes.

### **A PATH FORWARD**

Giant gartersnake conservation and recovery in the future can be fostered in several ways. The first is more widespread implementation of appropriate water management for giant gartersnakes. For the most part, water management to support giant gartersnake populations is well-known: they require surface water that persists throughout the active season and is available perennially. Critical habitat for giant gartersnakes, while formally undefined, is inextricably linked to water supply, distribution, and application across the Central Valley landscape. Limitations to water on the landscape related to water supply, cropping patterns, or wetland restoration can have large effects on giant gartersnake populations. The challenge in managing water for giant gartersnakes is like that for other water management issues in California, including increasing variability in precipitation patterns (Swain et al. 2018) and generally increasing demand for water for a variety of uses. Future water supply may further be affected by changes to how groundwater is managed in California given California's Sustainable Groundwater Management Act, which was passed by the legislature in 2014, and the potential uncertainty associated with local control of groundwater resources (Kiparsky et al. 2017).

Recent conservation efforts in the Sacramento Valley have focused on a floodplain restoration approach, which attempts to integrate the needs of fish, birds, and other wildlife (<https://www.biographic.com/raised-in-rice-fields/>). Understanding how wetland-dependent species like giant gartersnakes fit into multi-species conservation programs will be a challenge as different target species require water in different locations and seasons. Given the reliance of giant gartersnakes on inundated wetlands during the April–October active season, when water is present on the landscape is critical (Halstead et al. 2019). The potential to retain winter water for waterfowl later in the spring (i.e., through March and April) through water management in post-harvest rice fields could prove to be very beneficial to giant gartersnakes, which often emerge from brumation sites with little to no nearby flooded habitats (Halstead et al. 2019). Efforts to promote March and April water availability for migrating shorebirds also might benefit giant gartersnakes. The potential to have a greater number of post-harvest rice fields or associated ditches with water and food present offers

an opportunity for increased body condition, survival, and potentially reproductive success for giant gartersnakes. With proper planning and input, a multi-species strategy that better incorporates the life history needs of the snake could be developed.

Most state and federal refuges in the Sacramento and San Joaquin valleys are managed in large part for wintering waterfowl. This type of management involves seasonally flooding wetlands in the late summer and fall and then draining in the late winter to encourage growth of vegetation that provides food for waterfowl. This food production period results in a large extent of seasonally dry wetland areas primarily during the giant gartersnake active season. Although most of the refuges maintain some level of spring and summer flooded wetlands, it is usually less than 15% of managed habitats and often, depending on water availability, much less than that (Central Valley Joint Venture 2020). The lack of summer wetlands available in the Central Valley may be affecting the success of breeding waterfowl in California, which make up an important component of the state's waterfowl harvest (De Sobrino et al. 2017; Central Valley Joint Venture 2020). Thus, working within the state and federal refuge systems and helping secure water on the landscape during the spring and summer may provide a win-win for waterfowl and giant gartersnakes on public lands. Achieving giant gartersnake conservation and recovery goals will largely depend, however, on the cooperation of private landowners.

Encouraging private landowners to more fully utilize existing conservation incentives would almost certainly benefit giant gartersnakes. The vast majority of giant gartersnake populations occur on private lands, and it is unlikely that sufficient wetland acreage under public ownership could be acquired to support persistent giant gartersnake populations. Moreover, relying solely on public lands for giant gartersnake conservation ignores opportunities for reconciliation ecology (Rosenzweig 2003) and win-win solutions whereby rice farmers can continue their livelihood and perhaps enhance their lands for giant gartersnakes and other wetland-dependent wildlife. Programs like SHAs and HEPs exist, but only two examples of each program have been implemented in the Sacramento Valley, with at least one farm participating in the Central Valley HEP also enrolled in an SHA. Encouraging enrollment in these and other conservation programs will benefit giant gartersnakes in their Sacramento Valley stronghold.

Relatively small modifications to rice cultural practices could have large effects on giant gartersnake populations. For example, grading or removing vegetation from canal banks, particularly in winter when nearly all giant gartersnakes are brumating underground (Halstead et al. 2015), could kill large numbers of giant gartersnakes. Conducting these activities during the active season or on short stretches of a single side of a canal could minimize negative effects of canal management. Maintaining large, contiguous areas of rice agriculture to the extent possible would increase survival of adult giant gartersnakes and benefit giant gartersnake populations (Halstead et al. 2019; Rose et al. 2019). Finally, maintaining a mosaic of open water and emergent vegetation, especially tules, in canals can provide cover and foraging opportunities for giant gartersnakes. Encouraging these actions through SHAs and HEPs could benefit rice farmers and provide important habitat enhancements for giant gartersnake populations.

Habitat Conservation Plans and NCCPs also could play a more prominent role in giant gartersnake conservation. Large portions of the historical range of giant gartersnakes do not have HCPs that include giant gartersnakes, including key counties with extant snake populations (e.g., Colusa, Glenn, and most of Sutter counties in the Sacramento Valley and

Merced County in the San Joaquin Valley; Greco 2020). Moreover, many HCPs that are not also approved as an NCCP include only a jeopardy standard (i.e., stable populations or no net loss), rather than the recovery standard (i.e., positive population growth) adopted by NCCPs (Greco 2020). Habitat Conservation Plans and NCCPs often have a landscape scale approach, and in areas without HCPs, mitigation can be piecemeal without regard for larger conservation goals or spatial context (Greco 2020). The development of HCPs, however, is to offset incidental take, so in practice it is unclear how they would be implemented in areas without development pressure. Nonetheless, a regional approach to conservation that includes wider adoption of HCPs and NCCPs with recovery standards would likely benefit giant gartersnakes by promoting recovery (rather than loss prevention) and emphasizing connectivity among populations.

The modification of the landscape giant gartersnakes inhabit challenges our concept of what constitutes a giant gartersnake population. Although five genetic clusters largely corresponding with drainage basins are well-defined, it is unclear how populations are structured within drainage basins (Wood et al. 2015). Demographic studies of giant gartersnakes thus far have focused on treating populations as discrete units (e.g., Hansen et al. 2015; Rose et al. 2018c). Given the network of irrigation canals that make up a majority of giant gartersnake habitat, connectivity among populations is likely important for determining viability at the landscape scale (Greco 2020). For example, it is unknown whether certain high-quality habitats (e.g., wide, permanently inundated canals; restored wetlands) act as sources that support viable, growing giant gartersnake populations while others (e.g., narrow, seasonally inundated canals) are sinks or only transiently occupied. Studies that integrate capture-mark-recapture or occupancy data from many sites and explicitly model the effect of distance between populations (e.g., network distance in canal habitat) on survival, population growth, colonization, and extinction rates could inform future management and conservation actions. Spatial capture-recapture models can explicitly relate individual capture data to movement and habitat connectivity (Royle et al. 2018) and have the potential to reveal how landscape structure affects giant gartersnake population dynamics. If metapopulation dynamics are important for sustaining giant gartersnake populations in fragmented habitat, plans designed to manage aquatic and terrestrial habitats at the landscape scale will be necessary to ensure viability in the long term. Identifying the appropriate spatial scale(s) for management will be increasingly important as urbanization encroaches upon and fragments remaining habitat and increases road density and traffic creating vehicle strike risks (Brehme et al. 2018; USFWS 2020). Rice fallowing and reduction in the network of water-filled canals caused by drought and water transfers further reduce connectivity among populations, and in the near-term might have stronger effects than urbanization on most giant gartersnake populations.

The challenges and unknowns faced with maintaining extant giant gartersnake populations increase when conservation actions meant to improve and recover giant gartersnakes are considered. For example, optimizing the design and management of giant gartersnake habitat restoration projects remains an important unknown in giant gartersnake recovery. Although much has been learned in the past decade about giant gartersnake habitat selection (Valcarcel 2011; Halstead et al. 2016; Reyes et al. 2017), formal evaluation of giant gartersnake colonization and use of restored wetlands is incomplete. Optimum ratios of aquatic active season and terrestrial brumation habitat remain unknown, as does habitat selection of male, neonate, and juvenile snakes that are too small for current radio telemetry methods. A related question is whether giant gartersnake use of terrestrial environments is an artifact of

the contemporary landscape, where expansive marshes with dense, persistent tule patches are rare. The lower survival of giant gartersnakes in linear habitats, like canals, challenges restoration design to maximize water and emergent vegetation edge without creating potential population sinks where giant gartersnakes might be more susceptible to predators like otters (Halstead et al. 2012). Water management issues, such as whether groundwater is an acceptable substitute for surface water and what bathymetry best supports all life stages of giant gartersnakes while minimizing the suitability of marshes for introduced predatory fishes, also need quantitative evaluation. Finally, balancing timing and methods of canal and marsh management, such as pesticide use, drying for vegetation control and removal of aquatic invasive species, and dredging and grading to maintain water flow, among other practices, with giant gartersnake ecology remains largely anecdotal and awaits further detailed study.

Because giant gartersnake populations inhabit a region with well-established populations of invasive aquatic plants and animals, understanding the interactions of giant gartersnakes with invasive species and the effects of invasive species management on giant gartersnakes is essential. For example, invasive plants, like water hyacinth (*Eichornia* spp.) and water-primrose (*Ludwigia* spp.), prevent efficient water flow and left unchecked can completely cover canals. Although the open water-vegetation interface selected by giant gartersnakes (Valcarcel 2011) can be maintained with chemical and mechanical control, the effects of these practices on giant gartersnakes are not well understood (Halstead et al. 2016). In addition to habitat alteration by plants, two species of watersnakes from the eastern U.S. (southern watersnakes, *Nerodia fasciata*, and common watersnakes, *N. sipedon*) have been introduced to the Sacramento area and could compete with giant gartersnakes if their introduced ranges expand (Rose and Todd 2014). Other introduced aquatic animals like bullfrogs and fishes can be both prey for and predators of giant gartersnakes (Wylie et al. 2003), complicating the effects of invasive species management. The mix of positive and negative effects of invasive species on giant gartersnakes requires careful planning of both invasive species control regimes and habitat management for giant gartersnakes. For example, it is possible that improving wetland habitat or water management regimes for giant gartersnakes can simultaneously benefit aquatic invasive species, thereby slowing the recovery of giant gartersnakes. Therefore, reducing habitat suitability for invasive species, providing structural refuge for giant gartersnakes, or some degree of culling invasive predators and competitors in restored marshes might improve giant gartersnake demographic rates and the likelihood of a successful establishment at restored or enhanced sites. At sites where Sierran treefrogs persist, minimizing the effects of invasive species on these native frogs can facilitate the recovery of the snakes' preferred native prey. Synergistically pairing invasive predator removal with translocation has improved recovery of imperiled species such as the endangered Sandy Cay rock iguana (*Cyclura rileyi cristata*) and the endangered humpback chub (*Gila cypha*; Spurgeon et al. 2015; Hayes et al. 2016).

Understanding the tradeoffs resulting from invasive species as prey, predators, and competitors can be the key to understanding when and how invasive species control might bolster the outcome of ongoing conservation actions for giant gartersnakes. A stronger net negative effect of an invasive species on giant gartersnakes likely points to a higher utility of controlling the invasive species below a management threshold (Noonburg and Byers 2005). For example, the estimated annual loss of snake recruitment caused by bullfrogs (Wylie et al. 2003) might be overcompensated by the snakes frequently preying on pre-metamorphic and juvenile bullfrogs. Because giant gartersnakes prefer bullfrogs over invasive fish prey (Ersan

et al. 2020a, 2020b), it is possible that where Sierran treefrogs are rare or extirpated, bullfrogs may be an important prey source to sustain the snake population (Rose et al. 2018b, 2019).

Future studies on giant gartersnakes can aim to assess the tradeoffs between invasive species' benefit as prey and harm as competitors and predators, as demonstrated in theoretical studies (Schellekens and van Kooten 2012) and experimental studies on the endangered Mohave tui chub (*Siphateles bicolor mohavensis*) and invasive mosquitofish (*Gambusia* spp.; Henkanaththegedara and Stockwell 2014). These tradeoffs could be examined using mathematical models to compare, for example, projected population growth rate of the snakes under alternative invasive species control scenarios, including a "no control" scenario. Results from these studies could inform the degree to which controlling invasive species might facilitate the recovery of giant gartersnakes when paired with other ongoing conservation efforts.

Full recovery of giant gartersnakes will not be achieved without re-establishing populations where snakes have been extirpated using translocation (USFWS 2017). Translocation, or the human-assisted movement of individuals from one location to another, can be a useful tool for species conservation and can be of two different types: conservation translocations and mitigation translocations (Germano and Bishop 2009). Historically, translocations in snakes have been dominated by mitigation translocations to reduce human-animal conflicts, either to remove nuisance individuals or to remove animals from sites undergoing development as an impact minimization measure (Germano et al. 2015). Mitigation translocations often have unknown or variable success rates. Despite being well funded, mitigation translocations often fail to select translocation sites that meet the ecological requirements of the animals being moved and do not adequately monitor animals following translocation (Germano et al. 2015). This can result in animals returning to the area from which they were removed or failing to thrive in the less suitable location. Mitigation translocations are further complicated when the species biology directly opposes the construction objectives or timeline. For example, construction activities in irrigation canals and rice agriculture infrastructure are most feasible during the winter when water in canals is drawn down and fields are fallowed, but this timeframe coincides with the giant gartersnake inactive season when snakes are overwintering in burrows along the banks of canals and are most vulnerable to disturbance (Hansen 2013; Halstead et al. 2015). Even if special care is taken to translocate snakes out of the construction area, snakes that are moved during the inactive season will likely be more vulnerable to mortality than if they were disturbed during the active season. Such mitigation translocations are generally used to minimize mortality in extant snake populations, whereas conservation translocations have the explicit goal of species recovery.

Conservation translocations, which are planned to introduce populations to or augment them in suitable protected habitat, have the potential to be more successful than mitigation translocations because the objectives align with the biological needs of the species and are designed with species preservation and recovery in mind. Conservation translocation has not yet been attempted in giant gartersnakes, but the U.S. Geological Survey is currently implementing a small-scale translocation within the American Basin genetic cluster. The southern region of the American Basin has been heavily developed, and despite recent efforts to restore habitat in the region, the southern sub-population has failed to recover (ICF International 2018). Adults and captive-reared juveniles from the more abundant central sub-population are being translocated into the restored wetland complex in the southern basin with the goals of bolstering the southern sub-population and identifying best practices for

future translocations. If translocated giant gartersnakes survive, reproduce, and establish a growing population, the strategy could be used to re-establish populations in the San Joaquin and Tulare basins to address one of the nine recovery actions for the species (USFWS 2017), provided that threats are ameliorated and a reliable supply of water is available. Translocating adult giant gartersnakes might be most efficient because adult female survival has the greatest influence on giant gartersnake population growth rates (Rose et al. 2019), but removing these snakes could negatively affect donor populations. On the other hand, because giant gartersnake population growth rate also is sensitive to juvenile survival and somatic growth rates (Rose et al. 2019), captive rearing of giant gartersnakes could reduce effects to donor populations while augmenting small populations or establishing new populations. Successful translocation also opens the possibility to introduce new alleles to populations with high inbreeding or low genetic diversity (Wood et al. 2015) and assist in the colonization of restored wetland habitat in the future.

## CONCLUSION

Giant gartersnake conservation has undoubtedly benefited by listing under both the California and U.S. endangered species acts. Both acts brought attention to the extent of habitat loss experienced by the species and stimulated investment in research and habitat restoration. Although many unknowns remain, much has been learned about giant gartersnakes in the 50 years since listing under the CESA, and research has accelerated substantially in the past decade. Key elements of giant gartersnake recovery will be managing water to ensure it is available to giant gartersnake populations during the spring and summer, encouraging conservation on private lands, managing invasive species, addressing the challenges of climate change and changing land use, and restoring giant gartersnakes to portions of their range from which they have been extirpated. Thus, fully recovering giant gartersnakes will require substantial investment in habitat restoration, ensuring a reliable water supply, and research into conservation translocations and other management practices. Despite these challenges, we expect that the science and practice of giant gartersnake conservation will continue to improve in the next 50 years.

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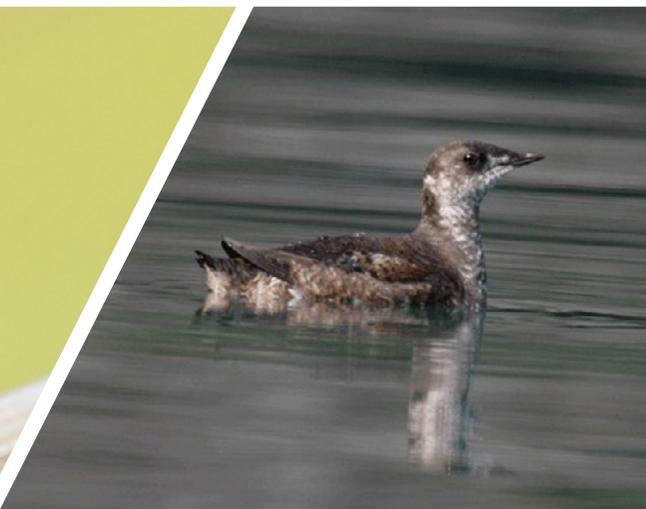
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# Birds



# Birds

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1. Bank swallow (*Riparia riparia*).  
Photo Credit: Shawn McCready, CC BY-ND 2.0
2. Marbled murrelet (*Brachyramphus marmoratus*).  
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3. Northern spotted owl (*Strix occidentalis caurina*).  
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4. California least turn (*Sternula antillarum browni*).  
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5. Greater sandhill crane (*Antigone canadensis tabida*).  
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6. Willow flycatcher (*Empidonax traillii*).  
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## FULL RESEARCH ARTICLE

**Owens Valley nesting willow flycatcher under pressure**LACEY GREENE<sup>1\*</sup>, ELSBETH OTTO<sup>1</sup>, AND CHRIS MCCREEDY<sup>2</sup><sup>1</sup> California Department of Fish and Wildlife, Region 6, 787 North Main Street, Bishop, CA 93514, USA<sup>2</sup> Atwell, LLC, 2 Towne Square #700, Southfield, MI 48076, USA

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Willow flycatchers (*Empidonax traillii*; WIFL) nest along the Owens River and Horton Creek in the Owens Valley. Migrating WIFL visit these sites as well as many other tributaries to both the Owens River and Mono Lake. We estimate there are approximately 35 WIFL territories in the Owens valley, or 5% of territories in California. Nesting WIFL in the Owens Valley are likely the federally endangered southwestern subspecies (*E. t. extimus*; SWIFL). The Chalk Bluff nesting site is particularly important as large nesting areas tend to be both rare and important for SWIFL and it contains more than half (63%) of all known WIFL territories in the region, which also represents 12% of all nesting SWIFL in California. Between 2014 and 2016, WIFL territory numbers declined from 37 to 27 across the three largest breeding sites. Territory numbers may have been influenced by drought conditions or brown-headed cowbird (*Molothrus ater*; BHCO) nest parasitism. In 2015 and 2016, comprehensive nest monitoring found nest parasitism rates were >40%, and nest success was lower in parasitized nests (16%; N = 5/31) compared with non-parasitized nests (60%; N = 31/52). BHCO management could potentially improve nest success for WIFL as well as many other open-cup nesting riparian birds in the Owens Valley.

**Key words:** brown-headed cowbird, callback surveys, *Empidonax traillii*, *Empidonax traillii extimus*, *Molothrus ater*, nest parasitism, nest success, Owens River, riparian birds, southwestern willow flycatcher, willow flycatcher

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Willow flycatchers (*Empidonax traillii*; WIFL) are a riparian obligate passerine that associate with both riverine and meadow habitat. Within California, all WIFL subspecies were classified as endangered in 1991 and protected under the California Endangered Species Act (CESA), and in 1995, SWIFL was specifically identified as endangered and protected under the Federal Endangered Species Act (U.S. Fish and Wildlife Service [USFWS] 2002). Although the southwestern subspecies designation has been supported by genetic, morphometric, and behavioral analyses, it is not possible to separate willow flycatcher

subspecies from visual observations. All three subspecies (*E. t. extimus*, *E. t. adastus*, and *E. t. brewerii*) may be encountered within Inyo and Mono counties during migration, and the boundary between the breeding distributions of *E. t. extimus* and *E. t. adastus* is not well-defined (Theimer et al. 2016). Here, we refer to territorial birds within the USFWS designated SWIFL critical habitat (Fig. 3 in USFWS 2002, 2013) as *E. t. extimus* (SWIFL), but otherwise do not specify a subspecies designation (WIFL).

In the last 30 years, WIFL breeding sites declined by 50% in the Sierra Nevada region of California (Loffland et al. 2014), including complete extirpation from Yosemite National Park (Siegel et al. 2008). Additionally, breeding populations have rapidly declined at two breeding sites for federally endangered SWIFL within California: along the Kern River (M. Whitfield, Southern Sierra Research Station, personal communication) and at Pendleton Marine Corps Base, which once held the second-largest breeding population of the subspecies in the state (Kus et al. 2017). These declines have been driven by habitat loss and degradation, BHCO nest parasitism (Fig. 1), and livestock grazing (USFWS 2002). Declines may be further exacerbated by the changing climate (Ruegg et al. 2018).

SWIFL nesting in the Owens Valley were first formally documented in 1944 (Grinnell and Miller 1944). In 1993, canoe surveys confirmed nesting SWIFL along the Owens River between Pleasant Valley Reservoir and Bishop (Laymon and Williams 1994). Between 2001 and 2006, 5 sites and 28 territories were identified in the Owens Valley (Durst et al. 2007; Rourke et al. 2004). Additionally, a small, breeding WIFL population existed near Mono Lake from 2000–2012, but the subspecies was not determined (McCreedy and Heath 2004).



**Figure 1.** Parasitized southwestern willow flycatcher nest with two brown-headed cowbird eggs (larger). Photo Credit: Dave Bell.

The Los Angeles Department of Water and Power (LADWP) identified SWIFL as a target species in their conservation planning for Inyo and Mono counties. The City of Los Angeles (LA) owns the majority of lower-elevation riparian habitat in Inyo and Mono counties including 125,450 ha (310,000 acres) of land, on which exist 724 km (450 mi) of natural waterways, 178 km (111 mi) of man-made waterways (ditches and canals), and 135 km (84 mi) of aqueducts. This land is managed by LADWP primarily for water gathering but also for power production. We implemented surveys to provide updated and more comprehensive information on the current distribution and abundance of WIFL in Inyo and Mono counties. We also implemented nest monitoring to determine if BHCO parasitism (Fig. 1) might be impacting the species in this region.

## METHODS

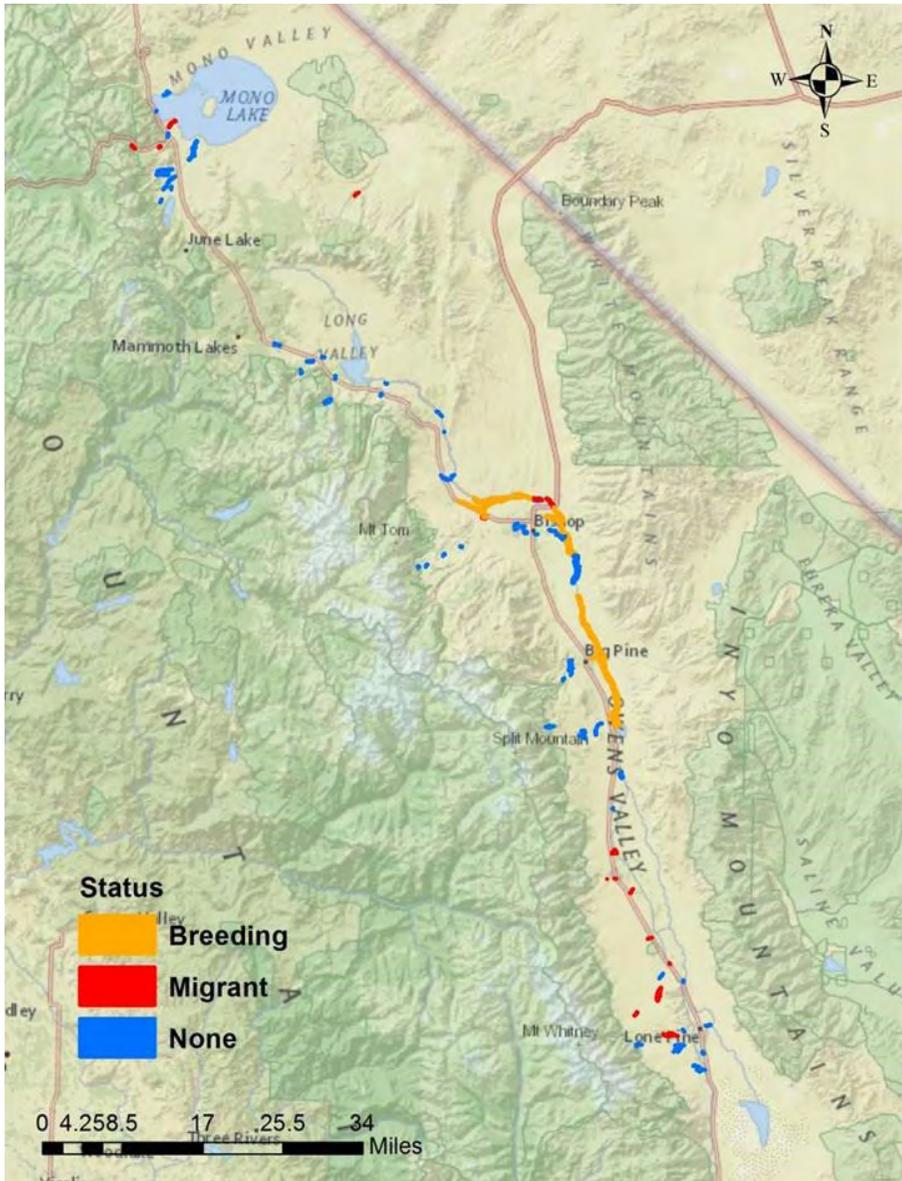
### Study Area

We surveyed all WIFL riparian habitat between 1120–2440m with slope  $< 20^\circ$  in Inyo and Mono counties. WIFL use this habitat from May to August (Fig. 2), when conditions are hot and dry, although micro-habitat within riparian areas is often cooler and moister than in surrounding upland. This land was predominantly owned by LA, but also included some areas owned privately or by the U.S. Forest Service, Paiute Tribe, or California State Parks. From May to August, the average high temperature in Bishop, California is  $33.3^\circ\text{C}$  with an average of 0.4 cm of precipitation per month. Habitat includes both diverse, multi-tiered riparian vegetation and near-monocultures of similar height. More complex habitat typically includes coyote willow (*Salix exigua*) and wild rose (*Rosa woodsii*) with red willow (*S. laevigata*) or Fremont's cottonwood (*Populus fremontii*) overstory. Non-native salt cedar (*Tamarix ramosissima*) and Russian olive (*Elaeagnus angustifolia*) are also present, but generally rare. Additionally, along the Owens River, there are long stretches that are near-monocultures of coyote willow and a few monoculture sections of salt cedar, particularly just north of Tinnemaha Reservoir. Higher-elevation tributaries with steeper gradients are dominated by water birch (*Betula occidentalis*). In addition to WIFL and BHCO, common riparian birds include Bewick's wren (*Thyromanes bewickii*), bushtit (*Psaltriparus minimus*), red-winged blackbird (*Agelaius phoeniceus*), song sparrow (*Melospiza melodia*), and yellow warbler (*Setophaga petechia*). Observed mammals within the area include beaver (*Castor canadensis*) and mink (*Neovison vison*).

### Study Design

We assessed the distribution and abundance of WIFL within the Owens Valley and Mono Basin below 2440 m using standardized callback surveys (Sogge and Sferra 2010). This included 36 sites (195 km of survey tracks) in 2014 and 36 sites (124 km of survey tracks) in 2015 of riparian habitat. A subset of nesting sites were also revisited in 2016 for further nest monitoring (Laue 2017). Most nesting SWIFL were associated with the Owens River and within USFWS designated critical habitat for the southwestern subspecies (Fig. 2).

We identified survey sites using a habitat suitability model that incorporated the presence of water, canopy closure, percent willow, habitat width, habitat patch size, and elevation (developed by S. Laymon for LADWP, unpublished). A few sites identified in the model were later excluded when field visits revealed they did not contain adequate habitat



**Figure 2.** Distribution of willow flycatcher based on 2014–2015 callback surveys in the Owens Valley and Mono Basin, CA, USA. Breeding birds were identified by their presence throughout the breeding season.

and a few sites were added based on local knowledge and desktop review (<5%). We classified sections of the Owens River as separate sites based on natural breaks in vegetation or by road crossings. Site size and complexity were variable; survey timing of individual sites ranged from <30 minutes to 80 hours per visit (visits occasionally involved multiple surveyors and multiple days).

Each site was visited at least three times between 14 May and 31 July (Sogge and Sferra 2010) either by foot, canoe, or both for wider habitat. As needed, survey timing was shifted up to 7 days from protocol-specified time periods, based on bird arrival and activity, weather, and staff capacity. Surveyors initially listened for WIFL for 1–2 minutes, and if not heard, broadcast 10–15 seconds of WIFL song recordings (“*fitzbews*” and “*britts*”) from handheld speakers with consistent amplification matching surround bird noise, from within WIFL habitat (Sogge and Sferra 2010). Listening and broadcasting were repeated every 30 meters throughout site. WIFL presence was confirmed by a “*fitzbeu*” and also occasionally confirmed by observing silent or “*whit-ting*” birds based on observation details and observer experience. WIFL were also confirmed from opportunistic observations unassociated with specific survey efforts.

Repeated observations from five breeding sites indicated that we were consistently able to identify the presence of breeding birds throughout the survey season. Therefore, we considered WIFL to be migrants if they were 1) observed only during a single visit OR 2) observed in the first survey window and then only again late in the third survey window, assuming this could represent separate spring and fall migration events. Within active breeding sites, it was not always possible to distinguish migrants from residents.

We conducted territory mapping (2014–2016) and nest monitoring (2015–2016; following Martin and Geupel 1993) at the three largest nesting sites, which included over 85% of the known breeding territories in the region. Territories were determined by the presence of an active nest or the persistent presence of birds in the breeding period. With careful and repeated behavioral observations (“*whit-ting*”, counter “*whit-ting*”, interactions, and sometimes nests), territories were identified as having a single male, pair, or polygynous groups (2 females, 1 male). Typically SWIFL will remain within a territory during a season, although some single males may move territories mid-season (Sogge and Sferra 2010). To avoid double counting, areas in which birds were observed ONLY prior to June 22 or ONLY after July 11 were not counted as territories unless there was an active nest.

Nest monitoring effort and survey area extent varied between years, with an initial trial in 2014 toward the end of the callback survey season and more consistent effort at the three largest nest sites (Chalk Bluff, Horton, S. Hwy 6) in 2015 and 2016. A nest was considered active only if a SWIFL egg or nestling was observed; partially built nests or nests that were only observed holding BHCO eggs or young were not considered active. Nests were considered parasitized if they either A) had a BHCO egg or nestling OR B) had damaged but uneaten SWIFL eggs or a SWIFL nestling on the ground, even if no BHCO was observed the in nest. Successful nests fledged at least one SWIFL. Typically fledglings were observed, but occasionally fledging was assumed if a nestling > 10 days old (Paxton and Owen 2002) was observed and the nest was found undisturbed after that time. If at least one fledgling was observed and there were no other signs of disturbance, we assumed all nestlings fledged. In 2015 and 2016, nests were monitored every 3–7 days to determine nest fate. We did not approach nests in the presence of BHCO to avoid increasing the risk of nest parasitism or predation.

## RESULTS

WIFL were present in one quarter of all sites, including 36% of Mono Basin sites, 0% of Long Valley sites (between Owens River headwater and Crowley reservoir), and 24% of Owens Basin sites (Fig. 2). WIFL were often detected only within a small portion of each

site, indicating there was often variable habitat quality within sites. All WIFL observations occurred in habitat dominated by native plant species, although some non-native species were occasionally present (both salt cedar and Russian olive). Migrants occupied more locations and more varied habitat than breeding birds, including smaller habitat patches and lower-order waterways in steeper terrain with faster-moving water. Migrants were observed using the same survey sites where SWIFL nesting territories were also located. However, because birds were not banded, it was not always possible to distinguish migrants from residents and identify the numbers of migrants in nesting areas.

Breeding SWIFL were found along the Owens River and nearby along one of its tributaries, Horton Creek (Fig. 2 and Table 1). In the overall region we estimate there are ~35 territories with the majority (~63%) of territories within the Chalk Bluff Site (Table 1). We did not find any nesting SWIFL along the Owens River between highway 6 and 5 Bridges Road, where they had previously been documented in 2001 (Rourke et al. 2004). In 2014, we did find a single “*whit-ting*” bird at this site during an early visit, but with no further observations during subsequent visits, we did not consider this a territory. We also did not find nesting SWIFL along Rush Creek near Mono Lake, where females had previously been documented from 2001-2011 (C. McCreedy, personal communication). Before these surveys, Horton Creek was not previously identified in the literature as a SWIFL nesting site.

Only the Chalk Bluff site consistently had >5 SWIFL territories (Table 1). With an average of 22 territories, the Chalk Bluff site has half of all known territories within the Basin and Mojave Recovery Unit for SWIFL (Durst et al. 2008). Most territories (31/38; 83%) found in 2015 were in the same location as territories documented in 2014, although without individually marked birds, it was not possible to determine individual site and territory fidelity. Of the 106 territories identified, most (85%) were in pairs of 1 female and 1 male while 9% were single males and 6% were polygynous groups with 2 females.

We identified the fate of 85 active SWIFL nests (Table 2). Overall nest success was 45% (N = 38), but it varied between years and sites (Table 2). The earliest first SWIFL egg was detected on 9 June, the latest was detected on 22 July, and the average date of laying the first egg was 26 June; the earliest hatch day was 23 June, the latest was 2 August, and the average was 11 July; the earliest fledgling was observed on 4 July, the latest on 16 August, and the average date of fledging was 27 July. Averages were calculated only using data from 2015 and 2016, when there was comprehensive nest monitoring throughout the breeding season.

For all active nests with known outcome, the nest parasitism rate was 37% (range 18–43%) between 2014 and 2016 (Table 2). The lower parasitism rate in 2014 was likely influenced by late and incomplete nest monitoring that year. BHCO were observed in all nest sites and parasitism was documented in all nest sites except for the Owens River South of Line Street. However, there was only a single nest with known fate at this site. At sites with  $\geq 5$  nests monitored, BHCO parasitism ranged from 33 to 67%. In addition, we documented 21 SWIFL nests that we did not consider active (no SWIFL eggs observed), that were abandoned with a BHCO egg. Typically, SWIFL seemed to abandon inactive parasitized nests shortly after BHCO eggs were laid, but one inactive nest did support a BHCO to the nestling phase before the nestling was later found dead in the nest. Parasitized nests had lower nest success (16%; N = 5/31) than non-parasitized nests (60%; N = 31/52; Pearson's  $\chi^2 = 13.236$ , df = 1, P < 0.001; Table 2).

**Table 1.** Results from territory and nest monitoring from all known nesting sites for southwestern willow flycatcher in the Owens Valley, CA, USA, 2014–2016. Includes nests at all stages of development.

Site	2014			2015			2016			Average		
	N	Territory	Nest*	N	Territory	Nest	N	Territory	Nest	N	Territory	Nest
Horton	9	5	(0)	11	5	9	8	4	9	9.3	4.7	9.0
Chalk Bluff	45	24	(16)	43	22	38	37	20	25	41.7	22.0	31.5
S Hwy 6	13	8	(4)	12	6	10	6	3	5	10.3	5.7	7.5
S Line	4	2	1	3	2	0	NS	NS	NS	3.5	2.0	0.5
Big Pine	2	1	1	2	1	1	NS	NS	NS	2.0	1.0	1.0
Tinnemaha	2	1	1	4	2	NS	NS	NS	NS	3.0	1.5	1.0
Totals	75	41	23	75	38	58	51	27	39	67	35	50

NS = not surveyed.

\*Reduced nest search effort in 2014 (not included in averages)

**Table 2.** Results from southwestern willow flycatcher nest monitoring in the Owens Valley, CA, USA, 2014–2016 from all active nests (SWIFL egg present) with known fate. Percentages reported because nest search effort and area covered was not consistent between years.

Year	Nests with Known Outcome	Nest Success (%)	Nest Parasitism Rate (%)*	Non-parasitized Nest Success (%)*	Parasitized Nest Success (%)*
2014 <sup>a</sup>	17	65	18	71	33
2015	40	45	42	64	13
2016	28	32	43	44	17
All Nests <sup>b</sup>	85	45	37	60	16

\*Does not include 2 nests in 2015 for which parasitism status was not determined.

<sup>a</sup>2014 nest monitoring began in the middle of the nesting season.

<sup>b</sup>Calculated from all data combined, not average across years.

## DISCUSSION

Although once a common bird in California (Unitt 1987), WIFL abundance and distribution have declined since 1970, and the current estimate is that there are 650 territories in the state (Schofield et al. 2021). Recent conservation efforts, particularly those targeting habitat restoration and BHCO management, have had mixed results and highlight the importance of local knowledge for effective management (USFWS 2002; Rourke et al. 2004; Schofield et al. 2021). We assessed the current distribution and abundance of WIFL in Inyo and Mono counties to inform management and performed nest monitoring to elucidate relevant local threats, particularly BHCO nest parasitism.

Due to the thorough geographic extent of our surveys, these results likely represent a census of SWIFL nesting sites within the Owens Valley and Mono Basin. Cooperation from many landowners (California State Parks, USFS, Bishop Paiute Tribe, private entities and most importantly, LADWP) enabled us to survey all WIFL habitat in the region. All nest sites, as well as most potential habitat in the region, are located on land owned by LA and managed by LADWP. In general, we found more nesting sites and more territories than previous surveys (Laymon and Williams 1994; Rourke et al. 2004). However this does not necessarily represent an increase in the overall population as past efforts were not as comprehensive, often covering smaller areas, including fewer visits per site, or surveying only by canoe or foot, instead of both. Our migratory WIFL observations do not likely represent a census of sites used by migratory birds because migrants may spend a short time at a site and the survey protocol is not intended to locate all migrants.

Our results highlight the importance of the Chalk Bluff site on the Owens River because it has persistently supported a large breeding population of SWIFL, averaging 22 territories per year from 2014–2016 (Table 1). More than 50% of all nesting SWIFL in the Owens Valley were located at the Chalk Bluff site and past surveys have repeatedly identified the area as having the most nesting birds in the region (Laymon and Williams 1994; Rourke et al. 2004). Throughout the range of SWIFL, sites with >20 territories are relatively rare, making up <5% of all sites (Durst et al. 2007). The Chalk Bluff site appears to be uniquely important for SWIFL persistence in the region and supports 12% of all known nesting SWIFL in California (22/190 territories; Durst et al. 2007).

Although we documented a decline in territory number across the three largest nesting sites between 2014 and 2016, we are reluctant to call this a trend due to the limited duration of the study. Skewed sex ratios have been identified in one declining SWIFL population (Kus et al. 2017) but we did not find any evidence of this. We documented some polygynous groups ( $N = 6$ ), but the overall sex ratio was nearly equally balanced with additional single male territories ( $N = 10$ ). To reduce impacts from livestock, livestock are removed from SWIFL habitat by 15 May (LADWP 2005; USFWS 2005) and our nest timing data support that this is an appropriate timeline to protect SWIFL nest sites.

Some SWIFL territories experienced intermittent inundation throughout the nesting season as a result of LADWP's managed flow regime. In addition, beavers were active at several nesting sites and may also have played a role in inundation. Although not quantified in this study, we observed that inundation seemed associated with some potentially positive elements including increased insect prey availability, decreased predator access, reduced temperature, and increased humidity (L. Greene, CDFW, personal observation). Other studies have found positive (Moore and Ahlers 2018) and negative (Ellis et al. 2008) correlations between inundation and SWIFL nest success. LADWP's ability to manage water flow from Pleasant Valley Reservoir into the Chalk Bluff site could potentially benefit SWIFL productivity through timely inundation, and possibly even counteract likely negative consequences caused by climate change.

In the Owens Valley, the climate is predicted to become warmer, with earlier runoff, and more extreme conditions, including drought (Morelli et al. 2011). Drought has been correlated with overall reproductive failure in other SWIFL populations (Durst et al. 2008). These surveys occurred during the most recent California drought (2012–2016). During 2016, the final and driest year of drought, we observed plant stress in the form of orange fungus (*Melampsora* sp.) and early leaf drop of coyote willow and wild rose that increased visual exposure of some SWIFL nests later in the season. This may have impacted nest

outcome, particularly for late season nests. This could be the beginning of increasing stress on SWIFL which were identified as the subspecies “most vulnerable to climate change” in an analysis combining projected changes in temperature and water availability with estimates of adaptability based on distribution and genetic diversity (Ruegg et al. 2018).

For years with comprehensive nest monitoring (2015 and 2016), we found high rates of BHCO nest parasitism (>40%), and relatively average rates of nest success (at least one SWIFL fledged; 45%) compared to other studies. Whitfield and Sogge (1999) compiled data from a series of short-term studies from the 1980s and 1990s across the southwest and found parasitism rates ranged from 0–66%, but only three sites had parasitism rates >40%. However, more recent and longer timescale studies have tended to find lower BHCO parasitism rates. Of 3,488 nests with known outcome along the middle Rio Grande in New Mexico from 1999–2017, 14% were parasitized (range across years 5–21%) with 43% nest success (range across years 25–75%), although these results did include some small-scale short-term BHCO removals. From 1999–2006 BHCO nest parasitism rates averaged 3% along Roosevelt Lake in New Mexico (N=892), although it varied by site and year reaching as high as 43% for a few sites in 2002, when drought conditions reduced vegetation cover (Ellis et al. 2008). Nest success varied from 57% pre-inundation (1996–2004, N = 680) to 45% during inundation (2005–2006, N = 212; Ellis et al. 2008). Along the lower Colorado River in Arizona and the Virgin River in Nevada, from 2003–2007, 23% were parasitized (range across sites 0–32%; N = 233 nests). High levels of BHCO nest parasitism on the Owens Valley, in combination with high nest success in non-parasitized nests (60%) indicate this may be a particularly good candidate for BHCO management.

BHCO parasitism rates above 20–30% can have significant impacts on SWIFL recruitment (USFWS 2002). Our result of lower nest success in parasitized nests is similar to other studies that have found parasitized SWIFL nests tend to fail, have lower hatching and fledging success (Whitfield and Sogge 1999) and have decreased daily nest survival (Stumpf et al. 2012). BHCO can also act as nest predators, removing host eggs and nestlings (Smith 1981) and we frequently observed the presence of a BHCO egg coincided with the reduction of a WIFL egg. Although BHCO are native to the United States, they have greatly increased and expanded with European settlement, including into California (Rothstein 1994) and they are known to be both nest parasites and nest predators (Latif et al. 2012; Lowther 2020) (*Thamnophis* sp.). BHCO nest parasitism on SWIFL was first documented in early 1900’s and nest collection data indicates it has increased steadily since then (Whitfield and Sogge 1999). BHCO nest parasitism has been documented on over 220 other open-nesting bird species, with 144 documented to have fledged a BHCO (Lowther 2020). In our study area, we documented BHCO parasitism on red-winged blackbird, blue-gray gnatcatcher, common yellowthroat, song sparrow and yellow warbler, a California species of special concern.

Despite a clear relationship between BHCO nest parasitism and reduced host nest survival, BHCO removals have had variable impacts. In New Mexico, localized BHCO removal decreased nest parasitism rates but had no impact on overall nest success rates (Moore and Ahlers 2018). In Arizona and Nevada, BHCO parasitism reduced individual nest success and the seasonal productivity of individuals, but longer term data analyses indicated it may not reduce the overall lifetime reproductive success of individuals, as individuals compensate by re-nesting and increasing egg production (Stumpf 2011). Although BHCO management has had variable results (USFWS 2002; Schofield et al. 2021), BHCO removal has been successful in the nearby Amargosa River drainage where BHCO trapping has essentially eliminated BHCO from the system and host productivity (fledglings/

brood attempt) has nearly tripled (McCreedy 2020). BHCO numbers may also be reduced more passively by reducing food availability at brood sites (e.g., switching to pellet feed at stables and corrals; Schofield et al. 2021). Additionally, lower levels of parasitism has been documented in taller trees (Brodhead et al. 2007), inundated habitat (Moore and Ahlers 2018), smaller habitat patches (Brodhead et al. 2007), and in habitat farther from the edge (Stumpf et al. 2012). These studies indicate that habitat management may be an effective way to reduce nest parasitism.

## Recommendations

We recommend continued nest monitoring of known SWIFL nest sites to determine if BHCO parasitism rates remain high outside of drought conditions. Further nest fate analyses should be conducted to identify the relative importance of nest predation and nest parasitism as well as habitat features that may help to mitigate these factors. At the Chalk Bluffs site, we consistently observed BHCO perched on snags within SWIFL nest areas and distance from snag might also be a factor driving parasitism rates. Additionally, the feasibility of BHCO management should be evaluated; banding BHCO present within SWIFL nest sites would be a first step in identifying BHCO roost sites that could be targets for passive or active management (Schofield et al., 2021). Further demographic and nest habitat analyses should be conducted to determine the impact of climatic and hydrologic conditions, livestock grazing, as well as fire (e.g., 2018 Pleasant fire) and non-native plants (e.g., *Tamarisk* sp.) on breeding SWIFL.

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Small Mammals



# Small Mammals

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1. Amargosa vole (*Microtus californicus scirpensis*).  
Photo Credit: Public Domain
2. Giant kangaroo rat (*Dipodomys ingens*).  
Photo Credit: Dagmar CC BY-NC-ND 2.0
3. Riparian brush rabbit (*Sylvilagus bachmani riparius*).  
Photo Credit: H. Grimes, CC-BY-NC-SA 2.0
4. Mohave ground squirrel (*Xerospermophilus mohavensis*).  
Photo Credit: Mark Allaback, Biosearch Environmental Consulting
5. Tipton kangaroo rat (*Dipodomys nitratoides nitratoides*).  
Photo Credit: California Statu University, Stanislaus, Endangered Species Recovery Program
6. San Joaquin antelope squirrel (*Ammospermophilus nelsoni*).

FULL RESEARCH ARTICLE

## Current status of the Mohave ground squirrel: an update covering the period 2013–2020

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The Mohave ground squirrel (*Xerospermophilus mohavensis*) is endemic to the western Mojave Desert of California. It is restricted to a small geographic area and is listed as Threatened under the California Endangered Species Act. Human development has resulted in loss and degradation of its desert habitat and climate change is believed to pose an additional threat. To determine its current conservation status and geographic distribution, I have assembled all available data from field studies during the years 2013–2020. These data confirm that the species is still present in 4 core areas and that it continues to be widespread in the northern and central portions of its historical range. However, the recent data also confirm earlier conclusions that the Mohave ground squirrel is now extirpated from the southernmost portion of its range. Recent surveys raise concerns about its status in other areas as well. A large-scale trail camera survey on the South Range unit of China Lake Naval Air Weapons Station in 2019 failed to detect the Mohave ground squirrel over much of this large installation. Recent trail camera surveys on Fort Irwin strongly suggest that the closely-related round-tailed ground squirrel (*Xerospermophilus tereticaudus*) has now replaced the Mohave ground squirrel over most of the base. There is additional evidence that the round-tailed ground squirrel is present in disturbed habitats to the west of Barstow and that hybridization with the Mohave ground squirrel is occurring there. It will be important to protect and conserve currently occupied Mohave ground squirrel habitat in view of this new information.

**Key words:** climate change, conservation, distribution, Mohave ground squirrel, Mojave Desert, renewable energy, threats, *Xerospermophilus mohavensis*

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The Mohave ground squirrel (*Xerospermophilus mohavensis*) is found only in a small area of the western Mojave Desert and is designated as a Threatened species under the California Endangered Species Act (CESA). It was one of the first species listed by the State of California, in 1971. While its historical range was about 20,000 km<sup>2</sup>, the Mohave ground squirrel is believed to occupy only about 65 percent of this area today. Mohave ground squirrels are only active aboveground in spring and early summer. As temperatures

rise and desert vegetation dries out in June and July, they accumulate body fat and then enter a lengthy dormancy (Best 1995). They emerge in February and, if conditions are right, females raise litters of 6–10 young. Mohave ground squirrel reproduction is extremely sensitive to year-to-year variations in winter rainfall. If winter rainfall is low, new growth of desert annual vegetation is much reduced and Mohave ground squirrels will fail to reproduce (Harris and Leitner 2004; Leitner et al. 2017). Thus, their abundance is greatly reduced during multi-year droughts. The species may be severely affected if climate change results in hotter and drier conditions in the California deserts (Inman et al. 2016). Human activities have already resulted in substantial loss and degradation of suitable habitat for the species (Inman et al. 2013). The California Department of Fish and Wildlife (CDFW) recently completed a conservation strategy for the Mohave ground squirrel that summarizes the available scientific information for the species and lays the foundation for its conservation and recovery (CDFW 2019).

In recent years, there has been great interest in the development of renewable energy in the California deserts. In 2016, the U.S. Bureau of Land Management (BLM) approved the Desert Renewable Energy Conservation Plan (DRECP) that streamlined the permitting process for solar, wind, and geothermal projects while providing for the conservation and management of native species on public lands (U.S. Bureau of Land Management 2016). BLM has identified 3 Development Focus Areas (DFA) within the Mohave ground squirrel range and has funded field studies to document the status of the species both in the DFAs and elsewhere in its range. Since 2013 there have also been 33 surveys on private lands proposed for solar development.

I have published two previous studies of the status of the Mohave ground squirrel throughout its range. The first report assembled data from all available sources covering the period 1998–2007 (Leitner 2008). These data strongly suggested that the species was absent from much of the southernmost portion of its historical range. The second study reviewed new information from 2008–2012, confirming this conclusion and also pointing to the lack of adequate data on the status of the species on two large military installations: Naval Air Weapons Station China Lake (China Lake) and Fort Irwin National Training Center (Fort Irwin) (Leitner 2015). The objectives of this paper are to (1) summarize information on Mohave ground squirrel occurrence from the period 2013–2020, and (2) synthesize this information to assess the current status and distribution of this species. This information can then be used to inform conservation actions for the Mohave ground squirrel.

## METHODS

I have assembled and analyzed all available data pertaining to Mohave ground squirrel distribution and occurrence over the 8-year period from 2013 through 2020. These data include 1) records from the California Natural Diversity Database (CNDDDB), 2) protocol trapping efforts required for proposed development projects, 3) reports from regional field studies sponsored by government agencies, and 4) incidental observations reported by field biologists. The closely-related round-tailed ground squirrel (*Xerospermophilus tereticaudus*) is widely-distributed through the eastern California deserts (Ernest and Mares 1987). It occurs in a number of locations along the eastern boundary of the Mohave ground squirrel range. Therefore, I have also collected recent occurrence data for this species along this contact zone.

The CNDDDB is a state-wide inventory of the status and geographic locations of

special-status plants, animals, and natural communities. This online catalog is managed and regularly updated by CDFW. It provides positive records of occurrence only. Prior to 2013 the CNDDDB listed 414 Mohave ground squirrel records and 20 new occurrences were entered during the period 2013–2020. These new occurrences have been recorded and mapped for this status update.

The CDFW requires that live-trapping surveys be conducted at proposed development sites within or adjacent to the range of the Mohave ground squirrel where suitable habitat is present. These surveys must follow a specific protocol and must be conducted by a qualified biologist who is permitted under a Memorandum of Understanding (MOU) with CDFW (CDFG 2003). Protocol surveys generally require 5 days of trapping on 100-trap grids. They must be repeated 3 times during the Mohave ground squirrel active period. All MOU-holders must file annual reports describing the results of any protocol surveys that they have carried out. I was able to access these reports and have incorporated the results of 218 protocol surveys into this status update. All protocol surveys were conducted on private land. They were concentrated in the southern and western portions of the historical range and were often required for proposed solar and utility projects.

During the past 8 years, a number of regional field investigations yielding important data on Mohave ground squirrel distribution and status have been sponsored by state and federal agencies. These surveys are conducted on public and military lands and are intended to support management and conservation actions. They have employed both live-trapping and camera-trapping techniques. Live-trapping efforts generally utilize grids of 100 traps and are conducted over 5 consecutive days. Sampling procedures for camera-trapping studies have been more variable, with 5–25 cameras per site and sampling periods ranging from 5–15 days. In 2013 and 2014, camera-trapping studies funded by CDFW helped to clarify the status of the Mohave ground squirrel on the western edge of its range in Kern County (Leitner 2014, Leitner and Delaney 2015). The Bureau of Land Management has supported extensive live-trapping and camera-trapping surveys from 2016–2020 in several important areas (Leitner 2020). These included the Little Dixie Wash and Coolgardie Mesa core areas, 3 Development Focus Areas, and the contact zone west of Hinkley where round-tailed ground squirrels have been recorded. In 2018, Edwards Air Force Base (EAFB) conducted camera-trapping at 25 sites to review the current status of the species at the installation (Lopez and Tautfest 2019). China Lake carried out an extensive camera-trapping survey in 2019 at 25 locations on its South Range, a large area where Mohave ground squirrel data were completely lacking (Vernadero Group Incorporated 2019).

I have also obtained 25 incidental Mohave ground squirrel records. These records are based on reports from biologists who have made visual observations or have detected them with traps or trail cameras incidental to other field activities.

I list the number of records obtained for this review from all 4 data sources (Table 1). For regional and protocol live-trapping surveys, a record was defined as a single trapping session (usually 5 days) at a specific grid location. A positive record refers to a 5-day trapping session in which one or more Mohave ground squirrels were captured. For regional camera-trapping surveys, a positive record means that at least one Mohave ground squirrel photograph was obtained during the sampling period, which can range from 5 to 15 days. Of course, all incidental records are positive.

**Table 1.** A summary of the data sources used in this 2013–2020 status review of the Mohave ground squirrel. Regional surveys are conducted on public and military lands and are sponsored by government agencies to collect data needed for management, while protocol surveys are carried out on private lands proposed for development to determine presence or absence of Mohave ground squirrels. Incidental observations are Mohave ground squirrel records obtained by qualified biologists incidental to other field investigations. A record represents a unique survey event; for live-trapping surveys the usual time period was 5 days, while camera-trapping surveys ranged from 5–15 days. The total number of records of each type is presented, along with the number of surveys that resulted in the detection of one or more Mohave ground squirrels. The sampling effort is indicated as trap-days, that is, the number of live-traps or camera traps used in a survey multiplied by the number of days that traps were operated.

Type of Data	Total Records	Positive Records	Trap-Days
Regional Live-Trapping Surveys	124	59	85,217
Regional Camera-Trapping Surveys	137	76	29,168
Protocol Live-Trapping Surveys	218	14	118,775
Incidental Observations	25	25	---
Totals	504	174	233,094

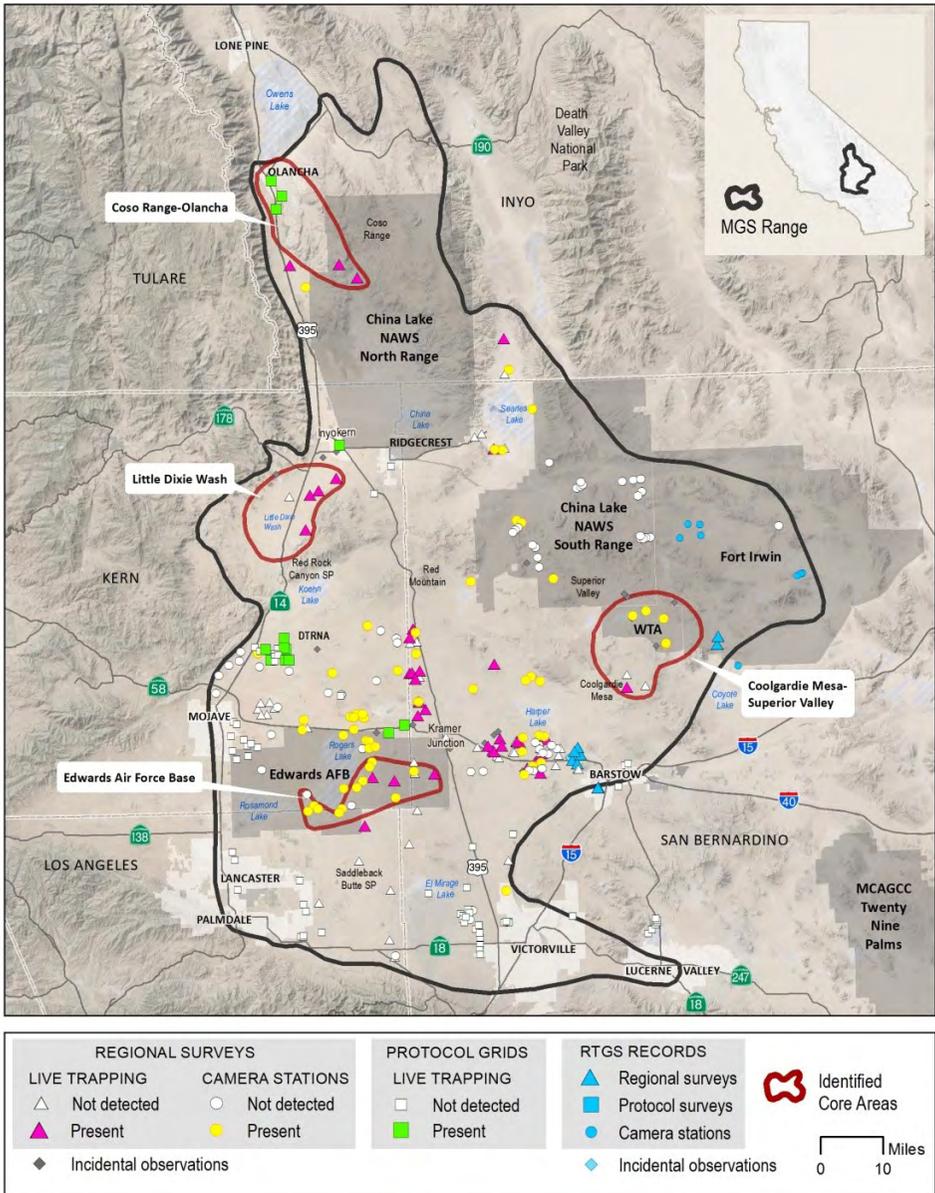
## RESULTS

### General Distribution

A great deal of new information on Mohave ground squirrel status and distribution has become available over the past 8 years. Figure 1 shows the general spatial pattern of both positive and negative records throughout the species range. The 4 core areas shown here were first identified in Leitner (2008) based on evidence that they had historically supported relatively abundant and widespread Mohave ground squirrel populations. Since 2013, Mohave ground squirrels have been detected in all 4 core areas (Fig. 1).

Survey data are available for most areas, except for the North Range of China Lake and for the area between Ridgecrest and Red Mountain. As in previous status reviews, regional trapping and camera surveys have yielded the greatest proportion of positive records (Leitner 2008, 2015). The most recent protocol surveys did not detect the species and were concentrated in the southern part of the range where there is more private land available for development (Table 1). The number of protocol surveys was much reduced as compared to the 1998–2012 period, but there has been increased use of cameras to supplement live trapping in these project-related surveys.

In general, the new data confirm the patterns documented in the 2 previous status reviews (Leitner 2008, 2015). There have been no recent occurrence records outside the historical range boundaries (Fig. 1). Furthermore, there continues to be clear evidence that Mohave ground squirrels are no longer present in large areas within the historical range. This is particularly striking in the southernmost part of the range where there are essentially no records from northeastern Los Angeles County and Lucerne Valley, with only a single detection near Victorville. A number of protocol, regional, and camera surveys have failed to detect the species on the western portion of Edwards Air Force Base or around the town of Mojave, as was noted for the period 2008–2012 (Leitner 2015). New camera data from Fort Irwin has revealed that round-tailed ground squirrels are now present on much of this military reservation, well within the historical Mohave ground squirrel range boundary. A

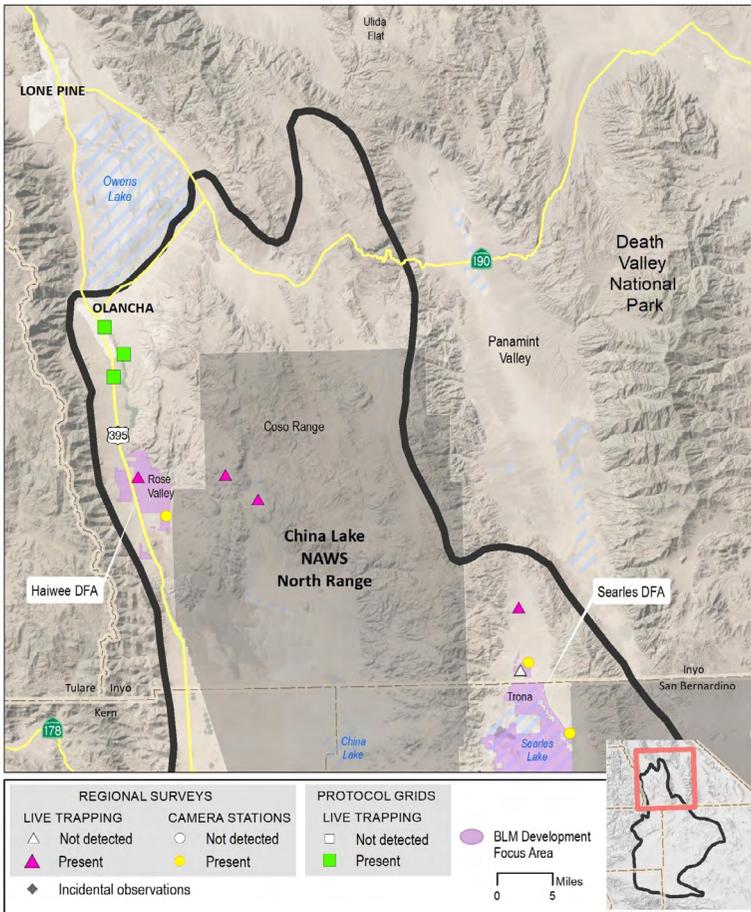


**Figure 1.** The geographic distribution of all Mohave ground squirrel records for the period 2013–2020. The solid black line denotes the historical boundary of the Mohave ground squirrel range. The 4 core areas outlined here continue to support Mohave ground squirrel populations. Occurrences of the round-tailed ground squirrel (RTGS) in the contact zone between the 2 species are also shown.

recent large-scale camera survey on the South Range unit of China Lake has failed to record Mohave ground squirrels over most of this large facility (Vernadero Group Incorporated 2019). The only positive records were found at a few sites on its extreme southern and western edges.

**Regional Analysis**

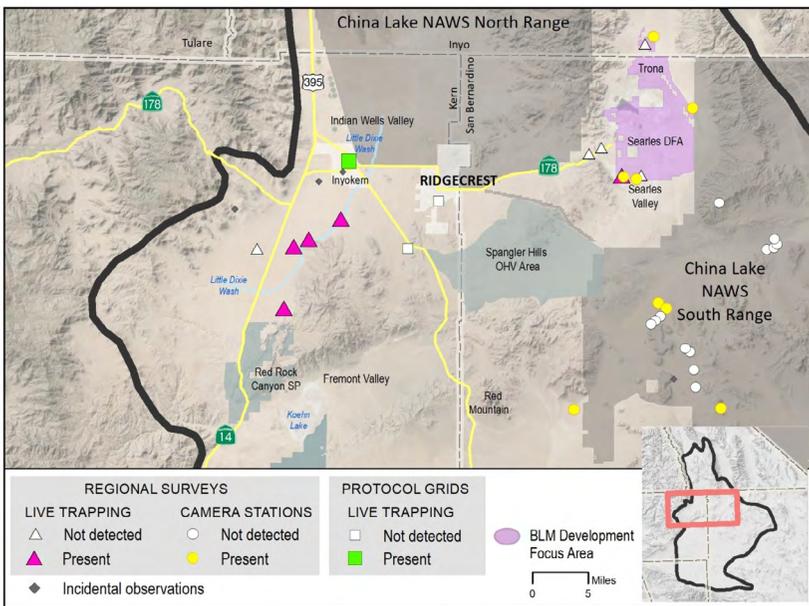
*Inyo County.*—The northernmost portion of the Mohave ground squirrel range is located in Inyo County just east of the Sierra Nevada (Fig. 2). Most of this area is made up of public lands administered by the US Bureau of Land Management (BLM) and military test ranges of China Lake. As in previous years, the species was captured regularly at 2 long-term monitoring sites in the Coso Range on China Lake (Leitner 2019). No surveys were undertaken elsewhere on the North Range unit of China Lake, so the status of the species is unknown over this extensive area. Mohave ground squirrels were detected regularly at 2 locations on public land in Rose Valley, which BLM has identified as the Haiwee Development Focus Area for geothermal energy under the DRECP. Protocol trapping at 3 proposed development sites near Olancha resulted in Mohave ground squirrel captures, demonstrating that the species is still extant at the historical northwestern corner of its range. To the east in Searles Valley, Mohave ground squirrels were detected at 2 locations, confirming that the species is still present in this area as well.



**Figure 2.** Mohave ground squirrel range within Inyo County, California. Symbols indicate locations of 2013–2020 records, both positive and negative.

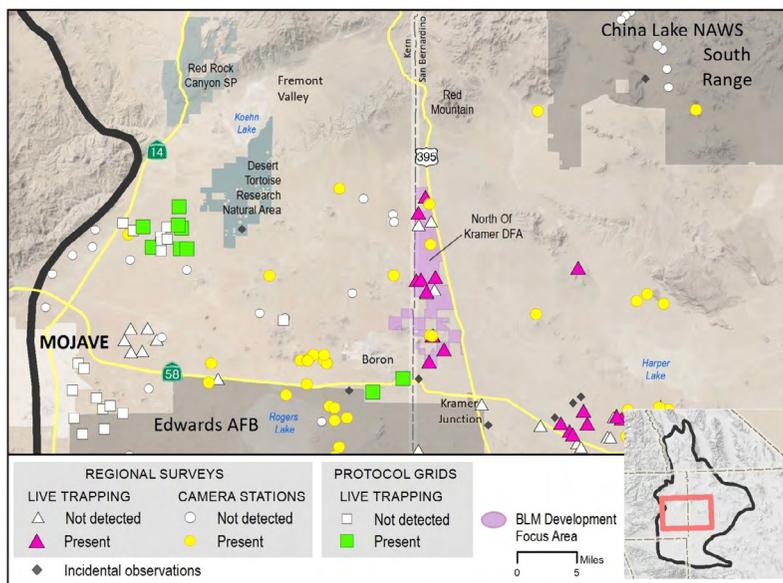
*Kern County—Ridgecrest area, Searles Valley, and Little Dixie Wash.* There is little evidence regarding the status of the Mohave ground squirrel in the Ridgecrest area during the period 2013–2020 (Fig. 3). Protocol trapping was carried out at 3 sites in this region and the species was detected only at one location near Inyokern. BLM has designated a large portion of Searles Valley as a DFA; Mohave ground squirrels were detected at 4 study sites on the margins of this proposed development area. There are no positive records available from south of Ridgecrest through the Spangler Hills Off-Highway Vehicle Area to Red Mountain and Fremont Valley.

The broad valley to the southwest of Inyokern known as Little Dixie Wash has been identified as a core area (Leitner 2008). Monitoring was conducted there by live-trapping at 4 regional survey sites from 2016–2019 (Leitner 2020). Mohave ground squirrels were present at 3 of the 4 sites, although numbers were quite low. The species was also documented in 2015 at a CDFW Ecological Reserve a few kilometers southwest of Inyokern.



**Figure 3.** Mohave ground squirrel range in the vicinity of Ridgecrest, Searles Valley, and in the Little Dixie Wash region. Symbols indicate locations of 2013–2020 records, both positive and negative.

*Kern County—Fremont Valley to Edwards Air Force Base.* Five large-scale studies have been carried out in the region from Fremont Valley south to Edwards Air Force Base (EAFB). In 2013 and 2014, CDFW sponsored camera studies here that sampled 15 sites dispersed throughout the area. Most of the study sites were on BLM land, with a few on private and CDFW properties. Mohave ground squirrels were detected at only 4 locations, all to the east and south of California City (Fig. 4). The BLM has funded a major sampling effort in the North of Kramer DFA. This project has collected data from 2018 through 2020 on 4 trapping grids and 4 camera sites located on BLM lands throughout this DFA. Mohave ground squirrels have been documented at all 8 of these sampling units (Fig. 4). Finally, 2 protocol surveys using both live-trapping and camera trapping have detected the

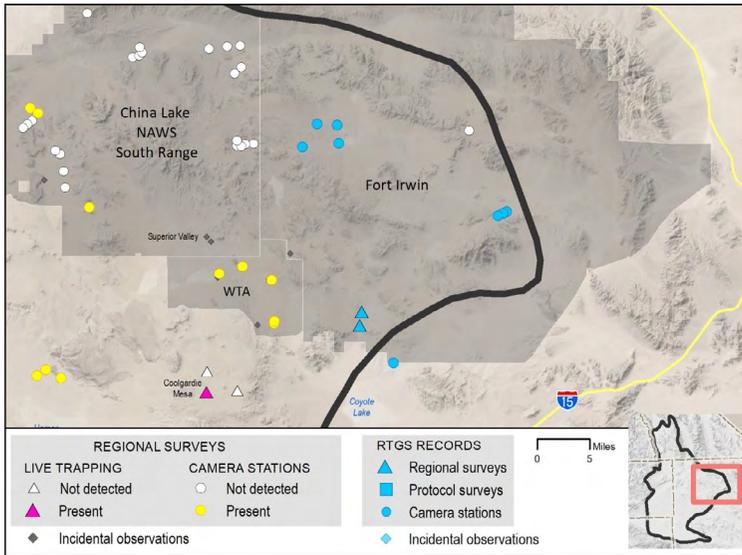


**Figure 4.** Mohave ground squirrel range extending from Fremont Valley and Red Mountain to Edwards Air Force Base. Symbols indicate locations of 2013–2020 records, both positive and negative.

species on potential development parcels west of the Desert Tortoise Research Natural Area (DTRNA) in 2019 and 2020. The DTRNA is a fully-protected conservation area located between Fremont Valley and Edwards Air Force Base and is known to support a significant Mohave ground squirrel population. It will be important to conserve population linkages between the DTRNA and EAFB.

*San Bernardino County—Coolgardie Mesa and Superior Valley, San Bernardino County.* Mohave ground squirrels were first documented on this extensive plateau north of Barstow through pioneering field work by Wessman (1977). Leitner (2008) reviewed a number of subsequent field studies in this region and proposed it as a core area based upon extensive occurrence records over a number of years. Live-trapping was carried out at 3 regional survey sites on Coolgardie Mesa from 2016–2019 (Leitner 2020). Although Mohave ground squirrels had been found at those locations during previous field studies (Scarry et al. 1994; Leitner 2010), the species was present at only one of the 3 sites during this recent trapping effort (Fig. 5). Most of Superior Valley in the northern portion of this core area has been incorporated into Fort Irwin and is now known as the Western Training Area (WTA). Camera monitoring in 2015 and 2016 by Fort Irwin environmental staff showed that Mohave ground squirrels were present at 5 sites throughout the WTA (Fig. 5).

*San Bernardino County—China Lake South Range and Fort Irwin.* I noted in the most recent status report (Leitner 2015) that data on the species were lacking for much of China Lake and Fort Irwin. Recent trail camera surveys have helped to clarify Mohave ground squirrel distribution on these 2 large military installations. In 2019, China Lake sponsored an extensive study at 25 locations throughout the very large South Range unit (Vernadero Group Inc. 2019). The species was detected at only 3 of these sites, all in the southwestern corner of the South Range (Fig. 5). Incidental observations during this project documented Mohave ground squirrels at a location near the camera detections and also in Superior Valley close to older records on the Fort Irwin WTA.

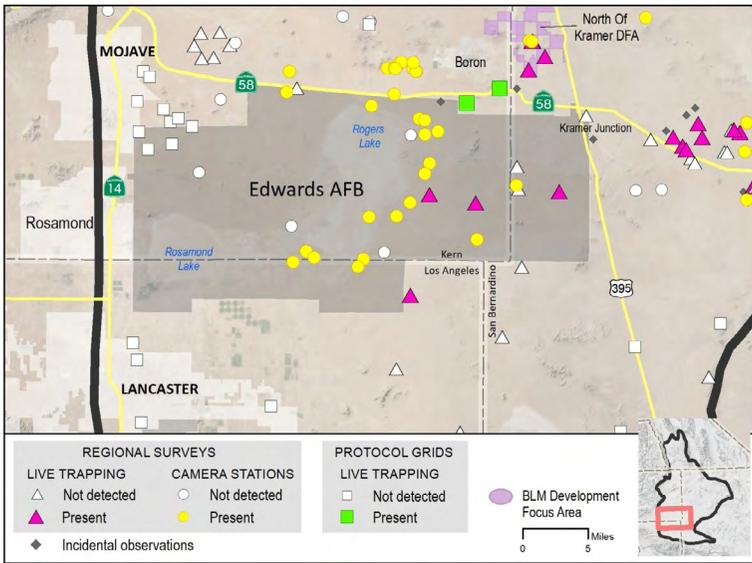


**Figure 5.** Mohave ground squirrel range from Coolgardie Mesa north to Fort Irwin and South Range of China Lake. Symbols indicate locations of 2013–2020 records, both positive and negative. Round-tailed ground squirrel (RTGS) records are also shown.

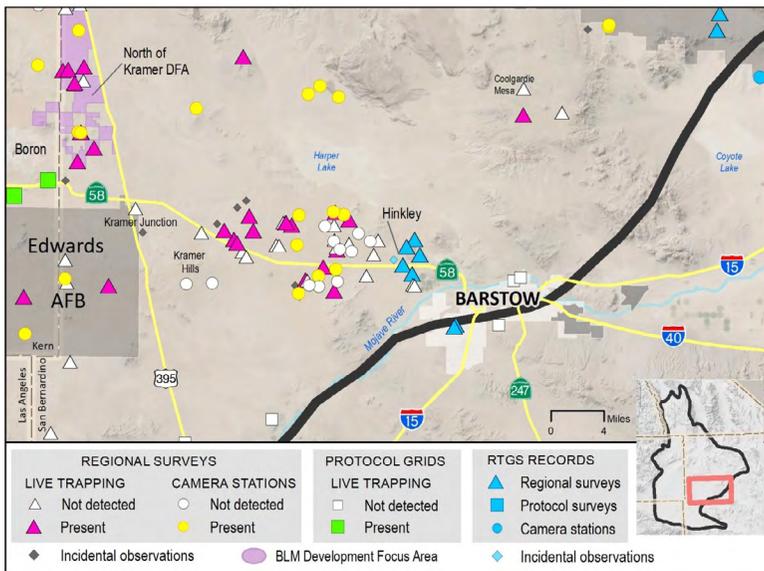
Camera studies were undertaken on Fort Irwin in 2018 in a region near the western boundary that had been proposed for a large training facility. This work documented the presence of round-tailed ground squirrels at a number of camera sites here (Fig. 5). This discovery was quite unexpected, as the area is approximately 30 km west of the historical eastern boundary of the Mohave ground squirrel range. Other recent camera results from an area on the north side of the Tiefert Mountains provide further evidence of round-tailed ground squirrel occupancy throughout much of Fort Irwin with the important exception of the WTA.

*Kern County—Edwards Air Force Base.* A large portion of EAFB was identified as a core area by Leitner (2008) based upon a number of Mohave ground squirrel records to the east and south of Rogers Dry Lake. The importance of this area has been confirmed by additional monitoring studies in recent years (Leitner 2015). In 2018, EAFB sponsored a camera-trapping survey at 25 sites (Lopez and Tautfest 2019) that has again documented the presence of the species throughout the eastern portion of the base (Fig. 6). However, it seems clear that that the western portion of EAFB does not support a Mohave ground squirrel population. A number of surveys since 1998 have consistently failed to detect the species there (Leitner 2008; Leitner 2015).

*San Bernardino County—Kramer Junction to Barstow.* There are a number of recent records of Mohave ground squirrels to the east of Kramer Junction, generally confirming that this area continues to support a widely distributed population (Fig. 7). However, trapping studies in the vicinity of Hinkley in 2013 resulted in the capture of round-tailed ground squirrels at several locations, a result confirmed by genetic analysis (Leitner et al. 2017). Since 2016, the BLM has sponsored extensive live-trapping and camera surveys in the region to the west of Hinkley to clarify the status of the Mohave ground squirrel there. It appears that this species is present at 12 sites here, although there is genetic evidence that hybridization with round-tailed ground squirrels is occurring here as well (Leitner and Leitner 2017).



**Figure 6.** Mohave ground squirrel range on Edwards Air Force Base and vicinity. Symbols indicate locations of 2013–2020 records, both positive and negative.

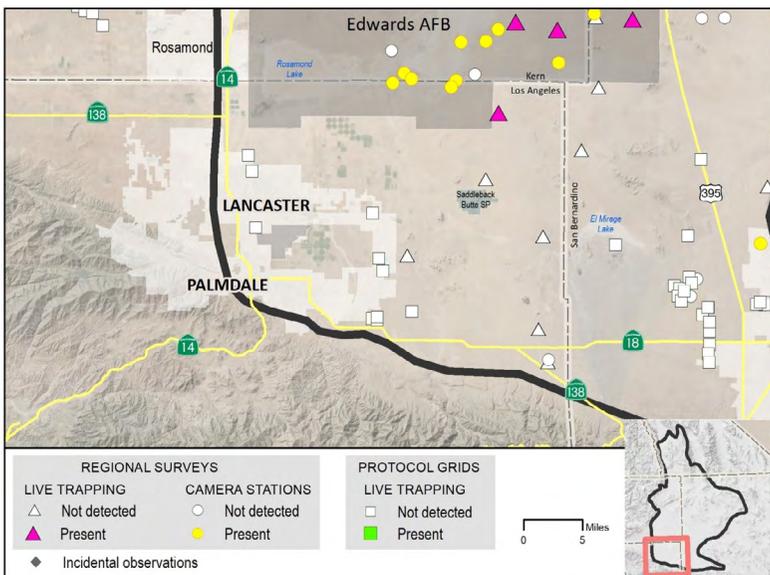


**Figure 7.** Mohave ground squirrel range from Kramer Junction east to Barstow. Symbols indicate locations of 2013–2020 records, both positive and negative. Round-tailed ground squirrel (RTGS) records are also shown.

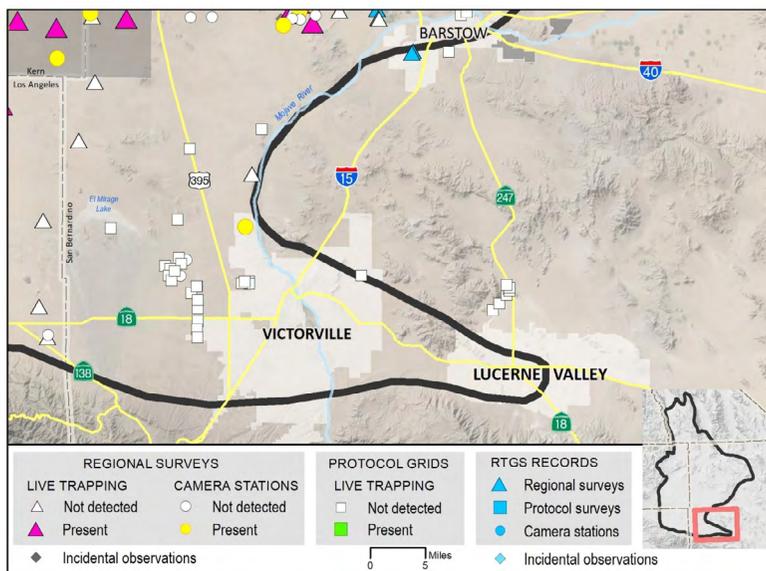
*Los Angeles County.*—Protocol and regional trapping efforts since 2013 have largely failed to document Mohave ground squirrel occurrence in Los Angeles County. There have been only 5 recent occurrences in the extreme northeastern corner of the county, on or adjoining EAFB (Fig. 8). These results are consistent with all survey efforts in recent years, strongly suggesting that the species is essentially extirpated in Los Angeles County (Leitner 2008; Leitner 2015).

*San Bernardino County—Mojave River to Lucerne Valley.* The Mojave River from Barstow to Victorville has historically been considered the eastern boundary of the Mohave ground squirrel range, with an easterly extension from Victorville to Lucerne Valley. There is no recent evidence that the species is present to the east of this reach of the Mojave River. Protocol surveys since 2013 in Barstow, Apple Valley, and Lucerne Valley have failed to detect Mohave ground squirrels, although round-tailed ground squirrels continue to be documented in the area around Barstow (Fig. 9).

*San Bernardino County—Victor Valley.* A great deal of urban development has occurred in the Victorville region during the past few decades, resulting in a current human population of approximately 400,000. Since 1998, protocol surveys have yielded only a few Mohave ground squirrel detections in this region. In June 2020, a camera trap captured the first record of the species here since 2011 (Fig. 9). Thus, there still appears to be a relict population in Victor Valley, but it is difficult to determine its exact status and geographic extent because almost all land there is in private ownership.



**Figure 8.** Mohave ground squirrel range in Los Angeles County, California. Symbols indicate locations of 2013–2020 records, both positive and negative.



**Figure 9.** Mohave ground squirrel range from Barstow to Victor Valley. Symbols indicate the locations of 2013–2020 records, both positive and negative. Round-tailed ground squirrel (RTGS) records are also shown.

## DISCUSSION

### Current Geographic Distribution

The boundaries of the range map (Fig. 1) provide the best representation of the historical Mohave ground squirrel distribution. Data for the period 2013–2020 show that the species is present throughout much of the northern and central portions of the historical range. The 4 core areas as mapped in Leitner (2008) still support extant Mohave ground squirrel populations (Fig. 1). Live-trapping and camera studies have also documented widespread populations in all 3 of the DFAs: Haiwee, Searles, and North of Kramer. There are also a number of recent records east of Kramer Junction along the SR58 corridor and in the vicinity of the DTRNA near California City. However, since 2013 there have been few documented records for the North Range of China Lake and for the public lands from Ridgecrest south to Red Mountain (Figs. 2 and 3). These areas should be a priority for future surveys to determine the status of the species there.

There is substantial evidence that Mohave ground squirrel populations are absent or critically diminished in several geographic areas within the historical range. In particular, the species appears to be extirpated from the southernmost portions of the range. The core area on EAFB is the only portion of the southernmost range that continues to support a widespread population. According to the CNDDDB, there were numerous records of the species throughout northeastern Los Angeles County in the 1970s and 1980s; however, the species has been almost completely absent from this entire region since 1991. The only current records from Los Angeles County are on or extremely close to EAFB. The recent large-scale camera study on EAFB has again confirmed that the species is not present on

the western portion of this large military installation (Lopez and Tautfest 2019). A single camera detection in Victor Valley in 2020 provides the only evidence of an extant population in that part of the historical range. All protocol surveys here since 2011 failed to capture or observe the species. Because the region consists almost entirely of private land, it is very difficult to conduct regional surveys that might identify potential conservation opportunities for this relict population.

Recent camera studies on military bases in the northern parts of the range also indicate that the species may be absent from significant areas within the historical range boundaries. Mohave ground squirrels were not detected at 22 of 25 camera sites on the China Lake South Range and were documented only along the southern edge of this large installation (Vernadero Group Inc. 2019). There is now evidence from camera data that round-tailed ground squirrels have replaced Mohave ground squirrels over almost all of Fort Irwin, a region where the latter were widely distributed in the 1990s (Krzysik 1994). In addition, live-trapping and genetic analyses have demonstrated that a round-tailed ground squirrel population is established west of Barstow in the Hinkley Valley (Leitner et al. 2017).

In conclusion, there is now concerning evidence that Mohave ground squirrels are absent from significant areas within the historical range. While the area of the historical range has been estimated at ~20,000 km<sup>2</sup>, it appears that only slightly more than 13,000 km<sup>2</sup> are potentially occupied.

## Threats

In the decades prior to the economic crisis of 2008, there was a significant amount of Mohave ground squirrel habitat loss due to urban development, especially in the southern part of the range. The number of protocol surveys for development projects on private land provides an index of development pressure. There was an average of 110 protocol surveys per year during the 15-year period from 1998-2012. Since 2013 there has been an average of only 22 protocol surveys per year, a clear indication that residential and commercial development activity is much reduced.

However, in recent years there has been a great deal of interest in developing renewable energy resources in the California deserts. The important wind resource area near the town of Mojave saw significant expansion on private land beginning about 2006 and continuing until about 2012. These projects were all located to the west of and outside of the Mohave ground squirrel historical range boundary and protocol surveys have never detected the species there. However, there have also been a number of large solar projects proposed on private land within the Mohave ground squirrel range. Protocol surveys were conducted at potential solar sites near Lancaster and Victorville but, as expected, the species was not detected. On the other hand, Mohave ground squirrels have been found at proposed solar projects near the DTRNA, which is well-known to support a viable population. Such development can be permitted if adequate mitigation is provided, usually in the form of dedicated conservation land.

The DRECP identified 3 sites on public land within the Mohave ground squirrel range as DFAs (US Bureau of Land Management 2016). The BLM has sponsored multi-year live-trapping and camera studies at these sites. The results of this research will be evaluated in 2021 to inform decisions about leasing these areas for development of renewable energy. Thus far, the evidence suggests that both the North of Kramer and Haiwee DFAs provide good Mohave ground squirrel habitat and support healthy populations (Leitner 2020). On

the other hand, the Searles DFA includes marginal habitat surrounding a saline playa. Mohave ground squirrels have been detected by camera trapping in a small isolated area at the north end of the DFA and there have been a few records of dispersing juveniles at the other margins of the DFA. Development of this DFA would have minimal impact on the species.

Approximately one-third of the historical Mohave ground squirrel range is located on large military reservations. Testing and training activities at EAFB and China Lake do not involve extensive ground operations, so there is relatively little impact to biological resources. In contrast, the mission at Fort Irwin is focused on realistic training of ground forces, which has resulted in considerable environmental impact. There are many Mohave ground squirrel records on Fort Irwin from 1973 through the mid-1990s (Krzysik 1994), but recent evidence indicates that round-tailed ground squirrels are now found throughout much of the facility. Round-tailed ground squirrels appear to be more tolerant of disturbance than Mohave ground squirrels, so military training activities may have favored their expansion. Public lands that make up a portion of the Coolgardie Mesa-Superior Valley core area were transferred to Fort Irwin a number of years ago. This area is now known as the Western Training Area and plans are being developed for training activities here. There could be significant impacts to an important Mohave ground squirrel population, depending upon the nature and location of military usage.

There is a growing consensus that climate change will result in continuing long-term warming and drying in the Southwestern deserts (Williams et al. 2020). Recent evidence from studies of desert faunal communities suggests that climate change is already having significant impacts. Iknayan and Beissinger (2018) undertook an extensive analysis of changes in the Mojave Desert avian community over the past century. Their analysis concluded that climate change, especially a long-term decline in rainfall, was the primary driver of a significant collapse in the species richness of Mojave Desert avian communities.

The Mohave ground squirrel appears to be highly susceptible to the predicted impacts of climate change in the Mojave Desert. Multi-year data from the Coso study area indicates that reproduction there occurs only when winter precipitation exceeds the threshold of 65 mm that appears necessary for the production of adequate spring herbaceous vegetation (Harris and Leitner 2004). The Mojave Desert underwent a prolonged drought from 1989-1991 and there was no reproduction in the Coso Mohave ground squirrel population for 2 years, resulting in severe population decline. It seems possible that the disappearance of Mohave ground squirrel populations throughout much of the southern range after 1991 was related to this drought. The overall effects of the more recent 2012-2016 drought are unclear. The species appears to still be present in all 4 core areas, although abundance may be somewhat lower (Leitner 2020).

### **Conservation Needs**

A number of actions will be required to adequately meet the conservation needs of the Mohave ground squirrel. These include 1) continuing field studies to document patterns of occurrence and to identify areas of concern, 2) careful siting of renewable energy projects to avoid loss of important habitat, 3) designing new military training sites on Fort Irwin to minimize significant impacts, and 4) prioritizing the protection of high quality habitat in areas on the northern parts of the range where winter rainfall is more likely to be adequate in the future.

It would be particularly useful to document the current status of Mohave ground squirrel populations on public lands south of Ridgecrest toward Red Mountain. Camera studies in 2011-2012 detected the species at a number of sites in this region (Leitner and Delaney 2014), but there are no recent data. A repeat of the 2011-2012 camera study would be desirable. Except for the long-term Coso study sites, there have been no systematic surveys on the North Range of China Lake. A large-scale camera survey there would provide important data and could be done without disruption of military testing operations. The 2019 camera study on the China Lake South Range suggested that MGS are absent from much of this region. There are plans to carry out a similar survey at other sites on this large military reservation, which should greatly increase our understanding of the Mohave ground squirrel distribution here.

Careful siting of renewable energy projects within the Mohave ground squirrel range can minimize their impacts. Large-scale solar projects on private land should focus on areas where Mohave ground squirrels are no longer present or on previously disturbed sites. It would be desirable to design large solar arrays to retain vegetation cover so that Mohave ground squirrels could more easily move through them. The North of Kramer and Haiwee DFAs on BLM lands appear to support viable populations, whereas there is a minimal amount of suitable Mohave ground squirrel habitat on the Searles DFA.

Fort Irwin is currently planning to initiate military training on the WTA, an area that is known to support an important population. It would be desirable to gain a better understanding of the extent of Mohave ground squirrel occurrence in different parts of this large area. With that knowledge, it should be possible to site high-intensity training to avoid the most serious impacts.

Climate change with increasing temperatures and diminishing winter rainfall can be expected to continue to impact the Mohave ground squirrel range in the western Mojave Desert. It seems reasonable that higher elevation and more northerly parts of the range will be most likely to continue to receive adequate rainfall for reproduction. It is also possible that Mohave ground squirrels may respond to climate change by extending their range northward into Owens Valley. Conservation efforts should focus on protecting all potential climate refugia from additional human impact.

There is concern that the round-tailed ground squirrel is expanding into significant areas along the eastern edge of the Mohave ground squirrel range. The round-tailed ground squirrel is widely distributed in the eastern California deserts and into Arizona. It appears to be well-adapted to disturbed habitats and to drier and warmer conditions. This species may be expected to continue to encroach on parts of the Mohave ground squirrel range at lower elevations and where significant anthropogenic disturbance has occurred.

## ACKNOWLEDGMENTS

The preparation of this review was funded by the CDFW. Several field studies supported by BLM and CDFW have contributed much-needed distributional data. I am very grateful to the many desert biologists who have provided records of their field activities. K. Cripe was responsible for production of the many maps. S. Phillips and Vernadero Group Inc. contributed GIS data for preparation of maps. Natural resource staff at China Lake, EAFB, and Fort Irwin were extremely helpful in providing information about Mohave ground squirrel occurrence on their installations. Finally, I would like to thank S. Osborn for his leadership on Mohave ground squirrel conservation and for encouraging me to prepare this paper.

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FULL RESEARCH ARTICLE

## Home range and movements of San Joaquin antelope squirrels in the San Joaquin Desert of California

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The San Joaquin antelope squirrel (*Ammospermophilus nelsoni*) is one of five species in the genus and has the most restricted range of the four mainland antelope squirrels, occurring only in the San Joaquin Desert of California. Despite being state-listed as Threatened since 1980, few studies have been conducted on *A. nelsoni*, especially ecological studies, which hampers recovery efforts. We conducted a radio-telemetry study in 2002 of 19 males on the Lokern Natural Area in the southwestern portion of the San Joaquin Desert. Based on 100% Minimum Convex Polygons (MCP), home ranges varied from 1.25–14.5 ha with a mean of 5.93 ha ( $\pm 0.90$  standard error). The average daily distance traveled by these 19 males was 128.5 m (range, 71.4–224.5) and the average greatest distance travelled in a day was 313.0 m, with some traveling  $> 0.5$  km. Our data are useful to further refine the estimates of home range and movements of this neglected protected species, but in the future, better home range studies are needed that span multiple years, include both sexes, and occur at sites across its range.

**Key words:** *Ammospermophilus nelsoni*, conservation, home range, Minimum Convex Polygons, movements, radio telemetry, San Joaquin antelope squirrel

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The San Joaquin antelope squirrel (*Ammospermophilus nelsoni*) is one of five species of antelope squirrels (*Ammospermophilus*) that occur only in the deserts of North America (Mantooth et al. 2013). *Ammospermophilus nelsoni* has the most restricted range of a non-

island antelope squirrel (Mantooth et al. 2013), occurring only in the San Joaquin Desert of California (Grinnell and Dixon 1918; Harris 1998; Germano et al. 2011). All antelope squirrels are small sciurids, weighing from 99 to 179 g (Kays and Wilson 2002), and have multiple adaptations for desert life (Eisenberg 1975; Ghobrial and Nour 1975; Chappell and Bartholomew 1981; Walsberg 2000). In particular, *A. nelsoni* exhibits a variety of adaptations, including (1) light colored pelage, (2) relatively large auditory bullae to compensate for low sound transmission in deserts (but see Mason 2016), (3) soles of the feet that are heavily furred, (4) sparse summer pelage and denser, darker winter pelage, (5) ability to behaviorally dump body heat to cool soil in the shade or in a burrow, and (6) a hypothalamus sensitive to body temperature that will decrease metabolic heat production when  $> 40^{\circ}\text{C}$  (Heller and Henderson 1976; Best et al. 1990).

In the San Joaquin Desert, *A. nelsoni* occurs with several other protected species (USFWS 1998; Germano et al. 2001), and was state listed as Threatened in 1980 because much of the natural lands in this region have been converted to agricultural, urban, and industrial uses (USFWS 1998). It once was widely distributed in arid shrubland and grassland habitats in the western and southern portions of the San Joaquin Valley from western Merced County south to Kern County and also on the Carrizo Plain (Fig. 1). Despite its protected status, few studies of the ecology of this species have been made, and even fewer have been published. In 1997 we began studying the effect that grazing by cattle has on populations of *A. nelsoni* and other protected species in the San Joaquin Desert (Germano et al. 2012). We censused *A. nelsoni* using trapping grids on the Lokern Natural Area study area beginning in 1997. We found that numbers of *A. nelsoni* were significantly higher on plots grazed by cattle during the 10-y study (Germano et al. 2012). Here we report the results of another study conducted in 2002 in which we radio-tracked *A. nelsoni* caught on grids to determine home ranges of the squirrels and if there were any differences in the sizes of home ranges on grazed and control areas of the study site. Estimates of home range size are also important metrics for understanding how development can affect species and can lead to better conservation and recovery actions for a species.

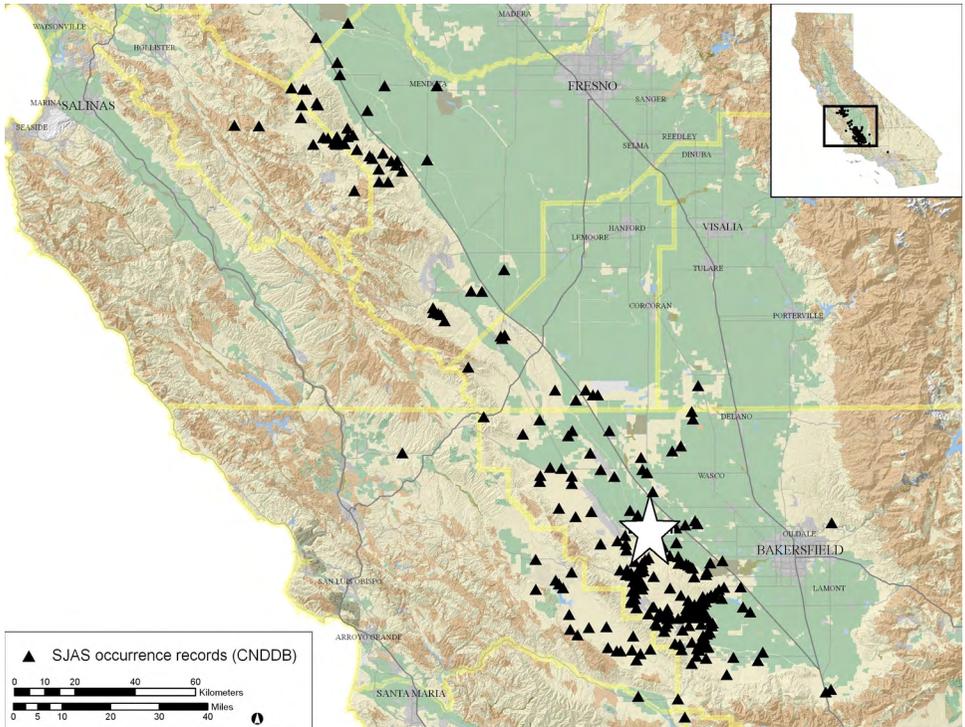
## METHODS

### Study Area

We studied *A. nelsoni* at the Lokern Natural Area (Fig. 1), which is in the southwestern end of the San Joaquin Desert (Germano et al. 2011), about 50 km west of Bakersfield in Kern County, California. The site ( $35^{\circ}22'24''\text{N}$   $119^{\circ}36'33''\text{W}$ , 158 m elevation) is a large and broad alluvial fan that is relatively undisturbed at the base of the Elk Hills, although the natural area is bordered by intensive agriculture, oil fields, and a large hazardous waste disposal site. The site is dominated by saltbush (*Atriplex* spp.), non-native annual grasses, and native annual forbs (Germano et al. 2012). Because the natural area is large (5,285 ha), relatively undisturbed, and mostly protected, other ecological studies have been performed there (Cypher et al. 2009; Germano et al. 2012; Germano and Rathbun 2016).

### Data Collection

We radio-tracked *A. nelsoni* in August and September 2002. We used Holohil Systems (Carp, Ontario, Canada) model MD-2C transmitters (4.0 g; 164 MHz) on the squirrels,



**Figure 1.** Occurrence records (black triangles) of the San Joaquin antelope squirrel (*Ammospermophilus nelsoni*) in the San Joaquin Desert of California. The approximate location of the Lokern Study Site (white star) is where we conducted a radio-telemetry study in 2002 on 19 male squirrels.

which we attached using wire and Tygon tubing collars (Holohil Systems). We recorded locations for squirrels on foot using a Communications Specialists (Orange, CA, USA) receiver (model R1000) and an H-Adcock or three-element Yagi receiving antenna and determined the UTM (Universal Transverse Mercator) coordinates of all loci with a GPS receiver (GeoExplorer 3; Trimble, Sunnyvale, CA, USA) with differential and real-time correction. With this unit, we measured a  $\pm 2$ -m variation ( $n = 7$ ) for a single location during the period of our radio tracking.

We attached radio-collars to 20 male antelope squirrels at the end of July 2002. To increase sample size because of limited numbers of transmitters, we only radio-tagged males to eliminate the confounding effect of the sex of the animals. Although we intended to only tag adults ( $\geq 130$  g in weight), we found it necessary to tag five subadults (106–129g) and four juveniles ( $\leq 105$  g) to achieve a sample of 20. Transmitters weighed 2.5–4.3% of the body mass of squirrels. Of the four square-mile sections (10.36 km<sup>2</sup>) on our study site (21, 27, 29, and 33), we did not tag animals on Section 27 because the high density of kangaroo rats in this area had resulted in minimal difference in residual dry matter between treatment (grazed) and control pastures. We, therefore, only collared animals on Sections 21, 29, and 33 (Fig. 2). We recaptured all radio-tagged squirrels and removed their collars 16–24 September 2002.

We attempted to radio-locate each antelope squirrel twice per day (except weekends), once in the morning and once in the afternoon. We tried to ensure that at least 4 h separated the two daily locations to eliminate auto-correlation between loci. We also haphazardly changed the order in which we located individuals to eliminate any temporal biases in locations. The diurnal antelope squirrels do not use a home burrow; meaning that each night they usually switch to a different burrow. The total number of night burrows is usually between five and 10 (G.B. Rathbun, unpublished data). During the day, *A. nelsoni* range widely over their home ranges and use many burrows as temporary refuges from disturbances, such as the biologists who radio-track them. Thus, we determined all our locations by homing in on animals in burrows rather than by triangulation (Kenward 2001).

### Home Range and Distance Analyses

We calculated home range size of *A. nelsoni* using the Minimum Convex Polygon (MCP) technique (Home Range Extension in ArcView 3.2; Esri, Redlands, CA, USA). We calculated home range size based on both 100% and 50% (core area) MCP. We collected data on 19 squirrels (one radio was lost within a few days), 12 of which were in plots grazed by cattle in earlier years (Germano et al. 2012) and seven of which were in non-grazed



**Figure 2.** Home ranges of San Joaquin antelope squirrel (*Ammospermophilus nelsoni*) based on 100% Minimum Convex Polygons (MCP; outer polygons) and 50% MCP (darker inner polygons) in treatment (grazed) and control (ungrazed) plots in 2002 at the Lokern study site in the southern San Joaquin Desert of California, USA. Numbers 21, 27, 29, and 33 are treatment pastures (Sections), small, imbedded squares in the corners of sections are control pastures (500 m on a side), and squares with dashed outlines are treatment and control plots.

plots. We collected between 17 and 48 locations for each squirrel (mean = 34.4, standard error = 2.35; Table 1). Data for 50% MCP home range sizes met parametric assumptions, and after square root transformation for 100% MCP, data of home range sizes were normal and homoscedastic. We compared home range sizes of untransformed data of 50% MCP and transformations of 100% MCP between control and treatment plots using One-Way ANOVA with  $\alpha = 0.05$ . We also compared 100% MCP and 50% MCP by age classes (adult, sub-adult, and juveniles) using the Kruskal-Wallis test ( $\alpha = 0.05$ ).

To determine distances that *A. nelsoni* moved between consecutive daily radio locations (ignoring distances from locations taken > 1 d apart), we used the ArcView Path With Distance and Bearing Extension, v. 3.2b. Data for average and greatest distance moved daily between treatment and control plots were normal and homoscedastic. We compared average and greatest distances moved between plot types with One-Way ANOVA ( $\alpha = 0.05$ ). We compared average and greatest distances moved by age classes using the Kruskal-Wallis test ( $\alpha = 0.05$ ).

**Table 1.** Identification number (ID), weight (g), age class (A = adult, S = sub-adult, J = juvenile), the number of radio-telemetry locations (No. Points), 100% and 50% Minimum Convex Polygon (MCP) home range sizes (ha), and average movement distances (AMD, in m) of male San Joaquin antelope squirrel (*Ammospermophilus nelsoni*) at the Lokern study site in the southern San Joaquin Desert of California, USA, in 2002.

ID	Weight (g)	Age Class	Sec/Treat	No Points.	100% MCP	50% MCP	AMD (m)
1	142	A	33T	46	4.438	0.594	114.1
2	140	A	33T	47	2.363	0.665	72.7
3	153	A	33T	45	5.206	1.730	114.1
4	159	A	33T	17	4.641	1.101	89.3
5	98	J	29T	42	14.48	2.056	224.5
6	111	S	29T	45	7.128	2.397	152.7
7	95	J	29T	48	6.530	0.667	135.3
8	111	S	21T	45	12.09	2.509	140.7
9	153	A	21T	42	12.69	1.963	131.4
10	103	J	21T	21	2.808	0.590	75.7
11	143	A	29T	19	10.74	1.218	156.0
12	112	S	21C	32	3.378	1.128	85.4
13	139	A	33C	28	5.901	1.529	174.4
14	134	A	33C	26	1.245	0.235	71.4
15	122	S	33T	29	7.097	0.754	222.1
17	105	S	29C	34	1.845	0.559	104.8
18	153	A	33C	28	2.888	0.490	130.6
19	139	A	33C	27	3.262	0.506	135.8
20	158	A	29C	33	4.024	0.916	110.6

## Between-Trap Distance Analysis

Using data collected in August 2002 during trapping at eight plots (four control, four treatment) for a grazing study on the Lokern (Germano et al. 2012), we calculated daily squirrel movements as the distances between trap locations on the X, Y stations of the trap grid. Plots consisted of an  $8 \times 8$  grid of 64 Tomahawk live traps with 40-m trap spacing. Trapping sessions lasted 6 d. In instances where squirrels did not move vertically or horizontally along trap lines, we calculated the diagonal distances between traps based on the hypotenuse of a right triangle. We only used trap locations of individual animals caught on consecutive days to calculate movement distances. We assumed data were normal based on the Central Limit Theorem ( $n > 30$  for all groups) and we determined that data were homoscedastic. We used Two-way ANOVA ( $\alpha = 0.05$ ) to compare distances moved daily between males and females, between control and treatment plots, and the interaction of these groups.

## RDM and Invertebrate Numbers

We measured the residual dry matter (RDM) on each plot in which we radio-tracked squirrels (Germano et al. 2012). This was done in August during trapping sessions using 30 quarter-m<sup>2</sup> quadrats placed randomly in the trapping grid. Although some RDM remained from the previous year, most of the RDM was due to herbaceous plant production for the current year, and we used this as a measure of current plant production. We also checked the 10 pit-fall traps arrayed along the edge of each plot each day during trapping (Germano et al. 2012). Pit-fall traps were 19.1 L plastic buckets that we had dug into the ground up to the edge of the bucket. Traps were open continuously during the 6 d of squirrel trapping. We averaged the number of invertebrates found in the traps as an estimate of arthropod abundance on each plot. We excluded the number of ants we found in invertebrate numbers as they are not typically food for *A. nelsoni* (Hawbecker 1947) and ant numbers were much higher than other invertebrates (Germano et al. 2012), which we suspected would skew our comparisons. For both RDM and invertebrate numbers, data were normal and homoscedastic. We compared separately the estimates of RDM and abundance of invertebrates between control and treatment plots using ANOVA ( $\alpha = 0.05$ ).

## RESULTS

The home ranges of *A. nelsoni* on the Lokern in 2002 varied in size and were spread across the treatment and control plots that we used (Fig. 2). The mean home range sizes (Table 2) did not differ significantly among age classes (100% MCP:  $H = 0.88$ ,  $df = 2$ ,  $P = 0.643$ ; 50% MCP:  $H = 1.32$ ,  $df = 2$ ,  $P = 0.516$ ). The average MCP home range size using 100% of loci for each individual was 3.48 ha on control plots and 7.38 ha on treatment plots (Table 2), which were significantly different ( $F_{1,17} = 8.38$ ,  $P = 0.010$ ). The average 50% MCP home range size was 0.77 ha on control plots and 1.35 ha on treatment plots (Table 2), but these differences were not significant ( $F_{1,17} = 3.61$ ,  $P = 0.075$ ). Irrespective of plot type, mean home range size was 5.93 ha for 100% MCP and 1.14 ha for 50% MCP (Table 2).

The mean average and greatest distances moved (Table 3) did not differ significantly among age classes (Average:  $H = 0.83$ ,  $df = 2$ ,  $P = 0.662$ ; Greatest:  $H = 1.24$ ,  $df = 2$ ,  $P = 0.538$ ). The average distance moved daily by squirrels on treatment plots was 135.7 m and

**Table 2.** Sample size (n), mean, standard error (SE), and range of 100% and 50% Minimum Convex Polygon (MCP) home range sizes (ha) of male San Joaquin antelope squirrel (*Ammospermophilus nelsoni*) by age class (adult, sub-adult, and juvenile) and by treatment (grazed) and control (ungrazed) plots in 2002 at the Lokern study site in the southern San Joaquin Desert of California, USA.

Age Class/ Plot Type	100% MCP				50% MCP			
	n	Mean	SE	Range	n	Mean	SE	Range
Adults	11	5.22	1.05	1.25–12.7	11	1.00	0.17	0.24–1.96
Sub-adults	5	6.31	1.78	1.85–12.1	5	1.47	0.41	0.56–2.51
Juveniles	3	7.94	3.44	2.81–14.5	3	1.10	0.48	0.59–2.06
Treatment	12	7.52	1.17	2.36–14.5	12	1.35	0.21	0.59–2.51
Control	7	3.22	0.57	1.25–5.90	7	0.77	0.17	0.24–1.53
Combined	19	5.93	0.90	1.25–14.5	19	1.14	0.16	0.24–2.51

**Table 3.** Sample size (n), mean, standard error (SE), and range of average and greatest daily movements (m) of male San Joaquin antelope squirrel (*Ammospermophilus nelsoni*) by age class (adult, sub-adult, and juvenile) and by treatment (grazed) and control (ungrazed) plots in 2002 based on radio locations at the Lokern study site in the southern San Joaquin Desert of California, USA.

Age Class/ Plot Type	Average				Greatest			
	n	Mean	SE	Range	n	Mean	SE	Range
Adults	11	118.2	9.73	71.4–135.8	11	286.3	26.6	164.4–443.0
Sub-adults	5	141.1	23.6	85.4–222.1	5	370.2	63.7	195.5–571.7
Juveniles	3	145.2	43.2	75.7–224.5	3	340.0	117.9	182.4–570.8
Treatment	12	135.7	14.2	72.7–224.5	12	353.4	38.8	164.4–571.7
Control	7	116.2	13.0	71.4–175.4	7	243.9	24.2	138.0–324.8
Combined	19	128.5	10.2	71.4–224.5	19	313.0	28.4	138.0–571.7

by squirrels on control plots was 116.2 m (Table 3) and these differences were not significant ( $F_{1,17} = 0.85$ ,  $P = 0.369$ ), nor were the greatest distances moved daily (353.4 m) on treatment plots or on control plots (243.9 m) by squirrels ( $F_{1,17} = 4.02$ ,  $P = 0.061$ ). The average distance moved daily irrespective of plot type was 128.5 m and the greatest mean distance moved daily was 313.0 m. Average daily movements of squirrels based on movements between traps (Table 4) did not differ significantly by sex ( $F_{1,169} = 0.14$ ,  $P = 0.707$ ) or plot type ( $F_{1,169} = 3.40$ ,  $P = 0.067$ ), nor was there a significant interaction ( $F_{1,169} = 0.93$ ,  $P = 0.337$ ).

We found that mean RDM on the control plots (1189.7 g/m<sup>2</sup>) was almost 14 times higher than mean RDM on the treatment plots (85.67 g/m<sup>2</sup>). The difference in RDM between plot type was significant ( $F_{1,4} = 57.91$ ,  $P = 0.002$ ). We did not find a significant difference in mean daily invertebrate numbers between control (1.70) and treatment (1.17) plots ( $F_{1,4} = 2.17$ ,  $P = 0.215$ ).

**Table 4.** Sample size (n), mean (m), 95% confidence interval (CI), and range of daily movement distances of male and female San Joaquin antelope squirrel (*Ammospermophilus nelsoni*) based on trapping in treatment (grazed) and control (ungrazed) plots in August 2002 at the Lokern study site in the southern San Joaquin Desert of California, USA. Movements are based on trap locations of squirrels on eight plots (four control and four treatment). Distances between traps were 40 m.

Plot Type/Sex	n	Mean	95% CI	Range
Control Males	37	52.2	8.06	0–215
Control Females	41	56.7	7.16	0–179
Treatment Males	45	73.7	6.94	0–240
Treatment Females	50	63.5	7.71	0–283

## DISCUSSION

It is surprising to us that a species that has been of conservation concern for almost 40 y has had so few studies of its ecology, such as reproduction, density, demographic structure, home range, and movements. Besides being useful for general comparisons of life histories across species in the genus, as well as comparisons among the Sciuridae, these ecological data are necessary for conservation and recovery of the species. *Ammospermophilus nelsoni* continues to face development and habitat loss within its range and agency personnel can use data such as home range and movements to better determine means to evaluate effects and mitigate impacts from these developments.

We found that the home range size of 19 male *A. nelsoni* on the Lokern varied greatly from 1.25–14.5 ha. We determined these home range sizes during August and September, a time when *A. nelsoni* are not reproductive (unpublished data). Home range sizes may differ in other seasons, especially when *A. nelsoni* are reproducing or when tending young. The overall mean home range size we found on the Lokern (5.93 ha) is higher than the 100% MCP estimates for male (3.73 ha) and female (2.29 ha) *A. nelsoni* made by Harris and Stearns (1991) on the Elkhorn Plain, but lower than estimates they made using a sample-size corrected MCP (males = 9.01 ha, females = 6.03 ha). We do not think that the sample-size corrected MCP estimates are a good comparison for our estimate. Although Harris and Stearns (1991) radio-tracked squirrels, of the three males for which they gave a home range estimate, one had a sample size of six locations. Of the five females, two had only seven and eight locations. They also gave estimates for seven juveniles, but all estimates were based on fewer than 15 locations. We recalculated 100% MCP estimates given by Harris and Stearns (1991, Table 8) for individuals with  $\geq 15$  locations and found average male home range size was 4.91 ha ( $\pm 2.88$  SD,  $n = 2$ ) and for females was 3.15 ha ( $\pm 1.41$  SD,  $n = 3$ ). Our home range estimate of 5.93 ha was not significantly different than the recalculated male estimate of 4.91 ha (Wilcoxon Signed Rank test;  $W = 107.0$ ,  $df = 1$ ,  $P = 0.644$ ) of Harris and Stearns.

The only other home range estimate for *A. nelsoni* is from Hawbecker (1958) but is based on trap locations. Although he gave several examples, his estimate of the home range of *A. nelsoni* was 4.45 ha (11 acres) based on one male captured over 1 year. This estimate also is not significantly different than our estimate of home range size ( $W = 120.0$ ,  $P = 0.324$ ). We did not radio-tag females, but our data on between trap movements is of some value for

what home ranges of females might be on our site. Although not a perfect analogue to home range, the lack of significant differences between the sexes in trap movement might mean that home range size is not different if we had followed females at our site.

There are two estimates of home range size for the congener white-tailed antelope squirrel (*A. leucurus*), but both are based on movements within a trapping grid. Using various methods to determine home range, Bradley (1967) estimated home range size as 3.24 ha, 6.03 ha, and 8.34 ha, but determined 6.03 ha to be the best estimate. Jorgensen and Hayward (1965) found home range sizes of males from 3.00 to 16.47 ha, but these are especially poor estimates because they are based on only 3.0 to 4.4 captures per male. Using radio-telemetry locations, Harris and Leitner (2004) found a home range size of 6.73 ha for 16 adult male Mohave Ground Squirrels (*Xerospermophilus mohavensis*) in the western Mojave Desert of California. Although in a different genus, *S. mohavensis* is similar in size to antelope squirrels with weights varying from 70–300 g (Kays and Wilson 2002).

We found that the home range estimates based on 100% MCP on the Lokern Natural Area differed significantly between grazed and ungrazed plots, with squirrels using almost twice as large an area where cattle had grazed the site. Both plant material and arthropods make up a large portion of the diet of *A. nelsoni* (Hawbecker 1947; Harris 2019). During 2002, we found that RDM (an estimate of plant food available to *A. nelsoni*) was significantly greater on the control plots than treatment plots, while the number of invertebrates we found in pit-fall traps was similar. This may account for the smaller home ranges of squirrels in the control plots. Squirrels on the treatment plots may have had to forage much more widely to meet dietary needs in 2002. Although denser vegetation on control plots may mean squirrels do not have to forage as widely as in grazed plots, squirrels may be more susceptible to predators if dense vegetation conceals the presence of predators better than in the more open grazed areas. Interestingly, over the course of the 10-y study of the effects of grazing on various protected species on the Lokern, numbers of *A. nelsoni* were significantly greater on treatment plots than on the ungrazed controls (Germano et al. 2012). Numbers varied widely over the 10 y, but ultimately sustained, high herbaceous cover depresses numbers of *A. nelsoni* (Germano et al. 2001, 2012).

Home range size gives information about how large an area is used by a species over an extended time period but does not indicate if an animal travels short distances to move within its home range, or if long daily distances are traveled. This also can be important to understanding the energetics of species and to assess the risk of an animal traveling into a project site in a short time. We found that the average daily movements of male *A. nelsoni* was almost 130 m, and the average greatest distance moved daily was 313 m, with some movements > 0.5 km in a day. Harris and Stearns (1991) did not report movement distances and Hawbecker (1958) reported various movements, sometimes over days and sometimes over years, so data are not comparable to what we found. Based on trap grid data for other antelope squirrels, Chew and Chew (1970) reported that the average movement distances of Harris's antelope squirrels (*A. harrisi*) was 274 m/individual, and for *A. leucurus*, Allred and Beck (1963) reported the greatest distance between captures was up to 129.5 m for males and 127.1 m for females, and Bradley (1967) gave the mean greatest distance between captures as 343.5 m. Although gathered in a different way than us, movement data among antelope squirrels appears to be similar.

Like other antelope squirrels, *A. nelsoni* is a highly social rodent that is very active during the day, and its recovery from threatened status will benefit greatly from additional and comprehensive ecological and natural history research. The few ecological studies that

have been conducted to date focusing on *A. nelsoni* are lacking in rigor, ours included. Only ours and the non-peer reviewed study by Harris and Stearns (1991) have used radio-telemetry to determine home ranges of antelope squirrels. Because rodents travel off grids, only radio-telemetry can accurately capture the full home range of individuals during an active season. Our study suffered from not including females, which we believed necessary at the time to increase sample size. Also, we only followed individuals for about 45 d. Our data are useful to further refine the estimates of home range and movements of this neglected protected species, but in the future, better home range studies would collect radio locations 3–4 times per week starting in the spring and following squirrels for 3–4 mo and at least for 2 y. We suggest repeating the studies on home range on the Elkhorn Plain and the Lokern using the guidelines above, and that additional studies should be carried out on the valley floor, perhaps at the Semitropic Ecological Reserve of the California Department of Fish and Wildlife, and at a site in the Panoche Valley. Multiple sites would give the full range of variation in home range and movements of *A. nelsoni*, which could guide recovery actions aimed at conserving sufficient habitat to sustain populations of the squirrel.

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FULL RESEARCH ARTICLE

## Population ecology and survivorship of San Joaquin antelope squirrels in grazed and control plots in the San Joaquin Desert of California

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The San Joaquin antelope squirrel (*Ammospermophilus nelsoni*) is endemic to the San Joaquin Desert of California. It has been listed as Threatened by the state of California since 1980 due to profound habitat loss, but a paucity of information could limit conservation efforts for this species. We examined data collected each August during 1997–2006 to determine whether *A. nelsoni* population attributes differed between grazed and ungrazed study plots. We found that sex ratios, mean weights, percentage of reproductive males, number of young, yearly survival, longevity, overall survivorship, and population growth trajectories all were similar between grazed and ungrazed plots. In general, sex ratios were even, males were heavier than females, some males were reproductive in August (although most females were not), and the number of young was inversely related to residual dry matter. We also found that we captured most individuals only once, but we captured a few squirrels for up to for 5 years, and the populations on both grazed and ungrazed plots were growing during the 10-year study. Our study was only the second long-term investigation of this species and the information is needed for further conservation and recovery efforts.

**Key words:** *Ammospermophilus nelsoni*, conservation, reproduction, sex ratios, survival, sex ratios

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The San Joaquin antelope squirrel (*Ammospermophilus nelsoni*; Fig. 1) is a small ground squirrel endemic to the San Joaquin Desert in central California (Best et al. 1990c; USFWS 1998; Germano et al. 2011). This species once was widely distributed in arid shrubland and grassland habitats throughout this region; however, extensive conversion of these habitats to agricultural, urban, and industrial uses has imperiled this species along with a suite of other co-occurring endemic species. Consequently, *A. nelsoni* was state listed as Threatened in 1980 (USFWS 1998).

Despite being a species of conservation concern, relatively few ecological investigations have been conducted on this species. Data on life-history attributes, distribution, and habitat relationships have been published by Grinnell and Dixon (1918), Hawbecker (1947, 1953, 1958, 1959), Otten and Cypher (1999), Cypher (2001), Harris (2019), and Germano et al. (2021). More germane to the conservation of *A. nelsoni*, Germano et al. (2012) assessed the effects of grazing on population trends of this species while Fiehler et al. (2017) assessed the effects of oil field activities. Additional information, particularly on factors affecting population dynamics, however, is necessary for conserving *A. nelsoni*.

From 1997–2006, we collected data on a suite of vertebrates, including *A. nelsoni*, on a site in the San Joaquin Desert to assess the effects of grazing on these species (Germano et al. 2012). Abundance trends for *A. nelsoni* were assessed on grazed and control plots. We found that numbers of *A. nelsoni* were much greater on grazed plots than control plots when the area warranted grazing and numbers were inversely correlated with amounts of herbaceous plant growth. Here, we further analyze these data to assess demographic attributes of *A. nelsoni*. Our objectives were to determine whether demographic attributes varied with respect to grazing, and whether attributes varied over time with fluctuations in annual environmental conditions.

## METHODS

### Study Area

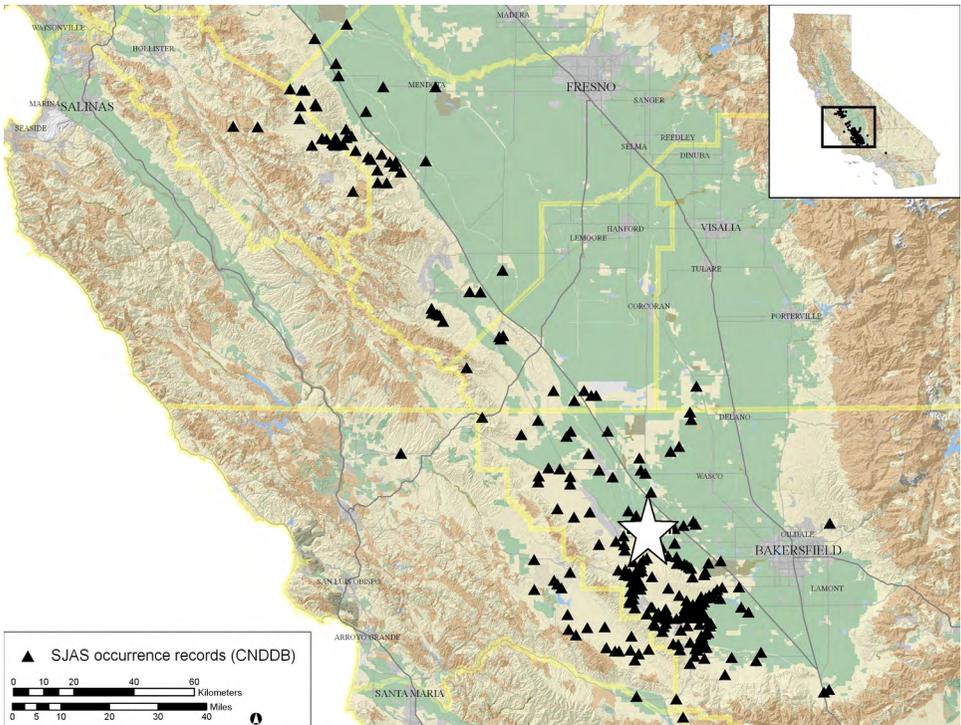
We studied *A. nelsoni* in a region commonly referred to as the Lokern Natural Area (Fig. 2), which is in the southwestern end of the San Joaquin Desert (Germano et al. 2011), about 50 km west of Bakersfield in Kern County, California. The site (35°22'24"N 119°36'33"W, 158 m elevation) is a large area of relatively undisturbed habitat situated on a broad alluvial fan at the base of the Elk Hills. The natural area is situated between intensive agriculture to the east and north and oil fields to the west and south. The site is dominated by saltbush (*Atriplex* spp.), non-native annual grasses, and native annual forbs (Germano et al. 2012). Because the natural area is large (5,285 ha), relatively undisturbed, and mostly protected, other ecological studies have been performed there (Cypher et al. 2009; Germano et al. 2012; Germano and Rathbun 2016).

### Data Collection

We caught *A. nelsoni* on grazed treatment plots and ungrazed control plots, with four replicates. Each treatment plot (2.6 km<sup>2</sup> or 1 mi<sup>2</sup>) was defined by section boundaries (Sections 21, 27, 29, and 33 of Township 29 South, Range 22 East). The treatments were adjacent to each other in a four-leaf clover pattern, with a fifth section enclosed in the middle (35.3762 N, 119.61614 W), which served as a pasture to temporarily place livestock while moving them into or out of the 4 surrounding treatment pastures (Fig. 3). The 4 control



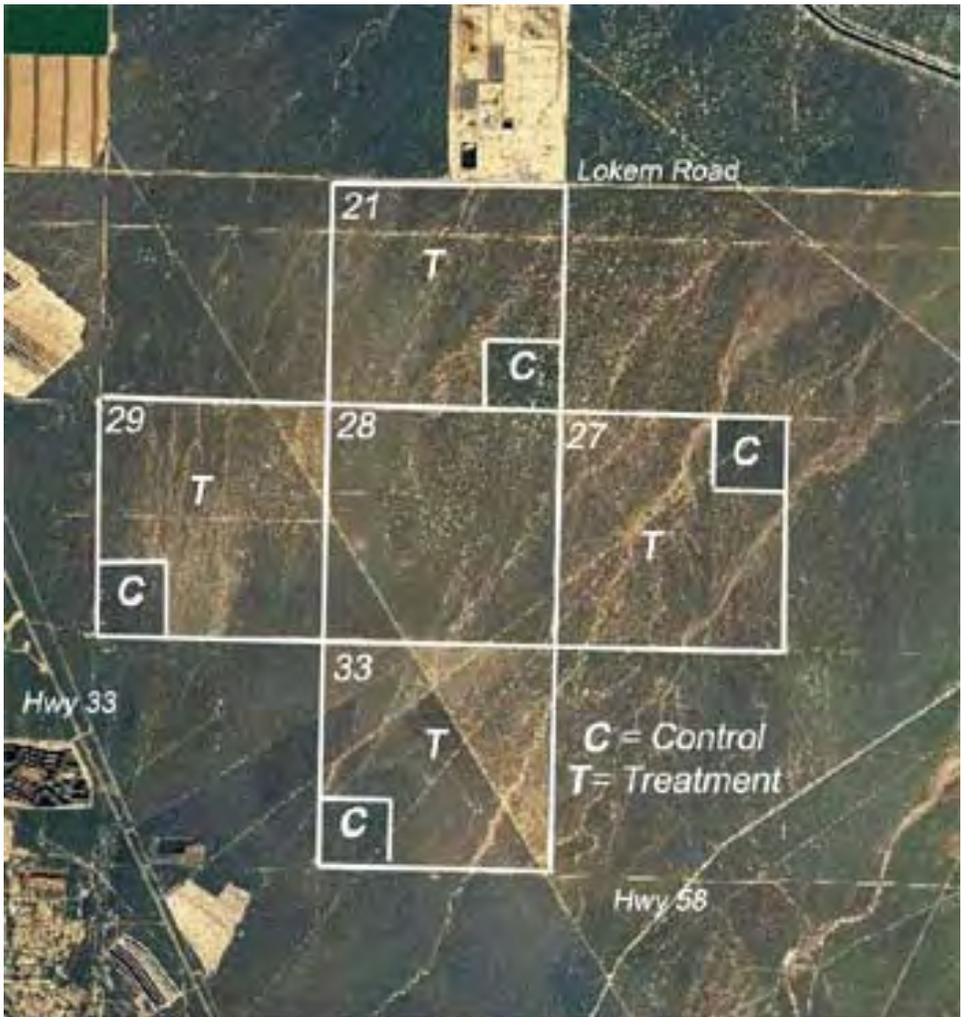
**Figure 1.** Adult female (left) and juvenile (right) San Joaquin antelope squirrel (*Ammospermophilus nelsoni*) photographed 23 April 2017 3.2 km south of Tupman, Kern County, California. (Photo Credit: Larry Saslaw).



**Figure 2.** Occurrence records (black triangles) of the San Joaquin antelope squirrel (*Ammospermophilus nelsoni*) in the San Joaquin Desert of California. The approximate location of the Lokern Study Site (white star) is where we conducted a 10-year study of antelope squirrels.

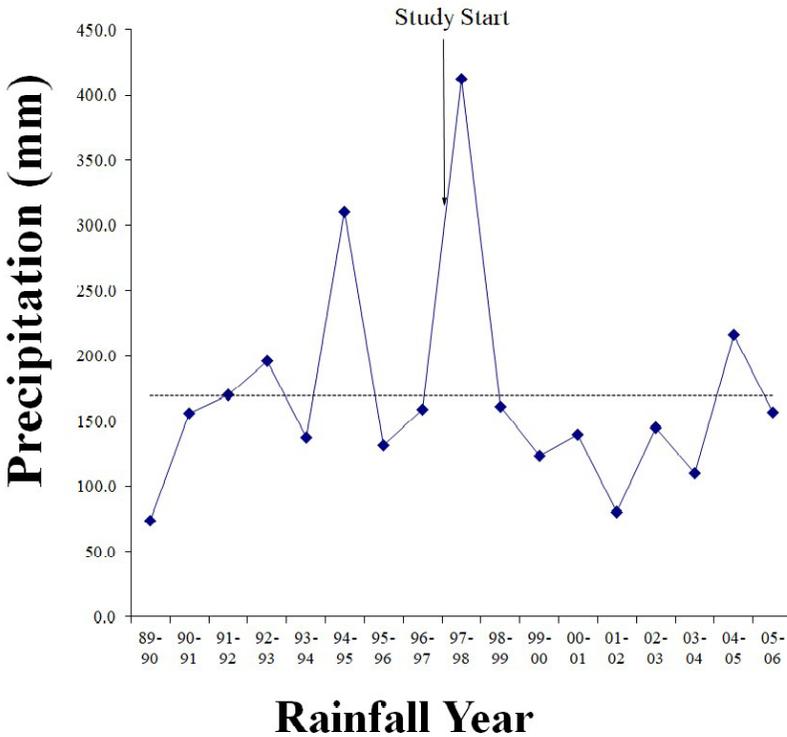
pastures were 25 ha (62 acres) each, and each square enclosure was located within a corner of a treatment pasture to reduce fencing costs (Fig. 3). Although control plots were within a smaller surrounding area than treatment plots, previous experience with the movements of squirrels indicated that the plot sizes were not likely to be a factor with our design, and this was confirmed based on sizes of home ranges of squirrels (Germano et al. 2021) and our extensive mark-recapture data associated with this project. Of the 1,265 individual squirrels we caught in 10 years of trapping, only 3 individuals switched between control and treatments plots, and 2 returned to their original plot.

We developed a cooperative agreement with the landowner (Chevron Production Company) and a local cattle operator (Eureka Livestock Company) to provide cattle graz-



**Figure 3.** Experimental design of the livestock grazing study in the Lokern Natural Area in western Kern County (T29S, R22E), California, where San Joaquin antelope squirrels (*Ammospermophilus nelsoni*) were trapped yearly on 4 grazed (T) and 4 control plots (C), 1997–2006. Each large block is 1 mi<sup>2</sup> and numbers are section numbers.

ing on the treatment plots. The stocking rate and timing of grazing was determined by our objective of maintaining about 560 kg of herbaceous vegetation per ha (500 lb per acre) at the end of the winter growing season. We aimed to start grazing by 1 December each year but would not turn out cattle unless there was at least 784 kg per ha (700 pounds per acre) residual dry matter or 5 cm (2 in) of new green growth. If grass growth did not attain the minimum standard in any year, then pastures were not grazed that year. Our objective was to attain the minimum dry mulch amount (or residual dry matter; RDM) by at least 1 April each year when the livestock were removed. During the 10-year study, rainfall varied from a high of 412.5 mm in the winter of 1997–1998 to a low of 80.5 mm in 2001–2002 (Fig. 4). Cattle were turned out onto the newly fenced treatment plots for the first time in February 1998. The yearly plot, vegetation, and animal sampling schemes were completed as planned in 1998, and the cattle were removed in July 1998, just prior to mammal trapping. In 1999, 2000, and 2001, a similar schedule was followed, although with progressively lower grazing intensity as conditions dried each successive year. Because rainfall was well below average and minimum forage was not available, cattle were not on the plots from 2002–2004. In 2005, rainfall was above average and was about average in 2006, so cattle



**Figure 4.** Rainfall in the Lokern area from 1989 to 2006. Data are from the Buena Vista Water District in Buttonwillow, California, about 11 km northeast of the study site. The dotted line is the Buttonwillow 20-year mean of 169 mm.

grazed the treatment plots for a brief period in both years. The duration of our study had to be long enough to encompass variation from several environmental factors, including the wild fire in 1997, the El Niño winter of 1997–1998, initial low populations of terrestrial vertebrates (Germano et al. 2012), and year-to-year variation in rainfall and numerous other environmental factors that are typical of a desert.

We established permanent  $8 \times 8$  live-trapping grids on each study plot consisting of 64 traps at 40-m intervals. This grid was superimposed on a transect grid set up for sampling lizards (Germano et al. 2012). We baited the traps with rolled oats and opened and checked the traps during 6 consecutive mornings once a year, between the end of July and the first of September. We monitored 4 grids at once, either 4 treatment plots or 4 controls, waited 1–2 weeks, and then trapped at the other 4 grids. Thus, the yearly trapping of treatment and control grids took 3–4 weeks.

We used collapsible single-door live traps (Model 13, Tomahawk Trap Co., Tomahawk, WI, USA), which were opened at dawn and closed at noon, or when ambient air temperature exceeded  $35^{\circ}\text{C}$  ( $95^{\circ}\text{F}$ ), whichever occurred first. We shaded each trap from the sun with burlap. While open, we checked the traps every 2 hours. For each squirrel captured, we recorded the trap location on the grid, its sex and weight, and we applied a unique mark on the fur with a black felt-tip pen (Sharpie Permanent Marker). We also tagged each squirrel with a passive integrated transponder (PIT) tag (Model TX1400 series, Biomark, Boise, ID, USA) inserted subcutaneously on the back with a hypodermic needle (Schooley et al. 1993).

### **RDM and Invertebrate Numbers**

We measured the RDM on each trapping grid in which we caught squirrels (Germano et al. 2012). This was done in August during trapping sessions using 30 quarter- $\text{m}^2$  ( $50 \times 50$  cm) quadrats placed randomly in the trapping grid. This gave us a measure of the herbaceous plant production for the year. We also checked 10 pitfall traps arrayed along the edge of each plot each day during trapping (Germano et al. 2012). Pit-fall traps were 19.1 L plastic buckets that we had dug into the ground up to the edge of the bucket. Traps were open continuously during the 6 days of squirrel trapping. We averaged the number of invertebrates found in the traps as an estimate of arthropod abundance on each plot for *A. nelsoni*. We excluded the number of ants we found in invertebrate numbers as they are not typically food for *A. nelsoni* (Hawbecker 1947) and ant numbers were much higher than other invertebrates (Germano et al. 2012), which we suspected would skew our comparisons.

### **Data Analyses**

We estimated sex ratios, mean adult weights, percentage of adults reproductive, and the number of young on control and grazed plots. To determine if there were significant differences in these life-history traits between control and grazed plots, we used General Linear Models (GLM) models. For sex ratios, we compared numbers of males and females by year, sex, and treatment with an interaction of year $\times$ sex $\times$ treatment.

We compared adult weights using year, sex, and treatment as main effects, RDM and number of invertebrates as covariates, and the interactions year $\times$ treatment, year $\times$ sex, treatment $\times$ sex, and year $\times$ sex $\times$ treatment. We determined adults to be any squirrel caught for the first time with a weight  $> 110$  g. Based on recent trapping we have done (2019–2020), it is possible that some of these individuals might have been young of the year born in

February or March, but a few squirrels that weighed 109–118 g in later years of trapping in this study had been caught the previous year. We did not include data for 1999 because there was only one datum for females on the control plots. All other groups for other years had a sample size of at least 4 (most > 15).

We judged male squirrels to be reproductive if they were scrotal (testicles fully descended). We found many males that we judged to be partially scrotal, but we could not be certain that testicles were descending or if they were regressing. For females, we determined an individual to be reproductive if she was pregnant (abdomen distended and high weight), lactating (enlarged nipples), or in estrous (swollen vagina). For comparing the percentage of adults that were reproductive on control and grazed plots, we could only test main effects of year and treatment because only two females (of hundreds of captures) showed any indication of being reproductive. Therefore, we only tested males.

We compared the number of young ( $\leq 110$  g at first capture) found on a plot by year and treatment with RDM as a covariate. Because of a low number of degrees of freedom, we could not include an interaction term. We also used a Pearson's Product Moment Correlation of the number of young to RDM using the number of young on control (combined) and grazed (combined) plots each year as separate points ( $n = 18$ ).

We estimated year to year survival on control and grazed plots by determining the number of individuals marked in one year that were caught in the next year or subsequent years. To determine if there were significant differences in 2 year survival of squirrels between control and grazed plots, we used a GLM model with year, sex, and treatment as main effects, RDM and the number of invertebrates as covariates, and an interaction of year $\times$ sex $\times$ treatment. We estimated longevity of male and female squirrels from recaptures across sessions. The number of sessions across which we found a given individual was used as the minimum longevity for the individual. For example, if we captured an individual in 2 consecutive years, then not in the next year, but again the following year, we estimated that this squirrel was at least 4 years old when we last caught it (adults when first captured were at least 1 y old). For all tests above,  $\alpha = 0.05$ .

*Survivorship.*—We calculated recapture and demographic vital rates of *A. nelsoni* using population encounter histories derived from individual encounter histories in Program MARK (White and Burnham 1999). We calculated population size ( $N$ ), apparent survival ( $\Phi$ ), and recapture rates ( $p$ ) using open population Cormack-Jolly-Seber (CJS) and POPAN models in Program MARK (Lebreton et al. 1992; White and Burnham 1999). We generated CJS model sets for both control and grazed plots based on group designation (female and male) to test whether  $\Phi$  or  $p$  was best estimated independent of group or time, by group or time, or with a group  $\times$  time interaction (generating 16 models). Model selection was based on Akaike Information Criterion ( $AIC_c$ ) values, with lower values denoting greater parsimony (Burnham and Anderson 2002) and we included  $\Delta AIC_c$  (difference between model  $AIC_c$  and lowest  $AIC_c$  in the model set),  $\omega$  (Akaike model weight),  $k$  (number of estimable parameters), and Deviance (measure of model fit). Apparent survival and recapture rates were based on model averaging.

Traditionally, encounter rates are used to calculate the probability that an individual will leave a population. If the encounter rates are reversed, then the probability of an individual entering the population can be estimated (Pradel 1996) where:  $\lambda$  (rate of individuals entering a population or cohort). Using Pradel models,  $\lambda$  estimates the realized growth rates of the age class from which the encounter rates were generated but is not necessarily equivalent to the growth rate of the population. Still, it provides an important metric of the

life-history characteristics of a population. Pradel's  $\lambda$  was estimated by Program MARK in conjunction with the CJS-model described above.

## RESULTS

From 1997 to 2006, the sex ratios of *A. nelsoni* on the Lokern varied from 0.79M:1.00F to 1.53M:1.00F, but these differences were not significantly different than 1M/1F (Table 1). There was a significant interaction of year, treatment, and sex on adult weights (Table 1), with males have significantly higher weights in most years in grazed and control plots, but no difference in 2002 on either plot type, and females heavier than males in 2000 and 2006 on control plots and heavier in 2004 on grazed plots (Fig. 5). Irrespective of year or treatment type, adult males averaged 136.2 g ( $\pm 0.680$  standard error [SE]; range, 111–196 g) and adult females averaged 126.5 g ( $\pm 0.568$  SE; range, 111–163 g). If most squirrels weighing 111–119 g are actually subadults ( $> 95\%$  in this weight range were first captures) and we designate adults as those weighing  $\geq 120$  g, then adult males averaged 140.6 g ( $\pm 0.642$  SE; range, 120–196 g) and adult females averaged 130.9 g ( $\pm 0.566$  SE; range, 120–163 g).

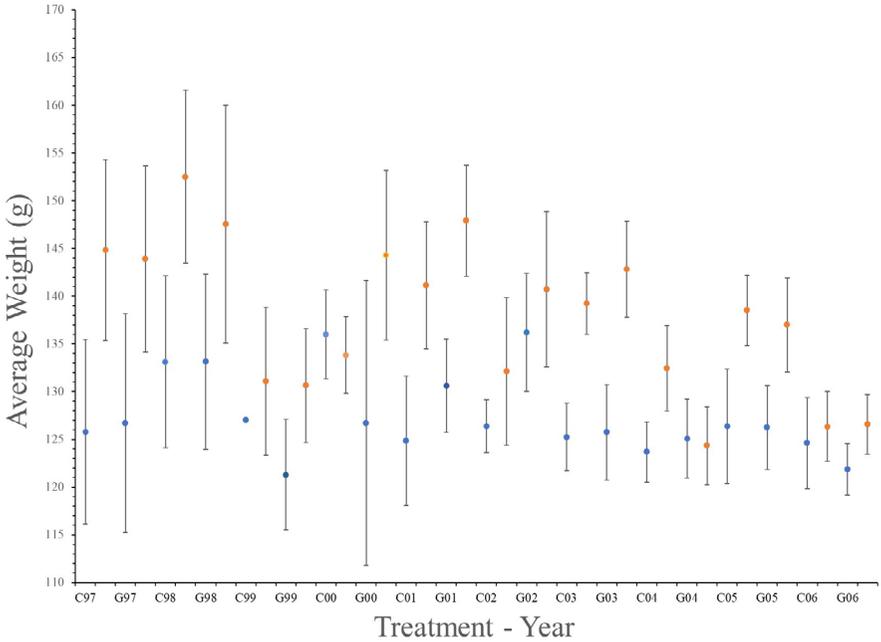
Of the 345 captures we made of adult females over the 10 summers of our study, we only found two females that we categorized as reproductive. In 1998 on a control plot, one female was lactating, and in 2002 on a grazed plot, one female was in estrous. In contrast, 16.1% of the 528 captures of males were scrotal (range, 0–66.7%; Table 2). The percentage of males that were reproductive differed significantly by year, but not by treatment type (Table 1). The number of young we caught on plots in the summer varied from one to 36 on control plots from 1997–2006 and from zero to 67 on grazed plots in those same years (Fig. 6). These differences in the number of young were significant by year but not by treatment type (Table 1). The number of young was significantly inversely correlated with RDM amounts ( $r = -0.567$ ;  $t = 2.753$ ,  $df = 16$ ,  $P = 0.014$ ).

Yearly survival of *A. nelsoni* was low in 1997 on both treatment types (0.056 on controls, 0.111 on grazed plots), reached a peak in 2000 (0.583 controls, 0.429 grazed), and then decreased into 2004 and 2005 (0.121 controls, 0.200 grazed), which coincided with a steady increase in population numbers from 2000 until 2005 on both treatment types (Fig. 7). Survival differed significantly by year, but not by sex, treatment, or the interaction of year $\times$ sex $\times$ treatment (Table 1). The majority of *A. nelsoni* (77.3%) we captured were found only once, but some squirrels were caught up to 3 y after first capture (minimum age of 4 y) and we caught one male and one female 4 y after first capture on control plots (Table 3), making them at least 5 y old at last capture. Longevity values based on recaptures were similar between males and females and between grazed and control plots (Table 3).

For *A. nelsoni* on grazed plots, the best models ( $\Delta AIC_c < 2$ ) to describe survivorship and recapture rates included survivorship by time and recapture rate by group (sex) and survivorship by group and recapture rate by time (Table 4). For *A. nelsoni* on control plots, the best models were survivorship by group times recapture rate by time and static survivorship times recapture rate by group (Table 4). Apparent static yearly survivorship for female (0.405) and male squirrels (0.368) on grazed plots did not differ significantly, nor did survivorship values for females (0.381) from males (0.317) on control plots, nor did these values differ significantly between grazed and control plots (Table 5). Yearly recapture rates also did not differ significantly between sexes within or between plot type (Table 5). Populations of male and female squirrels on both treatment types over the 10-year study were growing ( $\lambda > 1.0$ ) and did not differ significantly (Table 5).

**Table 1.** Results of General Linear Models tests of sex ratios, adult weights, percentage of adults reproductive, number of young captured, and yearly survival of San Joaquin antelope squirrel (*Ammospermophilus nelsoni*) in grazed and control (ungrazed) plots based on yearly trapping from 1997–2006 at the Lokern study site in the southern San Joaquin Desert of California, USA. Output includes degrees of freedom (df), adjusted sums of squares (Adj SS), adjusted mean squares (Adj MS), *F* value, and *P* value. Because of lack of reproductive sign of females, only males were tested in percentage of adults reproductive.

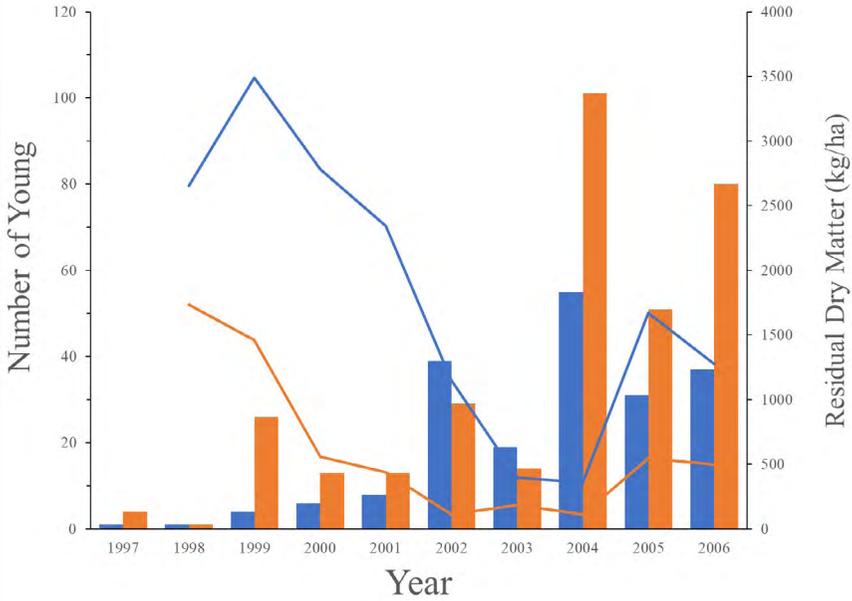
Source	df	Adj SS	Adj MS	<i>F</i>	<i>P</i>
<b>Sex Ratios</b>					
Year	9	14748.7	1638.75	17.33	< 0.001
Sex	1	0.0	0.03	0.00	0.987
Treatment	1	455.6	455.63	4.82	0.041
Year×Sex×Treatment	9	482.7	53.64	0.57	0.807
<b>Weights</b>					
RDM	1	339	338.9	2.11	0.147
Invertebrates	1	22	21.9	0.14	0.712
Year	8	16088	2010.9	12.52	< 0.001
Treatment	1	325	325.4	2.03	0.155
Sex	1	16707	16707.2	104.0	< 0.001
Year×Sex	8	5963	745.3	4.64	< 0.001
Treatment×Sex	1	28	27.8	0.17	0.678
Year×Treatment×Sex	8	2561	320.1	1.99	0.045
<b>Percentage Reproductive</b>					
Year	9	0.4215	0.0468	4.27	0.021
Treatment	1	0.0117	0.0117	1.06	0.329
<b>Number of Young</b>					
RDM	1	58.000	58.000	0.26	0.624
Year	8	7316.3	914.53	4.14	0.039
Treatment	1	492.40	492.38	2.23	0.179
<b>Yearly Survival</b>					
RDM	1	0.0209	0.0209	1.68	0.217
Invertebrates	1	0.0651	0.0651	5.24	0.039
Year	7	0.3509	0.0501	4.03	0.015
Sex	1	0.0480	0.0480	3.86	0.071
Treatment	1	0.0001	0.0001	0.00	0.948
Year×Sex×Treatment	7	0.0216	0.0031	0.25	0.964



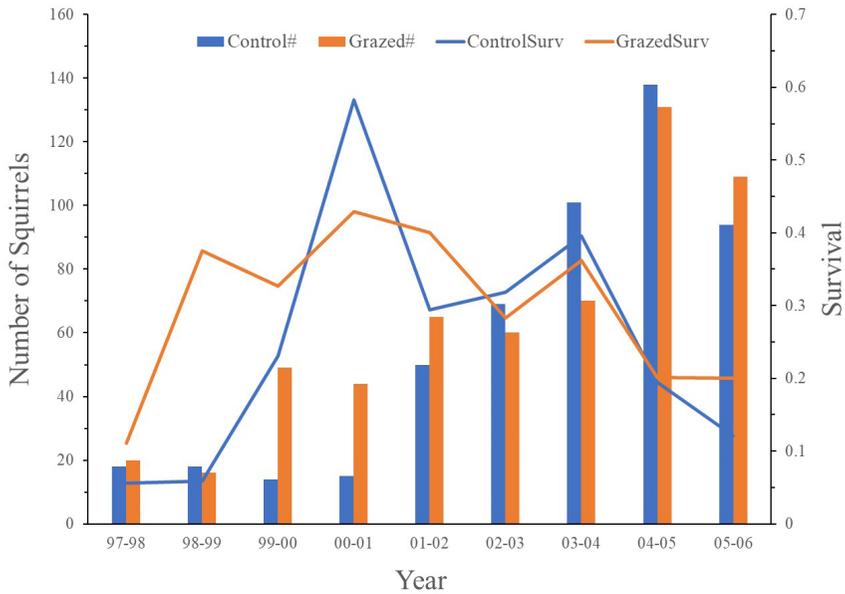
**Figure 5.** Weights of adult male (orange symbols) and female (blue symbols) San Joaquin antelope squirrels (*Ammospermophilus nelsoni*) in control (C) and grazed (G) plots from 1997–2006 at the Lokern study site in the southern San Joaquin Desert of California, USA. The symbols are the means and the vertical lines are the 95% confidence intervals.

**Table 2.** The percentage of male San Joaquin antelope squirrel (*Ammospermophilus nelsoni*) in grazed and control (ungrazed) plots that were scrotal from 1997–2006 at the Lokern study site in the southern San Joaquin Desert of California, USA.

Year	Control %Scrotal	Grazed %Scrotal
1997	18.2	20.0
1998	44.4	66.7
1999	33.3	0
2000	14.3	9.10
2001	7.10	16.1
2002	0	0
2003	9.4	3.0
2004	20.5	10.4
2005	35.0	13.7
2006	0	1.70



**Figure 6.** The number of young San Joaquin antelope squirrels (*Ammospermophilus nelsoni*) in control (blue bars) and grazed (orange bars) plots and residual dry matter (RDM) from 1997–2006 at the Lokern study site in the southern San Joaquin Desert of California, USA. RDM is shown as blue lines for controls and orange lines for grazed plots.



**Figure 7.** The number of all San Joaquin antelope squirrels (*Ammospermophilus nelsoni*) in control (blue bars) and grazed (orange bars) plots and yearly survival from 1997–2006 at the Lokern study site in the southern San Joaquin Desert of California, USA. Yearly survival is shown as blue lines for controls and orange lines for grazed plots.

**Table 3.** Longevity (number of individuals caught and proportion of captures below) of San Joaquin antelope squirrel (*Ammospermophilus nelsoni*) in grazed and control (ungrazed) plots based on yearly trapping from 1997–2006 at the Lokern study site in the southern San Joaquin Desert of California, USA. Minimum age is the number of years a squirrel was found on a plot.

Treatment	Sex	Minimum age (years)				
		1	2	3	4	5
Control	Females	120	31	16	1	1
	Proportion	0.710	0.183	0.095	< 0.001	< 0.001
	Males	168	28	10	0	1
	Proportion	0.812	0.135	0.048	0	< 0.001
Grazed	Females	153	35	20	7	0
	Proportion	0.712	0.163	0.093	0.033	0
	Males	172	36	11	3	0
	Proportion	0.775	0.162	0.050	0.014	0

**Table 4.** Cormack-Jolly-Seber model set (first 5 of 16 for each plot type) analyzing the effects of group (female, male) and time on apparent survivorship ( $\Phi$ ) and recapture rates ( $p$ ) of San Joaquin antelope squirrels (*Ammospermophilus nelsoni*) caught in grazed and control (ungrazed) plots from 1997 to 2006 at the Lokern study site in the San Joaquin Desert of California. Abbreviations are  $t$  = time,  $g$  = group,  $(.)$  = static value,  $AIC_c$  = Akaike Information Criterion,  $\Delta AIC_c$  = difference between model  $AIC_c$  and lowest  $AIC_c$  in the model set,  $\omega$  = Akaike model weight,  $k$  = number of estimable parameters, and Deviance = measure of model fit.

Model	$AIC_c$	$\Delta AIC_c$	$\omega$	$k$	Deviance
Grazed					
$\Phi(t) p(g)$	750.7443	0	0.48578	11	79.9346
$\Phi(g) p(t)$	752.2334	1.4891	0.23072	11	81.4236
$\Phi(t) p(.)$	753.5748	2.8305	0.11798	10	84.8450
$\Phi(g \times t) p(.)$	755.1651	4.4208	0.05327	19	67.4416
$\Phi(.) p(t)$	755.7443	5	0.03988	10	87.0145
Control					
$\Phi(g) p(t)$	545.6777	0	0.65998	11	59.6404
$\Phi(.) p(t)$	547.1749	1.4972	0.31219	10	63.2324
$\Phi(t) p(g)$	553.3156	7.6379	0.01449	11	67.2784
$\Phi(t) p(t)$	555.1468	9.4691	0.00580	17	56.3506
$\Phi(t) p(.)$	555.4574	9.7797	0.00496	10	71.5149

## DISCUSSION

Over a 10-year study in the Lokern Natural Area in the southern part of the range of *A. nelsoni*, we found that males and females occurred in equal numbers, adult males weighed about 10 g on average more than adult females, and females were not in reproductive condition in the summer, although some males were. Based on live-trapping over a 10-year period (1947–1956), Hawbecker (1958) also reported that the sex ratio for *A. nelsoni* was even but did not provide numbers. Interestingly, sexual dimorphism with males being slightly larger than females has been reported for *A. nelsoni* (Best et al. 1990c) and for a congener, the white-tailed antelope squirrel (*A. leucurus*; Belk and Smith 1991), but not for two other congeners, Texas antelope squirrels (*A. interpres*; Best et al. 1990b) and Harris' antelope squirrels (*A. harrisi*; Best et al. 1990a). The dimorphism reported in *A. nelsoni* was based on standard external morphological measurements. Our data constitute the first quantification of sexual dimorphism in mass for *A. nelsoni*.

We also found evidence of a second litter being produced based on the number of young-of-the-year squirrels (< 110 g) we found July–August on our grids, with numbers of second litter young steadily increasing over the course of the 10 years. First litters, and sometimes the only litter, are produced in March and young come above ground about the first week in April (Hawbecker 1958). Hawbecker (1958) stated that there is only one breeding season, which coincides with the one period of the year when green vegetation is present. We are quite sure that the small squirrels we found in August represent another litter. Even if some of the larger young (95–100 g) were born in March, we captured a number of young squirrels weighing < 95 g in August, some as small as 75 g. By 1 June, most *A. nelsoni* captured on the Elkhorn Plain, southwest of our study site, weighed 95–115 g, and by late August, the lightest squirrels weighed > 100 g (Williams et al., unpubl. report). In southern Arizona, Neal (1965) found that for *A. harrisi*, at 3 mo of age it is difficult under field conditions to distinguish young-of-the-year from adult. In *A. leucurus*, one relatively large litter is typically produced per year (Kenagy 1981; Kenagy and Bartholomew 1985), but Bailey (1931), working in New Mexico, found what he termed half-grown young *A. leucurus* in late August, and he suggested that a second litter is sometimes produced. Similarly, based on embryo counts, Davis (1978) found evidence that a second litter is produced in *A. interpres*.

**Table 5.** Apparent static yearly survivorship ( $\Phi$ ) and recapture rate ( $p$ ) based on model averaging, and lambda ( $\lambda$ ), including upper and lower 95% confidence intervals in parentheses for female and male San Joaquin antelope squirrels (*Ammospermophilus nelsoni*) caught in grazed and control (ungrazed) plots from 1997 to 2006 in the San Joaquin Desert of California. Comparisons within life-history traits (down a column) did not differ significantly (based on means not intersecting confidence intervals) among any comparisons.

Group/Sex	$\Phi$	$p$	$\lambda$
Grazed Female	0.405 (0.198, 0.698)	0.689 (0.343, 0.908)	1.24 (1.19, 1.30)
Grazed Male	0.368 (0.171, 0.668)	0.579 (0.255, 0.851)	1.24 (1.19, 1.30)
Control Female	0.381 (0.231, 0.588)	0.624 (0.411, 0.946)	1.21 (1.15, 1.28)
Control Male	0.317 (0.180, 0.548)	0.620 (0.399, 0.953)	1.21 (1.16, 1.27)

Annual survivorship over the 10-year study varied from 0.317 to 0.405 depending on the sex and treatment plot, but there were no significant differences among survivorship estimates. Despite these relatively low rates of survivorship overall, this was high enough to estimate lambda values well over 1.0 on both grazed and control plots, indicating an increasing population for males and females on either treatment type. Based on squirrels being recaptured on a plot, we found that some squirrels can live 3–4 years (up to 5 years), although we only captured most squirrels either once or twice, which may indicate a typical life span of 1–2 years. Based on periodic live-trapping from 1947 to 1956 at a site 40 km west of Fresno, California, Hawbecker (1958) stated that 80% of *A. nelsoni* do not survive from one year to the next, but he found 12 individuals that lived > 1 y, and 3 that lived to almost 6 years. Using static survivorship estimates (data integrated over all 10 years of our study; Table 5), longevity can be estimated based on yearly survivorship of squirrels (above). At the low end of longevity, only 10.8% of squirrels would live to 3 years and 3.2% to 4 years. At the high end, 16.4% live to 3 years, 6.6% live to 4 years, and 2.7% live to 5 years. These estimates are not very different from longevity estimated by recaptures on plots.

We did not find any consistent treatment effect on the traits that we studied in *A. nelsoni*, which we think is in part due to the high variability in weather over the 10-year study. We had high levels of RDM early in our study, grazing lowered this on treatments, and the area dried out from 2000–2004, which lowered the grass cover on the control plots despite no cattle grazing (Germano et al. 2012). High rainfall in 2005 and 2006 greatly increased herbaceous cover and grazing kept cover levels much lower on treatment plots and led to many more *A. nelsoni* on these plots (Germano et al. 2012). Although we also found many more young squirrels on grazed plots than on control plots, which added to the overall significantly higher numbers of all *A. nelsoni* on grazed plots (Germano et al. 2012), these higher numbers of young were not significantly greater than the number on control plots. Although we did not find a statistical effect of treatment on the number of young, numbers of young were negatively correlated with RDM, indicating that high levels of herbaceous cover are detrimental to this age group. Although we do not have evidence for this, it is possible that lower herbaceous cover allows both adult and young squirrels earlier detection of predators. This could lead to faster entrance into escape cover.

Our study of *A. nelsoni* population and life-history traits is only the second long-term data set for this species and compliments the pioneering work of Albert Hawbecker in the 1940s and 1950s. Like Hawbecker, we also have calculated home range sizes for male and female *A. nelsoni* (Germano et al. 2021). Unlike Hawbecker, though, our data are much more rigorous because we replicated our study design and therefore were able to statistically analyze trait values and trends, and our home range estimates were based on radio telemetry and not recaptures in traps. Combined, these data sets give important information about the biology of this threatened desert squirrel, which can be used to recover the species in the future.

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FULL RESEARCH ARTICLE

## **Conservation of threatened San Joaquin antelope squirrels: distribution surveys, habitat suitability, and conservation recommendations**

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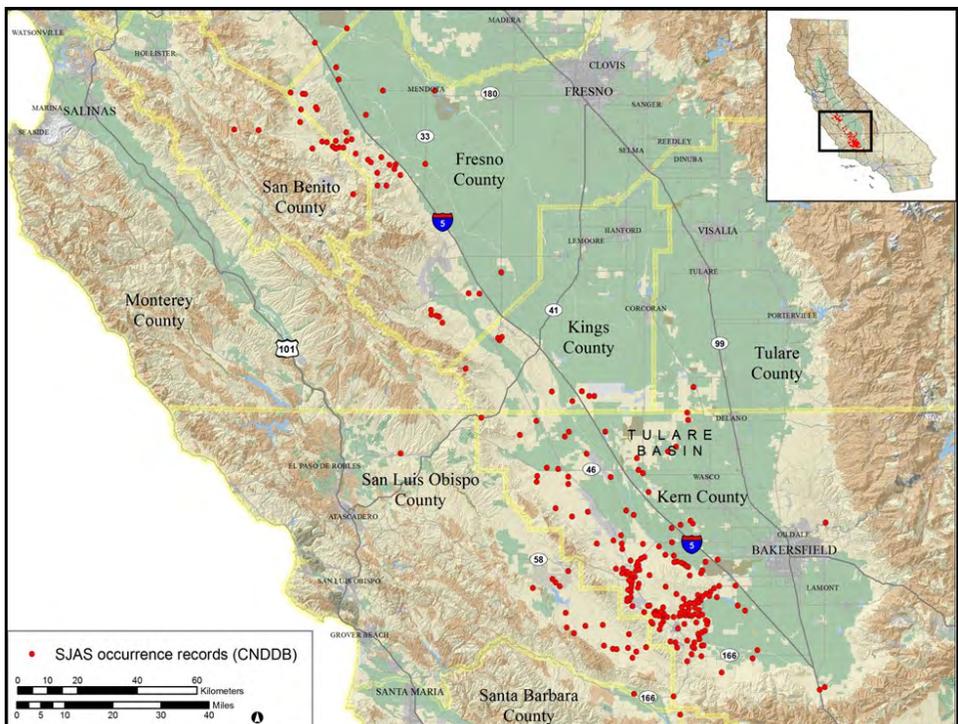
The San Joaquin antelope squirrel (*Ammospermophilus nelsoni*: SJAS) is listed as Threatened pursuant to the California Endangered Species Act due to profound habitat loss throughout its range in the San Joaquin Desert in California. Habitat loss is still occurring and critical needs for SJAS include identifying occupied sites, quantifying optimal habitat conditions, and conserving habitat. Our objectives were to (1) conduct surveys to identify sites where SJAS were present, (2) assess habitat attributes on all survey sites, (3) generate a GIS-based model of SJAS habitat suitability, (4) use the model to determine the quantity and quality of remaining habitat, and (5) use these results to develop conservation recommendations. SJAS were detected on 160 of the 326 sites we surveyed using automated camera stations. Sites with SJAS typically were in arid upland shrub scrub communities where desert saltbush (*Atriplex polycarpa*) or jointfir (*Ephedra californica*) were the dominant shrubs, although shrubs need not be present for SJAS to be present. Sites with SJAS usually had relatively sparse ground cover with >10% bare ground and Arabian grass (*Schismus arabicus*) was the dominant grass. SJAS were more likely to occur on sites where kangaroo rats (*Dipodomys spp.*) were present and burrow abundance was greater, but SJAS were less likely to be present on sites with California ground squirrels (*Otospermophilus beecheyi*). Based on our habitat suitability model, an estimated 5,931 km<sup>2</sup> of high or moderately high quality habitat and 4,753 km<sup>2</sup> of lower quality habitat remain. To conserve SJAS, we recommend (1) conducting additional SJAS surveys on sites not surveyed but with suitable habitat, (2) conserving unprotected lands with suitable habitat, (3) managing

vegetation on occupied sites if necessary, (4) restoring disturbed lands to increase suitability for SJAS, and (5) conducting translocations of SJAS to unoccupied sites with suitable habitat.

**Key words:** *Ammospermophilus nelsoni*, conservation, distribution, habitat suitability, San Joaquin antelope squirrel, San Joaquin Valley, threatened

The San Joaquin antelope squirrel (*Ammospermophilus nelsoni*: SJAS) is a small ground squirrel endemic to the San Joaquin Desert in central California (USFWS 1998; Germano et al. 2011). This species once was widely distributed in arid shrubland and grassland habitats in the western and southern portions of the San Joaquin Valley from western Merced County south to Kern County and also in the Carrizo Plain and Cuyama Valley (Fig. 1). Much of the habitat in this region has been converted to agricultural, urban, and industrial uses. Due to this profound habitat loss, the SJAS was state listed as Threatened by the California Fish and Game Commission in 1980 (USFWS 1998).

Adult SJAS weigh 130–170 g (USFWS 1998) and are considerably smaller than the ubiquitous and more familiar California ground squirrel (*Otospermophilus beecheyi*). Typical of ground squirrels, SJAS are diurnal and omnivorous (Best et al. 1990). They consume a variety of seeds, green vegetation, and a diversity of invertebrates (Hawbecker



**Figure 1.** California Natural Diversity Data Base occurrence records for the San Joaquin antelope squirrel (SJAS) in central California, USA.

1947; Harris 2019). Although they can excavate their own burrows, they readily use those of other species, particularly kangaroo rats (*Dipodomys spp.*; Hawbecker 1975; Harris and Stearns 1991). SJAS live in small familial colonies and home ranges are approximately 4 ha in size (Best et al. 1990). Reproduction occurs during late winter and early spring, and young (usually 6–11) are born between March and April (Best et al. 1990). Most SJAS live less than 1 year (Hawbecker 1975).

Kelly et al. (2005) estimated that by 2000, the area of grasslands and shrublands, the two types of habitat in which SJAS are primarily found, had been reduced by 65.1% and 63.7%, respectively, relative to pre-European settlement in the San Joaquin Valley. Habitat loss, fragmentation, and degradation are still occurring, and this continuing loss threatens to isolate and extirpate remaining populations. The distribution of SJAS has not been assessed since the 1980s. Also, optimal habitat conditions for this species are not well known. Additionally, the effects of competitors such as California ground squirrels are poorly understood (Harris and Stearns 1991; USFWS 1998).

We conducted surveys for SJAS at selected sites throughout their historic range. At each survey location, we quantified a suite of ecological attributes and correlated these with the presence of SJAS. This information was used to define preferred habitat conditions for SJAS and to prepare a habitat suitability model for the species. Finally, based on our results, we developed recommendations for conserving SJAS throughout their range.

## METHODS

### Study Area

This project was conducted throughout the historic range of SJAS (Fig. 1). The habitats in which work was conducted included annual grasslands, saltbush scrub, alkali sink scrub, and ephedra scrub (USFWS 1998), all of which are within the region known as the San Joaquin Desert (Germano et al. 2011). The regional climate is Mediterranean in nature, and is characterized by hot, dry summers, and cool, wet winters with frequent fog. Based on data from Buttonwillow, CA, mean maximum and minimum temperatures are 36.9°C and 18.5°C in July, and 14.0°C and 1.4°C in December. Annual precipitation averages 14.3 cm and occurs primarily as rain falling between October and April (WRCC 2020). Topography is diverse within the range of SJAS and varies from flat valley bottoms to steep-sloped mountain ranges with elevations ranging from ca. 100 m to 1,200 m. Loss of natural habitat within the historic range of SJAS has been profound due to agricultural and urban development. Extensive areas of remaining habitat are subject to disturbances including hydrocarbon (oil, natural gas) extraction, off-road vehicle use, and cattle grazing (USFWS 1998; Kelly et al. 2005).

### Surveys

We used automated camera stations to determine whether SJAS were present at a given site. We used Cuddeback (E3 Black Flash Trail Cameras; Non Typical, Green Bay, WI), Bushnell (models 119455, HD 119437, and HD 119477; Bushnell Outdoor Products, Overland Park, KS), and Reconyx (PC800 HyperFire Professional IR and Reconyx PC900 HyperFire Professional IR; Reconyx, Holmen, WI) field cameras. The cameras use an infrared sensor to detect movement and collect images at 5–20-megapixel resolution. At

each station, a 1-m t-post was hammered into the ground, and the camera was mounted on the post using a bracket and zipties. To attract squirrels to the camera stations, we placed an approximately 1-kg piece of Premium Wild Bird Block or Flock Block (Purina, Gray Summit, MO) about 2 m in front of each camera. The block consisted of a mixture of grains, seeds, molasses, and other ingredients pressed into a solid block. At some sites, we caged the block in chicken wire and staked it to the ground to prevent removal by other species, such as coyotes (*Canis latrans*) or cattle.

We conducted surveys primarily on public lands administered by the U.S. Bureau of Land Management (BLM) and the California Department of Fish and Wildlife (CDFW), and on conservation lands administered by the Center for Natural Lands Management (CNLM) and The Wildlands Conservancy. For a few locations, we received permission to establish stations on private lands. Up to 20 camera stations were established at a time, depending on the amount of habitat available. We spaced stations at least 350 m (ca. 0.25 mi) apart. This is the approximate diameter of a SJAS home range based on an estimated average home range size of 10 ha reported by Harris and Stearns (1991). This spacing substantially reduced the potential for detecting a given individual at more than one station. SJAS are semi-colonial and therefore are unevenly distributed even in suitable habitat (Grinnell and Dixon 1918; Hawbecker 1953; Best et al. 1990). Therefore, multiple stations were established in most areas, frequently as a long transect through a region.

Our goal was to operate stations for at least 7 days at each location. Images collected by each camera were carefully examined to determine whether stations had been visited by SJAS. Detections of other species were recorded as well, particularly visits by California ground squirrels. Also, we noted the day of first detection for SJAS for each station.

### Habitat Attributes

At each site where we established a camera station to survey for SJAS, we recorded a suite of habitat attributes (Table 1). We recorded information on shrubs, ground cover, topography, anthropogenic disturbances, kangaroo rat activity, California ground squirrel presence, and small mammal burrow (entrances  $\geq 5$  cm) abundance. Much of this information was qualitative so that a relatively large area (several hectares) could be characterized quickly (ca. 15 min). At each station, observations of kangaroo rat sign and of California ground squirrels and their sign were supplemented with detections of these species on the camera from that station.

We compared the proportional occurrence of each of the habitat attributes between stations with and without SJAS detections using contingency table analysis. For 2x2 analyses, a continuity correction was applied (Zar 1984). Some variables had more than two levels (e.g., shrub density, topography). For these variables, if the contingency table analysis indicated a significant difference in proportions, levels were compared pair-wise to assess which levels were different. A Cramer's V value and associated significance level were calculated along with each chi-square test to assess the strength of the association between the presence of SJAS and the presence of each habitat attribute. Cramer's V values range from 0 to 1 with "0" indicating no association and "1" indicating a strong association. We conducted statistical tests using IBM SPSS Statistics, Version 24 (IBM, Armonk, NY). We used an  $\alpha$  level of 0.1 as is increasingly common in ecological field studies to identify compelling trends that warrant further investigation (Gotelli and Ellison 2013).

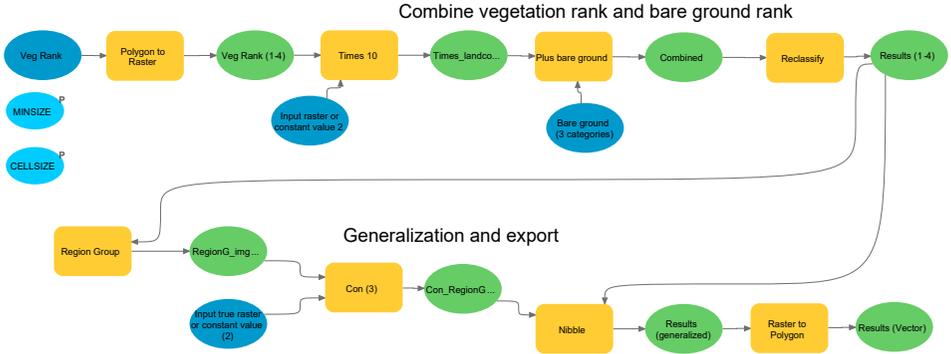
**Table 1.** Habitat attributes assessed on sites surveyed for San Joaquin antelope squirrels in the San Joaquin Desert, California, USA.

Attribute	Measure
Shrubs	Present/absent
Estimated shrub density if present	Dense: < 2 m apart Medium: 2–10 m apart Sparse: > 10 m apart
Shrub species	List of species present
Estimated ground cover density	Dense: < 10% bare ground Medium: 10–30% bare ground Sparse: > 30% bare ground
Ground cover species	List of species with > 10% cover
Alkali scalds	Present/absent
Topography	Generally flat Gentle slopes (< 10%) Steep slopes (> 10%) Wash within 100 m
Anthropogenic disturbance	Present/absent (e.g., oil field or OHV activity)
Kangaroo rat activity	Present/absent (based on burrows and scats)
California ground squirrels	Present/absent (based on squirrel observations and burrows)
Abundance of burrows (entrances $\geq$ 5 cm)	Low: 0–2 burrows visible Medium: 3–5 burrows visible High: 6 or more burrows visible

## Habitat Suitability Modeling

We produced a habitat suitability model for SJAS that incorporated results from the attribute analyses, particularly the dominant vegetation communities and the estimated ground cover density. For the model boundary, we used the southern portion of the San Joaquin Valley Recovery Planning area from the *Recovery Plan for Upland Species of the San Joaquin Valley* (Fig. 2 in USFWS 1998). For vegetation communities, we used a detailed vegetation layer from the CDFW Vegetation Classification and Mapping Program (VegCAMP) where available (CDFW 2010, 2015; CNPS 2013; California State University, Chico, Geographical Information Center 2016). Where VegCAMP data were not available, we used vegetation data derived from California Gap Analysis Project supplemented with newer land use data (University of California Santa Barbara Biogeography Lab 1998; CDOC 2014; California Council on Science and Technology 2015).

Using the most detailed vegetation classification available for a given location, we ranked upland vegetation communities from 1–4 (1 = best quality) based on habitat attribute data collected during the field surveys (Table 2). We tried to match habitat attributes on sites with SJAS to the descriptions of the vegetation classifications. We found that one vegetation classification (*Southwestern North American salt basin and high marsh*) was overly broad



**Figure 2.** GIS model for combining vegetation and percent bare ground rankings to assess habitat suitability for the San Joaquin antelope squirrel.

**Table 2.** Vegetation classification rankings used to model habitat suitability for San Joaquin antelope squirrels. Classification levels are unique to the sources cited in the footnote; each is essentially a vegetation community or habitat type.

Rank	Vegetation classification <sup>1</sup>	Classification level
1	<i>Atriplex polycarpa</i>	Alliance
	<i>Atriplex spinifera</i>	Alliance
	Chenopod scrubs	Supplemental data
	<i>Ephedra californica</i>	Alliance
	<i>Gutierrezia californica</i>	Provisional Alliance
	<i>Lycium andersonii</i>	Alliance
	<i>Monolopia (lanceolata)-Coreopsis (calliopsidea)</i>	Provisional Alliance
	North American Warm Semi-Desert Cliff, Scree, and Other Rock Vegetation	Macrogroup
	Southwestern North American salt basin and high marsh/Desert Scrub	Group/soil
	Xeromorphic Scrub and Herb Vegetation (Semi-Desert)	Class
2	<i>Ambrosia salsola</i>	Alliance
	<i>Amsinckia (menziesii, tessellata)</i>	Alliance
	<i>Atriplex canescens</i>	Alliance
	<i>Atriplex lentiformis</i>	Alliance
	<i>Atriplex vallicola - Lasthenia ferrisiae – Lepidium jaredii</i>	Provisional Association
	Barren	Supplemental data
	California Annual and Perennial Grassland	Macrogroup
	California annual forb/grass vegetation	Group
	<i>Centaurea (virgata)</i>	Provisional Semi-Natural Alliance
	Coastal scrubs	Supplemental data

Table 2 continued

Rank	Vegetation classification <sup>1</sup>	Classification level
2	<i>Encelia (actoni, virginensis)</i>	Alliance
	<i>Ephedra viridis</i>	Alliance
	<i>Ericameria linearifolia - Isomeris arborea</i>	Provisional Alliance
	<i>Ericameria linearifolia - Peritoma arborea</i>	Provisional Alliance
	<i>Ericameria nauseosa</i>	Alliance
	<i>Isocoma acradenia</i>	Provisional Alliance
	<i>Krascheninnikovia lanata</i>	Alliance
	<i>Lasthenia californica - Plantago erecta - Vulpia microstachys</i>	Alliance
	<i>Lepidospartum squamatum</i>	Alliance
	<i>Lupinus albifrons</i>	Alliance
	<i>Poa secunda</i>	Alliance
	Southwestern North American salt basin and high marsh/Grassland	Group/soil
	subshrub scrubs	Supplemental data
Valley and foothill grasslands	Supplemental data	
3	<i>Allenrolfea occidentalis</i>	Alliance
	<i>Corethrogyne filaginifolia</i>	Provisional Alliance
	<i>Eriogonum (elongatum, nudum)</i>	Provisional Alliance
	<i>Eriogonum fasciculatum</i>	Alliance
	Great Basin scrubs	Supplemental data
	Interior dunes	Supplemental data
	Mediterranean California naturalized annual and perennial grassland	Group
	<i>Nassella cernua</i>	Provisional Alliance
	Riverine, Barren	-
	<i>Salvia carduacea</i>	Provisional Alliance
	<i>Salvia leucophylla</i>	Alliance
	<i>Salvia mellifera</i>	Alliance
	Southwestern North American salt basin and high marsh/Alkali sink	Group/soil
	<i>Suaeda moquinii</i>	Alliance
	4	<i>Arctostaphylos glauca</i>
<i>Artemisia californica</i>		Alliance
<i>Artemisia californica - Eriogonum fasciculatum</i>		Alliance
<i>Artemisia tridentata</i>		Alliance
<i>Baccharis pilularis</i>		Alliance
Californian mixed annual/perennial freshwater vernal pool/swale/plain bottomland		Group
Central and south coastal California seral scrub		Group
Central and South Coastal Californian coastal sage scrub		Group
<i>Cercocarpus montanus</i>		Alliance

**Table 2** continued

Rank	Vegetation classification <sup>1</sup>	Classification level
4	Chaparral	Supplemental data
	<i>Elymus glaucus</i>	Alliance
	<i>Frangula californica</i>	Alliance
	<i>Prunus fasciculata</i>	Alliance
	<i>Quercus john-tuckeri</i>	Alliance
	<i>Ribes quercetorum</i>	Provisional Alliance

<sup>1</sup> Vegetation classifications based on CDFW 2010, 2015; CNPS 2013; California State University, Chico, Geographical Information Center 2016; U.C. Santa Barbara Biogeography Lab 1998; California Department of Conservation, Farmland Mapping and Monitoring Program 2014; California Council on Science and Technology 2015.

and included vegetation alliances that should be ranked differently. (An alliance is a category of vegetation classification which describes repeating patterns of plants across a landscape [CNPS 2013]). To solve this problem, we used a supplemental layer of historical vegetation based on reconnaissance-level soil surveys (Fig. 3 in Phillips and Cypher 2019) to identify which locations were generally in areas of *Valley saltbush scrub* (Rank = 1), *Grasslands* (Rank = 2), or other upland communities such as *Alkali Sink* (Rank = 3). In Table 2, these divisions are identified as Classification level = *Group/soil*.

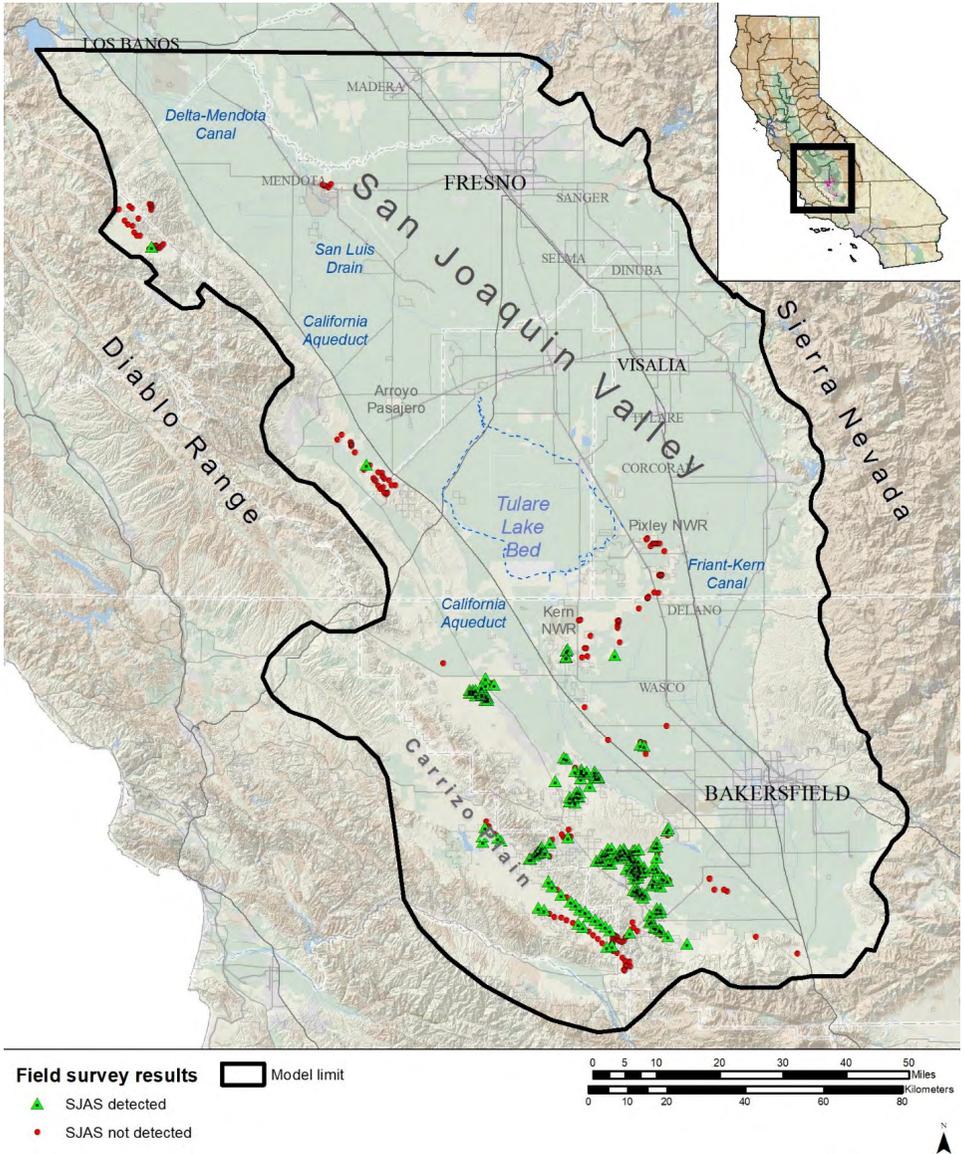
For percentage of bare ground, we used a GIS layer derived from satellite imagery (USGS 2013). Based on the field surveys we grouped percentage of bare ground into three categories: 1 = > 30% bare ground, 2 = 10-30% bare ground, 3 = < 10% bare ground. We used GIS software (ArcGIS Pro *ModelBuilder*) to create a sequence of steps (Fig. 2) to combine the vegetation rankings with the three categories of bare ground. We then organized these into four categories of habitat quality (Table 3) from best (Rank 1) to worst (Rank 4). Data in the model were represented as a grid (or raster) of cells that were 90 x 90 m. To reduce small patches or thin, linear features in the output, we replaced cells that were in groupings of < 50 cells (40 ha) with the value of cells in neighboring, larger patches. This smoothing procedure provided a more meaningful representation of the data by eliminating fragments too small to influence SJAS presence.

## RESULTS

### Surveys

We established camera stations at 326 locations to determine if SJAS were present. The surveys were conducted from 13 December 2017 to 28 May 2019. The majority of the locations were in western Kern County and eastern San Luis Obispo County (Fig. 3). Additionally, there were a few stations (< 20) in each of southeastern Tulare County, western Kings County, western Fresno County, and eastern San Benito County. The mean number of days that stations were operational was 9.0 d (SE = 0.16, range 3–30) with a mode of 8 d. SJAS were detected at 160 locations (Fig. 3). Mean latency to first SJAS detection was 2.6 d (SE = 0.17, range 1–14) with a mode of 1 d.

SJAS were frequently detected at the stations in San Luis Obispo County and Kern County. Indeed, all but 2 of the stations with SJAS detections were in these two counties with one detection being recorded each in southwestern Fresno County and eastern San Benito County.



**Figure 3.** Results from camera stations (n = 326) established to survey for San Joaquin antelope squirrels (SJAS) in the San Joaquin Valley, California, USA.

**Table 3.** Habitat quality categories for San Joaquin antelope squirrels that combine vegetation rankings with categories of percentage of bare ground.

Habitat quality rank	Attributes
1 (highest quality)	Vegetation rank 1 <sup>1</sup> , > 30% bare ground
2 (moderately high quality)	Vegetation rank 1, 10–30% bare ground Vegetation rank 2, > 30% bare ground
3 (moderately low quality)	Vegetation rank 1, < 10% bare ground Vegetation rank 2, 10–30% bare ground
4 (low quality)	All other upland vegetation

<sup>1</sup>Vegetation ranks from Table 2.

### Habitat Attributes

Habitat attribute data were collected at 319 locations surveyed for SJAS (Table 4). SJAS were not associated with shrubs in general or with shrub density, but when shrubs were present SJAS were associated with specific species. SJAS presence was strongly associated with small-leaved saltbushes, which primarily were desert saltbush (*Atriplex polycarpa*) but occasionally included spiny saltbush (*A. spinifera*). When saltbush was present, it usually was the dominant shrub. Other species observed where SJAS were detected included joint-fir (*Ephedra californica*) and matchweed (*Gutierrezia californica*). SJAS were negatively associated with iodine bush (*Allenrolfea occidentalis*), sinkweed (*Sueada* spp.), and alkali goldenbush (*Isocoma acradenia*).

Areas with SJAS were more likely to have sparse to medium ground cover (>10% bare ground) while areas without SJAS were more likely to have dense ground cover (0–10% bare ground) (Table 4). Arabian grass (*Schismus arabicus*) was present more frequently at locations where SJAS were detected compared to locations where SJAS were not detected, and when present at sites where SJAS were detected it tended to be a dominant species. Conversely, wild barley (*Hordeum* spp.) was present more frequently at locations where SJAS were not detected compared to locations where SJAS were present. The presence of red brome (*Bromus madritensis* ssp. *rubens*), amsinckia (*Amsinckia* spp.), and red-stemmed filaree (*Erodium cicutarium*) was similar between sites with and without SJAS.

Topography did not appear to influence the presence of SJAS. Sites with and without SJAS had similar proportions of flat, rolling, gentle slope ( $\leq 10\%$ ), and steep slope ( $> 10\%$ ) terrain (Table 4). Presence of washes also was similar between sites with and without SJAS. However, alkali scalds were less likely to be present on sites where SJAS were detected. Presence of habitat disturbances (e.g., oil field activities, off-road vehicle use) was similar between sites with and without SJAS. Presence of grazing also was similar, but when grazing was present on sites where SJAS were detected, it was much more likely to be by sheep than by cows.

Finally, kangaroo rats were more likely to be present on sites where SJAS were detected (Table 4). Also, burrows sufficiently large to permit entry by kangaroo rats and SJAS were more abundant on sites where SJAS were detected. Lastly, California ground squirrels were not present on most of the sites surveyed, but when they were present, SJAS were detected less frequently.

**Table 4.** Habitat attributes on sites with and without San Joaquin antelope squirrel (SJAS) detections during surveys conducted in the San Joaquin Valley, CA. Chi-square tests assessed attribute equality between sites with and without SJAS and Cramer's coefficient assessed the strength of the association.

Attribute	Sites w/ SJAS (n = 158)	Sites w/o SJAS (n = 161)	Chi-square test and Cramer's coefficient
Shrubs	Present: 114 (72.2%) Absent: 44 (27.8%)	Present: 112 (69.6%) Absent: 49 (30.4%)	$\chi^2 = 0.15$ , 1 df, $p = 0.70$ $C = 0.028$ , $p = 0.61$
Shrub density	Dense: 27 (17.1%) Medium: 67 (42.4%) Sparse: 64 (40.5%)	Dense: 17 (10.6%) Medium: 62 (38.5%) Sparse: 82 (50.9%)	$\chi^2 = 4.66$ , 2 df, $p = 0.10$ $C = 0.121$ , $p = 0.10$
Iodine bush	Dominant: 1 (0.6%) Not dominant: 2 (1.3%) Absent: 155 (98.1%)	Dominant: 9 (5.6%) Not dominant: 7 (4.3%) Absent: 145 (90.1%)	$\chi^2 = 9.48$ , 2 df, $p < 0.01$ $C = 0.172$ , $p < 0.01$
	Present: 3 (1.9%) Absent: 155 (98.1%)	Present: 16 (9.9%) Absent: 145 (90.1%)	$\chi^2 = 7.82$ , 1 df, $p < 0.01$ $C = 0.170$ , $p < 0.01$
Sinkweed	Dominant: 4 (2.5%) Not dominant: 6 (3.8%) Absent: 148 (93.7%)	Dominant: 10 (6.2%) Not dominant: 14 (8.7%) Absent: 137 (85.1%)	$\chi^2 = 6.17$ , 2 df, $p = 0.05$ $C = 0.139$ , $p = 0.05$
	Present: 10 (6.3%) Absent: 148 (93.7%)	Present: 24 (14.9%) Absent: 137 (85.1%)	$\chi^2 = 5.29$ , 1 df, $p = 0.02$ $C = 0.139$ , $p = 0.01$
Saltbush	Dominant: 87 (55.1%) Not dominant: 16 (10.1%) Absent: 55 (34.8%)	Dominant: 44 (27.3%) Not dominant: 11 (6.8%) Absent: 106 (65.8%)	$\chi^2 = 31.17$ , 2 df, $p < 0.01$ $C = 0.313$ , $p < 0.01$
	Present: 103 (65.2%) Absent: 55 (34.8%)	Present: 55 (34.2%) Absent: 106 (65.8%)	$\chi^2 = 29.48$ , 1 df, $p < 0.01$ $C = 0.310$ , $p < 0.01$
Goldenbush	Present: 5 (3.2%) Absent: 153 (96.8%)	Present: 21 (13.0%) Absent: 140 (87.0%)	$\chi^2 = 9.12$ , 1 df, $p < 0.01$ $C = 0.181$ , $p < 0.01$
Ground cover density	Dense: 15 (9.5%) Medium: 77 (48.7%) Sparse: 66 (41.8%)	Dense: 38 (23.6%) Medium: 66 (41.0%) Sparse: 57 (35.4%)	$\chi^2 = 11.46$ , 2 df, $p < 0.01$ $C = 0.190$ , $p < 0.01$
	Dense: 15 (9.5%) Med-Sparse: 143 (90.5%)	Dense: 38 (23.6%) Med-Sparse: 123 (76.4%)	$\chi^2 = 10.46$ , 1 df, $p < 0.01$ $C = 0.190$ , $p < 0.01$
Brome	Dominant: 80 (50.6%) Not dominant: 32 (20.3%) Absent: 46 (29.1%)	Dominant: 69 (42.9%) Not dominant: 35 (21.7%) Absent: 57 (35.4%)	$\chi^2 = 2.09$ , 2 df, $p = 0.35$ $C = 0.081$ , $p = 0.35$
Arabian grass	Dominant: 54 (34.2%) Not dominant: 45 (28.5%) Absent: 59 (37.3%)	Dominant: 10 (6.2%) Not dominant: 28 (17.4%) Absent: 123 (76.4%)	$\chi^2 = 56.69$ , 2 df, $p < 0.01$ $C = 0.422$ , $p < 0.01$
	Present: 99 (62.7%) Absent: 59 (37.3%)	Present: 38 (23.6%) Absent: 123 (76.4%)	$\chi^2 = 48.06$ , 1 df, $p < 0.01$ $C = 0.394$ , $p < 0.01$
Wild barley	Present: 9 (5.7%) Absent: 149 (94.38%)	Present: 33 (20.5%) Absent: 128 (79.5%)	$\chi^2 = 15.28$ , 1 df, $p < 0.01$ $C = 0.219$ , $p < 0.01$

Table 4. continued

Attribute	Sites w/ SJAS (n = 158)	Sites w/o SJAS (n = 161)	Chi-square test and Cramer's coefficient
Fiddleneck	Present: 14 (8.9%) Absent: 144 (91.1%)	Present: 23 (14.3%) Absent: 138 (85.7%)	$\chi^2 = 1.79$ , 1 df, $p = 0.18$ $C = 0.085$ , $p = 0.13$
Red-stemmed filaree	Dominant: 14 (8.9%) Not dominant: 97 (61.4%) Absent: 47 (29.7%)	Dominant: 11 (6.8%) Not dominant: 95 (59.0%) Absent: 55 (34.2%)	$\chi^2 = 0.98$ , 2 df, $p = 0.61$ $C = 0.055$ , $p = 0.61$
Topography	Flat: 79 (50.0%) Rolling: 34 (21.5%) Gentle slope: 21 (13.3%) Steep slope: 24 (15.2%)	Flat: 78 (48.4%) Rolling: 37 (23.0%) Gentle slope: 21 (13.0%) Steep slope: 25 (15.5%)	$\chi^2 = 0.13$ , 3 df, $p = 0.99$ $C = 0.020$ , $p = 0.99$
Washes	Present: 29 (18.4%) Absent: 129 (81.6%)	Present: 19 (18.8%) Absent: 142 (88.2%)	$\chi^2 = 2.19$ , 1 df, $p = 0.14$ $C = 0.092$ , $p = 0.10$
Scalds	Present: 7 (4.4%) Absent: 151 (95.6%)	Present: 38 (23.6%) Absent: 123 (76.4%)	$\chi^2 = 22.63$ , 1 df, $p < 0.01$ $C = 0.275$ , $p < 0.01$
Disturbance	Present: 97 (61.4%) Absent: 61 (38.6%)	Present: 101 (62.7%) Absent: 60 (37.3%)	$\chi^2 = 0.02$ , 1 df, $p = 0.90$ $C = 0.014$ , $p = 0.81$
Grazing	Cow: 28 (17.7%) Sheep: 53 (33.5%) No grazing: 77 (48.7%)	Cow: 51 (31.7%) Sheep: 18 (11.2%) No grazing: 92 (57.1%)	$\chi^2 = 25.26$ , 2 df, $p < 0.01$ $C = 0.281$ , $p < 0.01$
	Grazing: 81 (51.3%) No grazing: 77 (48.7%)	Grazing: 69 (42.9%) No grazing: 92 (57.1%)	$\chi^2 = 1.94$ , 1 df, $p = 0.16$ $C = 0.084$ , $p = 0.13$
Kangaroo rats	Present: 152 (96.2%) Absent: 6 (3.8%)	Present: 119 (73.9%) Absent: 42 (26.1%)	$\chi^2 = 29.27$ , 1 df, $p < 0.01$ $C = 0.312$ , $p < 0.01$
Burrow density	High: 62 (39.2%) Medium: 34 (21.6%) Low: 62 (39.2%)	High: 32 (19.9%) Medium: 27 (16.8%) Low: 102 (63.4%)	$\chi^2 = 20.11$ , 2 df, $p < 0.01$ $C = 0.251$ , $p < 0.01$
	High-Med: 96 (60.8%) Low: 62 (39.2%)	High-Med: 59 (36.6%) Low: 102 (63.4%)	$\chi^2 = 17.61$ , 1 df, $p < 0.01$ $C = 0.241$ , $p < 0.01$
California ground squirrels	Present: 4 (2.5%) Absent: 154 (97.5%)	Present: 16 (9.9%) Absent: 145 (90.1%)	$\chi^2 = 6.24$ , 1 df, $p = 0.01$ $C = 0.153$ , $p = 0.01$

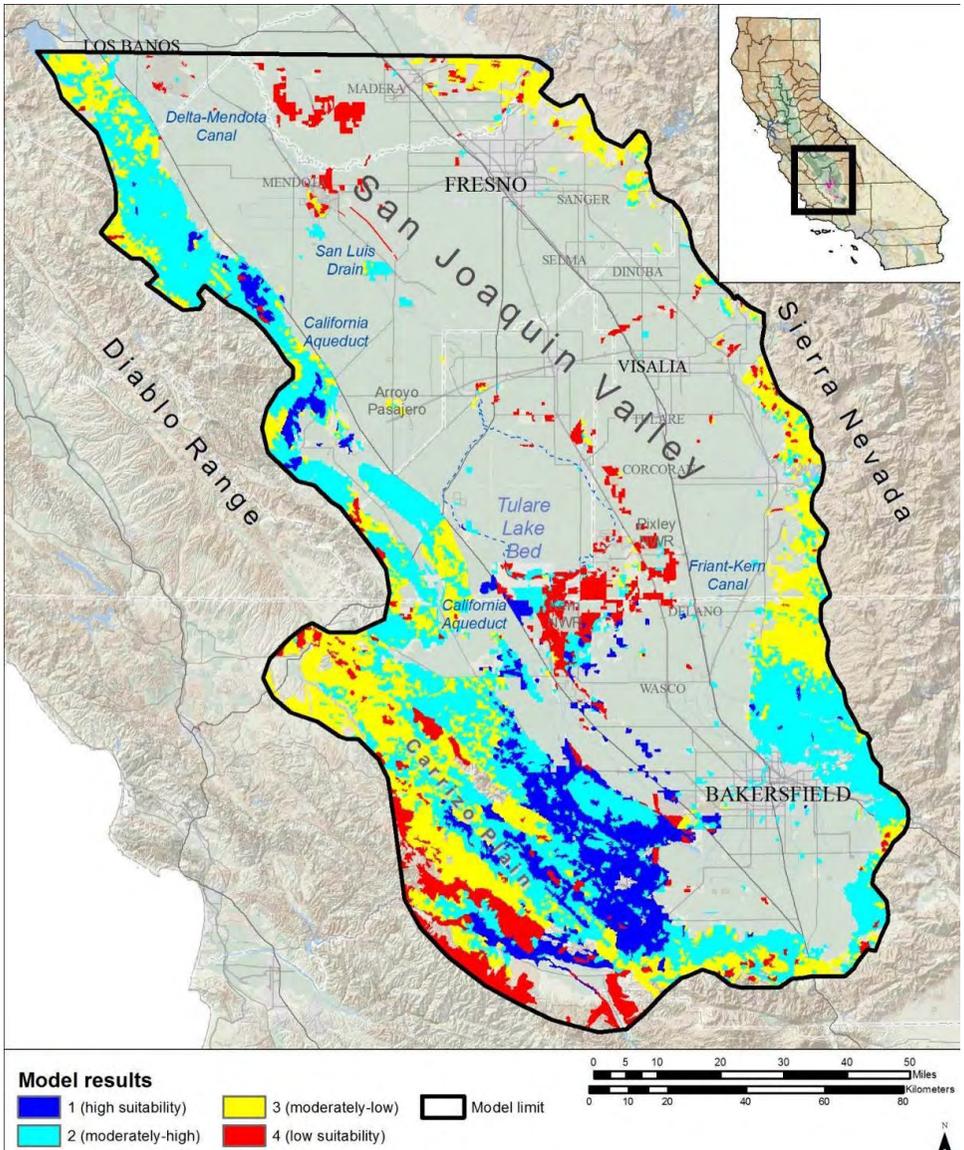
### Habitat Suitability Modeling

Within the SJAS habitat suitability model boundary, we identified approximately 1,348 km<sup>2</sup> of high-quality habitat, 4,583 km<sup>2</sup> of moderately high-quality habitat, 3,388 km<sup>2</sup> of moderately low-quality habitat, and 1,365 km<sup>2</sup> of low-quality habitat (Fig. 4). When we compared the results from field surveys with output from the model, we found that 58% of sites where SJAS were detected were in the highest quality habitat and 32% were in moderately high-quality habitat. The remaining 10% were in moderately low- or low-quality habitat, and in most cases these locations were in ecotone zones near higher quality habitat.

DISCUSSION

SJAS Survey Technique

Automated camera stations appeared to be an effective technique for detecting SJAS presence. The stations were easy to install and we found that about 20 stations could be established in the course of a day, depending upon station spacing. A location was surveyed continuously during the period that the station was operational. Continuous camera operation



**Figure 4.** Results of habitat suitability modeling analysis for the San Joaquin antelope squirrel (SJAS) in California, USA.

over multiple days helps avoid false-negative determinations resulting from variations in SJAS activity levels due to time of day or daily weather. For example, SJAS may reduce activity for several hours during mid-day on days when temperatures exceed about 32° C and for entire days when temperatures fall below about 10° C (Best et al. 1990). The strategy of deploying multiple cameras in a given general area is prudent as even within suitable habitat, the distribution of SJAS can be patchy (Grinnell and Dixon 1918; Hawbecker 1953; Best et al. 1990).

Visual encounter surveys conducted by foot or vehicle can cover large areas, but detections are limited to the brief time that observers are searching a given location. Thus, the potential for false-negative determinations is much higher than that for cameras. Live-trapping is another common survey technique for SJAS. However, trapping is labor intensive as traps must be set and then are typically checked multiple times per day to avoid heat stress to captured animals. The effective survey period is limited to the time that the traps are open. Also, as with any live-trapping, there always is some degree of risk of injury or death to animals during trapping.

The camera station survey approach does entail an initial investment in cameras, but the cost is generally not prohibitive. Cameras that can operate continuously and reliably for at least a week are readily available and can be purchased for under \$150 each. Other costs (approximately \$15-\$20) include posts and attachment materials, batteries, SD cards, and bait block. The posts, SD cards, and possibly some of the attachment materials can be used multiple times. Also, we commonly recovered and reused all or some of the bait block, particularly from stations where SJAS were not detected. We considered the camera stations to be an effective and cost-effective strategy for detecting SJAS in a given area.

### **Habitat Attributes**

The habitat attribute data warrant certain caveats. As mentioned in the methods, the protocol for assessing habitat attributes at each camera station location was designed such that the information could be collected rapidly, usually within about 15 min. Most attributes were characterized as present or absent, or were assigned to one of 3-4 ordinal bins. Thus, the data essentially are coarse scale in nature. Another caveat is that the camera station detection data potentially included some false-negative determinations. SJAS sometimes were detected at some stations but not at other nearby stations with seemingly similar habitat conditions. The reasons for these non-detections are unknown but could include a temporarily vacant home range, camera stations unknowingly placed too far from escape cover, or some other habitat attribute that we did not recognize as important to SJAS. Also, as mentioned previously, the distribution of SJAS even within suitable habitat can be patchy (Grinnell and Dixon 1918). Consequently, the habitat attributes from any stations with false-negative findings would have been grouped with stations without SJAS detections, thereby increasing the difficulty of detecting significant differences between stations with and without SJAS detections.

Despite the caveats above, several significant differences were found between stations with and without SJAS detections. Shrubs were absent on over a quarter of the sites where SJAS were detected. SJAS use shrubs for escape cover and thermal regulation in hot weather, but can use burrows for the same purposes when shrubs are not present. Harris and Stearns (1991) found that SJAS densities on the Elkhorn Plain actually were considerably higher

in areas without shrubs and that giant kangaroo rat (*D. ingens*) burrows were abundant in these areas. Dense shrubs may actually exclude SJAS (Grinnell and Dixon 1918; Hawbecker 1975). Shrubs may not be a required habitat feature for SJAS, although Hawbecker (1975) suggested that females using burrows under shrubs may have higher reproductive success.

When shrubs were present, overwhelmingly they were desert saltbush or jointfir. These species are the dominant shrubs in arid saltbush scrub and ephedra scrub communities that occur on the more well-drained sandier soils preferred by SJAS (Hawbecker 1975). Conversely, SJAS were infrequently detected in areas with iodine bush, sinkweed, and alkali goldenbush. These are the dominant shrubs in alkali sink communities. These results are consistent with range-wide habitat characterizations by Grinnell and Dixon (1918) and Hawbecker (1975) who reported that SJAS were closely associated with desert saltbush and jointfir.

SJAS also were detected more frequently in areas with lower ground cover. Over 90% of detections were in areas with > 10% bare ground and over 40% of detections were in areas with > 30% bare ground. SJAS are relatively small animals and have difficulty moving through dense ground cover (Germano et al. 2001). In particular, they rely on speed to reach cover and elude predators, and predation risk likely increases with herbaceous ground cover density. At two separate study sites in Kern County, SJAS abundance increased with decreasing ground cover (Cypher 2001; Germano et al. 2012). Arabian grass was a common dominant ground cover in locations with SJAS detections. This grass forms a low, sparse cover and prefers more arid sites where it is not outcompeted by species that require more mesic conditions, such as wild barley. Wild barley tends to form a dense cover and SJAS were rarely detected at locations where this species was present. The lack of association with red brome, amsinckia, and red-stemmed filaree likely was due to these plants being ubiquitous throughout the San Joaquin Valley region.

The lower SJAS detection rates in locations with iodine bush, sinkweed, alkali goldenbush, wild barley, and alkali scalds all indicate that alkali sink habitats, where these species and features are commonly found, are not optimal habitats for SJAS. We found that this habitat was typically only used where it was in close proximity to arid upland scrub habitat, or more commonly, locations that were in transition zones between arid upland scrub and alkali sink habitats. Our results are consistent with and further confirm those of previous researchers that also noted the suboptimal nature of alkali sink habitat for SJAS (Grinnell and Dixon 1918; Hawbecker 1953, 1975; Harris and Stearns 1991). Areas with alkali sink communities tend to occur in more low lying areas of the San Joaquin Valley with heavy clay soils where burrowing may be more difficult, the water table commonly is just a few centimeters below the surface, soils are saturated during the winter rainy season, and periodic flooding occurs. Consequently, SJAS were only detected on the valley floor in two locations (Semitropic Ridge area and Buttonwillow Ecological Reserve), both of which have habitat transitional between alkali sink and arid upland scrub habitat growing on slightly higher areas (e.g., sand ridges, hummocks).

Topographic ruggedness and slope did not appear to influence SJAS presence. However, the locations where we established camera stations did not have slopes exceeding 30%, and it is possible that locations with steeper slopes may be less suitable for SJAS (Hawbecker 1975). Harris and Stearns (1991) found SJAS on slopes of up to 20 degrees. Also, topography may influence SJAS in other ways. In particular, vegetation characteristics can vary with elevation and aspect with ground cover being denser at higher elevations and on more northerly facing slopes (Cypher 2001).

Presence of SJAS did not appear to be affected by habitat disturbances. These disturbances consisted primarily of infrastructure related to oil and gas production, such as pipelines and well pads. However, in the areas where we established camera stations, these features typically affected < 10% of the habitat and an abundance of intact habitat remained available. In a study of oil field effects on vertebrate communities in the southwestern San Joaquin Valley (Fiehler et al. 2017), SJAS continued to be present on plots with about a third of the habitat disturbed by oil field features (e.g., roads, well pads, pipelines, storage tanks, and other facilities).

Presence of SJAS also did not appear to be affected by grazing. When grazing was occurring on sites where SJAS were detected, the grazers usually were sheep, although this may have been due to a sampling bias. To some extent, we avoided areas where cattle were abundant as these animals, out of curiosity, commonly investigate and disturb camera stations, sometimes to the point of destroying them. However, on a site near Blackwell's Corner in northern Kern County that was being grazed by cattle, SJAS were abundant and were detected on 16 out of 20 camera stations operated on that site. Harris and Stearns (1991) also observed SJAS in areas that were heavily grazed by cattle. Germano et al. (a,b) found that SJAS home range size and demographic attributes were similar between grazed and ungrazed areas. Hawbecker (1975) even suggested that SJAS might benefit from the presence of cattle by feeding on the abundant insects attracted to cattle excrement.

The association between SJAS presence and kangaroo rat presence was not surprising. Kangaroo rats also are arid-adapted rodents that prefer areas with sparser ground cover (Goldengay et al. 1997; Cypher 2001; Germano et al. 2012). Thus, kangaroo rats and SJAS share similar habitat preferences. Furthermore, SJAS may benefit from the presence of kangaroo rats. Although SJAS can create their own burrows (Grinnell and Dixon 1918), Hawbecker (1947, 1953) reported that SJAS mostly use burrows created by kangaroo rats. Hawbecker (1953) concluded that the presence of SJAS was likely strongly influenced by the presence of kangaroo rats, particularly Heermann's kangaroo rats (*D. heermanni*) and giant kangaroo rats. These are larger kangaroo rats and SJAS can fit into their burrows with little or no modification (Hawbecker 1947). Harris and Stearns (1991) also reported an association in occurrence between SJAS and giant kangaroo rats. Consistent with these observations, we found that burrow abundance was typically higher in areas where SJAS were detected and that most of these burrows were made by kangaroo rats. Also, SJAS do not necessarily use a single "home burrow," but instead use multiple burrows as they forage throughout their home range (Hawbecker 1975). Thus, higher burrow abundance benefits SJAS.

The negative association between SJAS and California ground squirrels also was not surprising as this relationship has been noted previously (Taylor 1916; Grinnell and Dixon 1918; Hawbecker 1975; Harris and Stearns 1991). The nature of this negative association is not well understood. In areas where the two species co-occur, California ground squirrels may locally displace SJAS. Harris and Stearns (1991) observed California ground squirrels simply moving into SJAS burrow complexes and the resident SJAS moving to other nearby burrows. No aggression was observed. Similarly, we observed both species feeding together on the bait block at one of our stations. The two species may have different habitat preferences with SJAS preferring more arid areas with sparser vegetation and California ground squirrels preferring more mesic areas with denser vegetation (Grinnell and Dixon 1918; Jameson and Peeters 1988). Also, anthropogenic habitat disturbance appears to favor California ground squirrels as evidenced by their abundance in agricultural areas, urban areas, and even highly disturbed oil field areas (Fiehler et al. 2017). Such disturbance may

allow this species to colonize areas that traditionally were SJAS habitat, as reportedly occurred in the Panoche region (Hawbecker 1975).

### **Suitability Modeling**

In developing our SJAS habitat suitability model, we used the best available information (e.g., Hawbecker 1975; Best 1990; Harris and Stearns 1991; USFWS 1998) along with preferred habitat attributes based on findings from our surveys. However, we caution that as with any suitability model, the results do not guarantee that SJAS are present or absent at any given location. Instead, modeling results should be viewed as an estimate of the potential for SJAS to occur on given lands; higher suitability rankings indicate a higher probability of SJAS occurrence. Surveys to determine the presence of SJAS or at least to assess habitat conditions should be conducted on any parcel prior to initiating conservation (e.g., acquisition) or habitat-disturbing activities.

Williams (1981) estimated that the historic range of the SJAS encompassed approximately 1,398,600 ha and that by 1979 just 274,200 ha remained, of which only 41,300 ha (15%) was fair to good quality habitat. These estimates were for the San Joaquin Valley proper. Our habitat suitability modeling effort indicated that approximately 593,100 ha of high or moderately high-quality habitat were still present within the historic range of SJAS, which includes the San Joaquin Valley, Carrizo Plain region, and Cuyama Valley. An almost equal quantity (475,300 ha) of low and moderately low-quality habitat also was present. Possibly, some of this lower quality habitat could be enhanced to improve suitability for SJAS.

The largest quantities of remaining high and moderately high-quality habitat are located in western Kern County and eastern San Luis Obispo County. Considerable high and moderately high-quality habitat also occurs in a band along the western edge of the San Joaquin Valley from the Kern County line north into western Merced County. Significant areas of high-quality habitat occur in the Coalinga area and also on the eastern toe of the Coast Ranges south of the Panoche region. Also, a large area of mostly moderately high quality habitat occurs along the southeastern margin of the San Joaquin Valley from about Poso Creek just north of Bakersfield down to about Pastoria Creek in the very southeastern corner of the valley on Tejon Ranch lands. A number of small fragments of high-quality habitat occur on the valley floor, primarily toward the drier west side. Many of these fragments are < 40 ha and may be too small to support a self-sustaining population of SJAS.

### **Distribution**

The historic range of the SJAS is described as extending from western Merced County south along the western side of the San Joaquin Valley, across the southern valley in Kern County, north along the eastern side of the valley to southern Tulare County, and in the Carrizo Plain and Cuyama Valley (Williams 1981; USFWS 1998). Harris and Stearns (1991) reported that the current range was still similar in extent to the historic range, but that less of the range was occupied due to habitat loss. Williams (1981) concluded that SJAS had mostly been extirpated on the floor of the San Joaquin Valley. The Carrizo Plain and Elkhorn Plain in eastern San Luis Obispo County and the Elk Hills-Lokern area in western Kern County were considered strongholds for remaining SJAS populations (Grinnell and Dixon 1918; Harris and Stearns 1991).

During our survey effort, SJAS were commonly detected at the stations in the Carrizo Plain region. The Carrizo Plain is recognized as a core area for imperiled arid upland spe-

cies, including SJAS, that are endemic to the San Joaquin Desert region (USFWS 1998). The Temblor Range bounds the Carrizo Plain on the east and roughly follows the border between San Luis Obispo County and Kern County. We ran transects of cameras over this range along Crocker Grade Road located about mid-range and along Elkhorn Grade Road at the southern end of the range, primarily in an effort to determine the effect of rugged topography on SJAS. We obtained few detections of SJAS along the Elkhorn Grade transect, but SJAS were detected at most of the camera stations on the Crocker Grade transect. SJAS seemed less limited by terrain along this transect and more limited by the denser vegetation, particularly non-native grasses, which were present on north and east facing slopes. In western Kern County, SJAS were detected at most of the stations established in the Midway Valley, Buena Vista Valley, Buena Vista Hills, Lokern area, and Blackwells Corner area. All of these areas are within a second core area identified in the recovery plan for imperiled arid upland species (USFWS 1998).

At the southern end of the San Joaquin Valley, SJAS were only detected in a saltbush scrub community in the northwestern corner of the Wind Wolves Preserve where they also had been detected in the past (Cypher et al. 2011). Most of the remaining habitat at the south end of the valley is on the toe of the Transverse ranges and has dense grass, or is alkali sink habitat. SJAS were only detected at 1 of 26 sites surveyed in the Kettleman Hills on the west side of the San Joaquin Valley at the border between Fresno County and Kings County. These sites were characterized by moderate to dense ground cover of primarily non-native grasses.

The Panoche Valley region is recognized as a third core area for listed species in the recovery plan for upland species of the San Joaquin Valley (USFWS 1998). SJAS were only detected at 1 station out of 25 in this region. The location was on the Silver Creek Ranch that is now part of the Panoche Valley Preserve managed by CNLM. CNLM staff report that SJAS are abundant and widespread on the Preserve (B. Teton, CNLM, personal communication). SJAS also were observed at a location approximately 10 km north of Silver Creek Ranch in 2017 (B. Cypher, personal observation).

Near Kern National Wildlife Refuge in northern Kern County, SJAS were mostly detected on an approximately 4-km long sand ridge, called Semitropic Ridge, south of the refuge. This ridge is a relictual dune complex that is 1–3 m higher than the surrounding land and has sandier soil and supports a saltbush scrub vegetation community. Otherwise, SJAS were detected at only one of the numerous other survey sites to the east and northeast up to Pixley National Wildlife Refuge. These sites are in lower lying areas that primarily support intact or degraded alkali sink vegetation communities and also are more prone to occasional flooding.

Similarly, SJAS were detected on the Buttonwillow Ecological Reserve in Kern County. Similar to Semitropic Ridge, this area was ecotonal between alkali sink and saltbush scrub communities, but saltbush was the dominant shrub at sites where SJAS were detected. SJAS were not detected at any other sites on the valley floor.

SJAS potentially are present in other areas that were not surveyed during this project, primarily due to lack of access (i.e., private lands). One such area is the Cuyama Valley in southeastern San Luis Obispo County. Harris and Stearns (1991) conducted surveys in this area in 1988 and commonly detected SJAS on the north side of the Cuyama River along the base of the Caliente Range. A thin band of high-quality saltbush scrub habitat still remains and SJAS may still be present. North of Kern County, a mostly continuous band of good

quality habitat extends north along the western margin of the San Joaquin Valley. Based on our model, relatively large areas with highly suitable habitat are present west and north of the city of Coalinga, and just southeast of Panoche Valley between the Coast Ranges and the California Aqueduct. The band of suitable habitat continues up into western Merced County.

Scattered patches of saltbush scrub habitat persist along the southeastern margin of the San Joaquin Valley and SJAS were present at one time in this area (Hawbecker 1975). Grinnell and Dixon (1918) reported seeing SJAS “in grain fields at the base of the Tehachapi Mountains” and 35 individual SJAS were collected from a location northeast of Bakersfield in 1911. However, no extant populations of SJAS are currently known from the eastern side of the San Joaquin Valley. We did not conduct surveys in this region primarily because biologists have had access to much of this area in recent years and there have been no reports of SJAS sightings.

### **Conclusions and Recommendations**

Based on our survey results supplemented with recent opportunistic observations, SJAS are present in the Carrizo Plain region and along the western margin of the San Joaquin Valley from the southwestern corner of the valley north to about the Merced County line. They are locally abundant in the Carrizo Plain, western Kern County, and Panoche Valley regions, all of which have been identified as core areas for rare arid upland species including SJAS (USFWS 1998). SJAS primarily occur in locations with arid upland shrub scrub communities, typically with saltbush or jointfir as the dominant shrubs (although the presence of shrubs is not required) and with sparse ground cover. Alkali sink habitat appears to constitute suboptimal habitat for SJAS. SJAS are present at only a few locations on the floor of the San Joaquin Valley because so little natural habitat remains, and most is alkali sink habitat. These valley floor populations are relatively small and isolated, and therefore they are at increased risk of extirpation from catastrophic or stochastic events.

SJAS currently persist in a metapopulation structure consisting of populations of varying size and connectivity. Goals for SJAS conservation should include conserving as much of the remaining unprotected higher quality habitat as possible, expanding buffers around occupied habitat, and increasing connectivity between habitat patches to facilitate genetic and demographic flow, all of which will help maintain more optimal metapopulation dynamics and reduce extinction risk. In light of the continuing loss of habitat within the range of the SJAS, continued protections for this species under the California Endangered Species Act are warranted.

We offer the following recommendations based on our results:

1. Conduct additional surveys for SJAS, particularly on lands that have not been surveyed previously.
2. Conserve any unprotected lands where SJAS have been detected or that contain high quality habitat based on suitability modeling.
3. Manage vegetation if necessary (e.g., grazing) to reduce dense herbaceous ground cover and improve suitability for SJAS.
4. Identify strategies for restoring habitat on previously disturbed lands to render them suitable for occupation by SJAS.
5. Translocate SJAS from appropriate source populations to restored habitat or unoccupied conserved parcels with suitable habitat, if effective translocation strategies can be identified.

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FULL RESEARCH ARTICLE

## Development of a morphological key for the southern salt marsh harvest mouse using genetically verified individuals

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The salt marsh harvest mouse (SMHM; *Reithrodontomys raviventris*) is a state and federally listed endangered species endemic to the coastal marshes of the San Francisco Estuary of California. Of two subspecies, the southern (*R. r. raviventris*) is most endangered and lacks reliable morphological field tools to distinguish from the sympatric western harvest mouse (WHM; *R. megalotis*). We trapped and collected genetic samples and morphological data from 204 harvest mice from 14 locations from across the range of the southern SMHM. Genetic species identification indicated these to be composed of 48 SMHM and 156 WHM, which we compared at ten morphological characters. Most continuous characters overlapped between species. Color characters were significantly differentiated and we identified a number of species-specific diagnostic pelage categories in both species. A random forest analysis indicated that ventral coloration of the abdomen and the ventral tail hair color were the most useful for differentiating between species. We used these two morphological characters to develop a decision tree which correctly classified 94% of harvest mice to species with 99% accuracy. These findings suggest that our decision tree can be used to reliably identify the species of most harvest mice in the range of the southern SMHM, with a small proportion (6% in our study) needing genetic confirmation. The decision tree should be tested on additional harvest mice that were not used in its development, particularly from novel locations across the range.

**Key words:** decision tree, endangered species, field identification, genetic identification, morphology, random forest, *Reithrodontomys megalotis*, *Reithrodontomys raviventris*, salt marsh harvest mouse, western harvest mouse

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Fundamental to monitoring, management, and conservation of endangered species is the ability to identify them in the field. Correct identification is essential for accurate characterization of the species' range, habitat, abundance, demography, population trends, and dietary requirements (Smith et al. 2018; Sustaita et al. 2018; Quinn et al. 2019; Statham et al. 2019). Erroneous identification leads to a false characterization of these parameters, which in turn invalidates conservation assessments and other scientific conclusions.

The salt marsh harvest mouse (SMHM, *Reithrodontomys raviventris*) is an endemic species restricted to the tidal and brackish marshes of the San Francisco Estuary (including San Francisco, San Pablo, and Suisun Bays; Fisler 1965; Shellhammer 1989). Fragmentation and loss of  $\geq 75\%$  of the species' habitat through reclamation of tidal areas led to it being listed as endangered under both the California and U.S. endangered species acts (Shellhammer 1982, 1989; USFWS 1970). The species is composed of two genetically and morphologically distinct subspecies: the southern *R. r. raviventris* of San Francisco Bay, and northern *R. r. halicoetes* of San Pablo and Suisun Bays (Fisler 1965; Statham et al. 2016).

Across much of its range the SMHM is sympatric with the morphologically similar non-endangered western harvest mouse (WHM; *R. megalotis*). In contrast to the SMHM, the WHM is abundant and ranges across a vast area comprising much of western U.S. and extending into Canada and Mexico (Jameson and Peeters 2004). While the WHM is primarily a grassland species, it also inhabits the edges of salt and brackish marshes in the San Francisco Estuary (Fisler 1963). Most biologists have considerable difficulty distinguishing between the two species (Shellhammer 1984), which has led to a number of efforts to provide morphological tools to facilitate differentiation.

Shellhammer (1984) described a method for the differentiation of the species based on the work of Fisler (1965). Shellhammer (1984) assigned values to tail traits: diameter 20 mm from the base of the tail, tail color pattern (i.e., bicolored, unicolored, or intermediate), ventral tail hair color (i.e., tan, intermediate, white to grayish-white), and tail tip (i.e., blunt, intermediate, or pointed). Different sets of tail characters were examined in the northern part of the species range (i.e., San Pablo Bay, Suisun Bay) versus the southern (i.e., San Francisco Bay). Based on the total score animals were assigned to SMHM, WHM, or intermediate/unknown. Although other characters were recorded (i.e., behavior, ventral coloration, tail to body length ratio, and presence of orange ear tufts) they were not included in the scoring system for species assignment, resulting in some confusion regarding application of these criteria in differentiation of the species.

More recently, Sustaita et al. (2018) examined morphological characters of harvest mice from Suisun Bay (in the range of the northern SMHM subspecies) that were genetically confirmed to species. Using these data, they identified tail length, body length, and tail diameter as the most useful characters for distinguishing species. They then built a multiple logistic regression model that correctly classified 90.1% of individuals. However, the validity of these characters for the identification of harvest mice beyond Suisun Bay was untested.

Recently, different subsets of morphological measurements have been recorded by agency (i.e., U.S. Fish and Wildlife Service [USFWS], California Department of Fish and Wildlife [CDFW]) personnel working on harvest mice in the San Francisco Estuary. Use of genetic species identification revealed that morphological assignment methods were inadequate in the range of the southern SMHM, where only  $\sim 50\%$  were correctly identified (Statham et al. 2016). This was the case regardless of whether mice were being identified using the tail scoring method (Shellhammer 1984; USFWS and others), or the multiple logistic regression (Sustaita et al. 2018; CDFW). This finding essentially invalidated cur-

rent morphological methods for the identification of harvest mice species within the range of the southern SMHM, leaving the identification reliant solely on genetic analyses. The finding also threw into doubt historical records of species abundance and distribution, as well as scientific study reliant on correct identification, thus hampering recovery efforts.

The objectives of this study were to (1) assess whether morphological characters could be used to reliably distinguish between harvest mice species within the range of the southern SMHM, and (2) develop a morphological method for the identification of harvest mice species. To achieve these aims, we collected morphological and genetic data, and used both single variable and multivariable analyses to determine if morphological characters could be used to reliably differentiate species.

## METHODS

### Study Area

We conducted our study at 14 tidal and adjacent diked saline marsh locations across the south and central San Francisco Estuary of California (Fig. 1; 37.6° N, 122.1° W). These marshes encompass the putative geographic range of the southern SMHM. The marshes generally contained middle and high marsh where the vegetation cover was dominated by pickleweed (*Salicornia pacifica*).

### Fieldwork and Genetic Sample Collection

Animal trapping, handling, and genetic sampling procedures were approved by UC Davis Institutional Animal Care and Use Committee and authorized by the CDFW and the USFWS. We collaborated closely with partners (USFWS, CDFW, H. T. Harvey & Associates) to obtain harvest mouse morphological measurements and genetic samples. Data and samples from many of the mice were obtained during ongoing trapping and monitoring efforts. Additional sites that were not part of ongoing monitoring activities were chosen to provide a diverse set of sampling locations spread across the range of the southern SMHM (Fig. 1). We used ~70-130 Sherman live traps (H.B. Sherman Traps, Tallahassee, FL) spaced at ~10-m intervals, although the exact number of traps and the layout depended on the wetland shape. We baited traps with mixed bird seed and ground walnut, added cotton or polyester batting for warmth, set the traps at dusk, and checked them at dawn. When harvest mice were captured and processed we plucked hair as a source of DNA. Prior to sampling from an individual, we physically wiped down the forceps with a clean tissue, sterilized the forceps in a 2% bleach solution, rinsed with water to remove the bleach, and dried the forceps with a second tissue (Statham et al. 2016). We stored the hair in 95-100% ethanol until DNA extraction.

### Morphological Measurements

We collected a suite of morphological measurements for each harvest mouse, including those considered previously (Shellhammer 1984; Sustaita et al. 2018). The majority of the morphological data was collected by experienced CDFW and USFWS personnel, although >10 observers of varying skill levels contributed to data collection. Specifically, we recorded the Sex, Reproductive Condition, Mass, Total Length, Tail Length, Body Length (i.e., calculated by subtracting the tail length from the total length), Tail Diameter (20mm from base

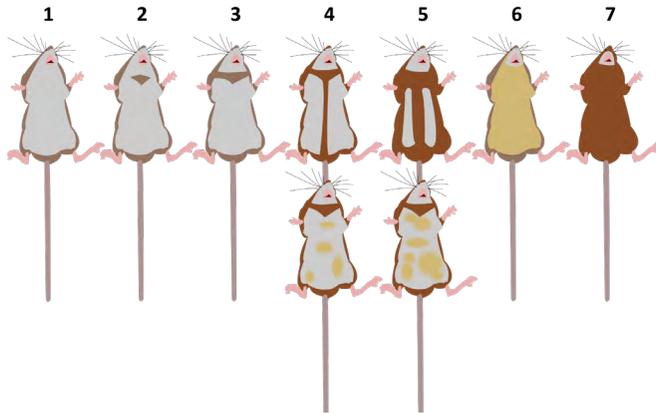


**Figure 1.** Map of genetically identified harvest mice. Numbers in the light-colored boxes are western harvest mouse (WHM; *Reithrodontomys megalotis*), numbers in the orange boxes are salt marsh harvest mouse (SMHM, *R. raviventris raviventris*). Red circles indicate trapping locations. Green highlighting indicates coastal wetlands.

of tail), Ventral Coloration (Fig. 2), Dorsal Hair Color(s), Tail Pattern (taking into account the hair color on both the dorsal and ventral surface of the tail [Fisler 1965] and categorized as bicolored, intermediate, unicolored), Ventral Tail Hair Color (i.e., all white, intermediate [few white hairs], tan [no white hairs]), Tail Tip Shape (i.e., pointed, intermediate, blunt), and Behavior (i.e., docile, intermediate, active). We did not conduct statistical analysis of Behavior or Dorsal Hair Color due to the relatively small number of records and the subjective nature of assigning animals to one category or another.

### Genetic Species Identification

We used DNA sequence data to discriminate between harvest mouse species following the methods of Statham et al. (2016). Briefly, we extracted DNA from the mouse hair samples and then PCR-amplified and sequenced a 426 bp fragment of the cytochrome *b* gene and a small section of tRNA-Glutamate using the primers MVZ-05 and MVZ-04R (Smith and Patton 1993; Brown 2003). SMHM and WHM form well defined reciprocally monophyletic clades (Statham et al. 2016) at this gene region. We used the associated >10% sequence divergence between SMHM and WHM to discriminate between the species with certainty.



**Figure 2.** Ventral Coloration categories in harvest mice from Central and South San Francisco Estuary. The categories are as follows: (1) white or greyish white; (2) cinnamon pectoral spot; (3) pectoral band (i.e., spot extended across chest between forelimbs); (4) pectoral band with center stripe extending down belly or  $\frac{3}{4}$  of the ventral surface white with mottled light cinnamon; (5) wider ventral stripe than 4 or  $\frac{1}{2}$  of the ventral surface white with mottled light cinnamon; (6) light cinnamon wash, paler than 7, does not have a band, stripe, or spot; (7) all cinnamon (with rare trace of pale color) with no abrupt color change from lateral (sides) to ventral (belly). Spots and stripes can vary in color intensity. Example photographs of each category are provided in the [accompanying document](#). The depiction and description of Ventral Coloration categories are based on descriptions by Fisler (1965) and Shellhammer (1984), with new clarifications and additional description.

## Statistical Analyses

For each of the continuous morphological characters (e.g. Body Length, Mass), we used t-tests to compare means. For categorical characters (e.g. Ventral Coloration, Tail Tip Shape) we used  $\chi^2$  tests of independence. Statistical analyses and graphing were conducted using R v.3.6 (<https://www.r-project.org/>). We conducted a multiple variable analysis to examine the importance of each of the morphological characters for the differentiation between species using Random Forest (Breiman 2001) implemented in the R package randomForest (<https://cran.r-project.org/web/packages/randomForest/index.html>). Random forest is a machine learning approach for creating classification trees. Decision trees are generated for each bootstrapped sample (i.e., a random sampling of individuals with replacement) where a random subset of predictor variables is assessed at each node. The best performing predictor variable is chosen for use at each node. Each decision tree attempts to classify data points at each of the nodes and assesses the information gain. This process continues with additional nodes until there is no further information gain. The information gain at each node in each tree can then be used to assess which predictor variables are important (most informative) for the correct classification. Individuals are then run through each of the trees, and the aggregation decision (the majority) is used to classify them (termed ‘bagging’).

Our analysis was limited to adult individuals with no missing data, resulting in a dataset of 129 individuals. We randomly assigned 70% of individuals to a training dataset and 30% of individuals to a test dataset. We created a random forest model with the training data set, generating 500 trees (sampling individuals from the dataset with replacement), and randomly choosing 6 (of 10) predictor variables (i.e., morphological characters) at each split. Based on the model (containing all 500 decision trees) we assessed the classification error overall and for each species. We then assessed how well the model predicted species in the test dataset.

We calculated the importance of each of the 10 characters in the model using two different parameters: ‘mean decrease in accuracy’ and ‘mean decrease in Gini’. The resolving power of a character was calculated as the ‘mean decrease in accuracy’ when a character is left out. The additive value of individual characters in the model is assessed using ‘mean decrease in Gini’ when that character is left out. Gini is effectively a measure of how homogenous or pure a group is at the end of the tree. The results of the single-variable and multivariable analyses were used to design a decision tree for the identification of harvest mice species in the field. We also took into consideration how subjective assignment to categories were for morphological characters.

For comparison to our southern-specific method, we also assigned these mice using the tail character scoring method of Shellhammer (1984), which was developed for both subspecies, and the multiple logistic regression method, which was developed on a population of the northern SMHM subspecies (Sustaita et al. 2018). Only adult mice with the full set of necessary characters were considered for each analysis.

## RESULTS

### Genetic Species Identification

We collected morphological data and genetic samples from 204 harvest mice from 14 sites within the putative range of the southern SMHM (Fig 1; Table 1). Using mitochondrial DNA sequence analysis, we identified 48 SMHM and 156 WHM. Despite catching 30 and 31 WHM respectively at Point Pinole Regional Shoreline (Contra Costa County) and

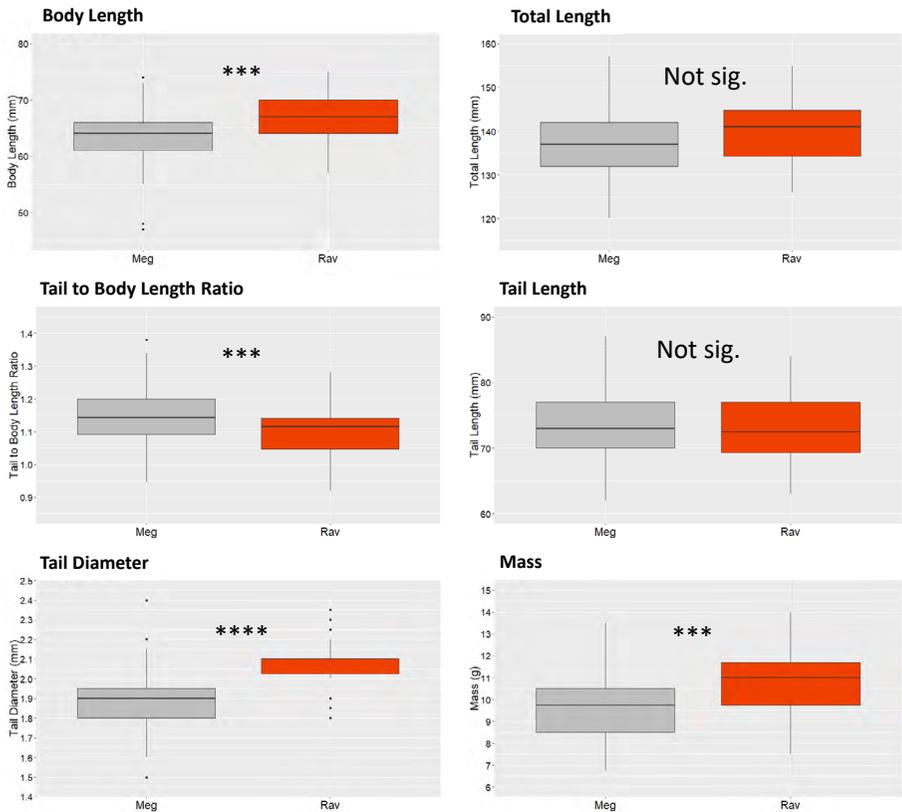
**Table 1.** Numbers of genetically identified salt marsh harvest mouse (SMHM; *Reithrodontomys raviventris raviventris*) and western harvest mouse (WHM; *R. megalotis*) from each location. Only mice that were included in statistical analyses are listed.

Site	WHM	SMHM
Audubon Marsh (Alameda County)	4	3
Bothin Marsh (Marin County)	29	0
Calaveras Marsh (Alameda County)	0	9
Eden Landing Ecological Reserve (Alameda County)	0	26
Dumbarton Marsh (Alameda County)	3	3
LaRiviere Marsh (Alameda County)	2	0
Mayhews Landing (Alameda County)	21	1
Milpitas - Coyote Creek Upland Ruderal Field (Santa Clara County)	10	0
Milpitas - Triangle Marsh (Santa Clara County)	0	2
Milpitas - Coyote Creek Reach 1A (Santa Clara County)	3	1
Milpitas - Lower Coyote Creek (Santa Clara County)	5	0
Newby Island (Santa Clara County)	36	0
Faber Marsh (San Mateo County)	4	1
Point Pinole Regional Shoreline (Contra Costa County)	31	0
Total	148	46

Bothin Marsh (Marin County), we did not catch any SMHM. All the genetically identified SMHM were restricted to the southern end of San Francisco Bay. We verified the continued existence of SMHM at Eden Landing (Alameda County), Dumbarton Marsh (Alameda County) Audubon Marsh (Alameda County), Mayhew's Landing (Alameda County), Triangle Marsh (Santa Clara County), and Milpitas (Santa Clara County) on the east shore of the South San Francisco Bay, and at Faber Marsh (San Mateo County) on the west shore of the South San Francisco Bay.

### Single-variable Analyses

We obtained largely complete sets of measurements on 186 adult harvest mice (140 WHM; 46 SMHM). Preliminary analyses did not identify significant differentiation between the sexes within species. Therefore, we grouped by species for subsequent analyses. We identified overlapping ranges of measurement for both species for all six continuous variables (Fig. 3). Tail Length and Total Length did not differ significantly between the species



**Figure 3.** Box plots displaying the range of measurements for each of the continuous variables in harvest mice from the Central and South San Francisco Estuary. The significance of a t-test is indicated: WHM = western harvest mouse (*Reithrodontomys megalotis*), SMHM = salt marsh harvest mouse (*R. raviventris raviventris*), Not sig. = not significant, \*\*\* = significant at  $P < 0.001$ , \*\*\*\* = significant at  $P < 0.0001$ .

(Table 2). Body Length, Tail to Body Length Ratio, Tail Diameter, and Mass all differed significantly between species (Table 2). Tail Diameter was the most significantly differentiated continuous character between species, where SMHM averaged 2.1 (SD 0.12) mm and WHM averaged 1.9 (SD 0.14) mm.

We identified significant differences among categorical measurements between harvest mice species (Table 3). For Tail Tip Shape and Tail Pattern, all categories were shared between species, although the proportion of each species in each category differed (Fig. 4). For Ventral Tail Hair Color one of three categories was restricted to a single species. Specifically, all harvest mice with white Ventral Tail Hair Color were WHM, thus making this a diagnostic character for the species. For Ventral Coloration five of seven categories were restricted to a single species (Fig. 5). All mice with the lightest Ventral Coloration (categories 1, 2, and 3) were WHM, and all mice with the darkest Ventral Coloration (category 7) were SMHM. Category 5 was rare and only identified in SMHM. Together the four most common Ventral Coloration categories were diagnostic for species in 86% (143 of 166 mice) of harvest mice with Ventral Coloration data.

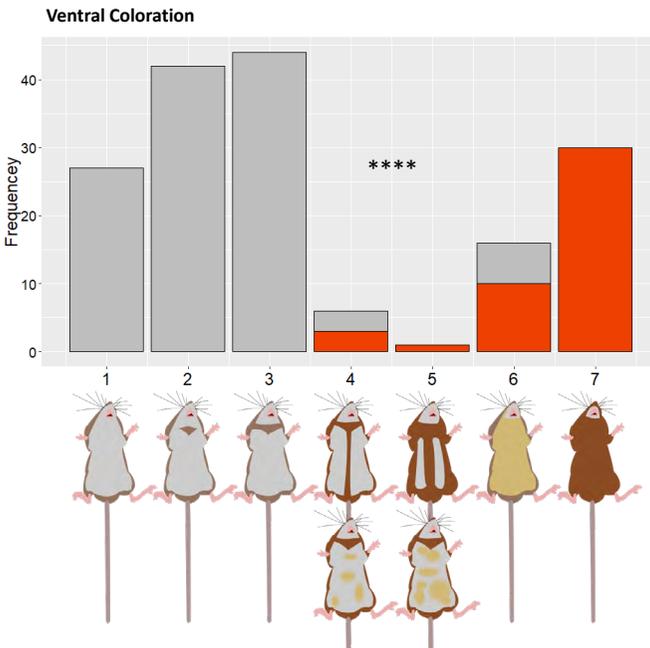
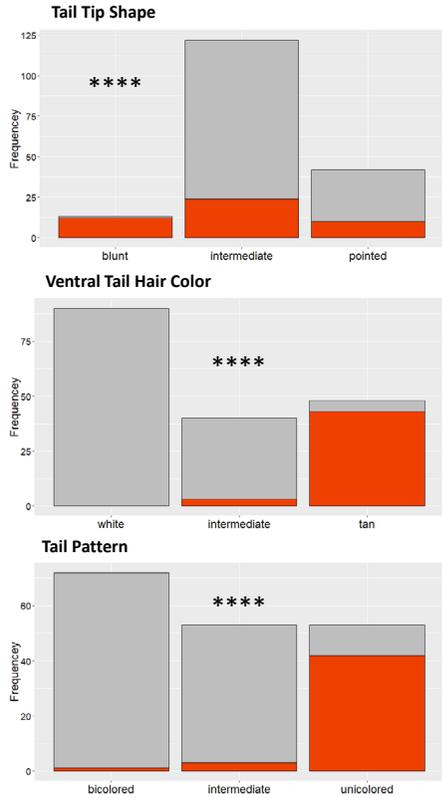
**Table 2.** Analysis of continuous morphological characters for salt marsh harvest mouse (SMHM; *Reithrodontomys raviventris raviventris*) and western harvest mouse (WHM; *R. megalotis*) in the South San Francisco Estuary. df and P are from a t-test between SMHM and WHM for each morphological character.

Character	SMHM mean	SMHM SD	WHM mean	WHM SD	df	P
Tail length (mm)	73.00	4.44	73.30	4.52	75	0.690
Body Length (mm)	66.46	4.18	63.75	4.61	81	< 0.001
Total length (mm)	139.46	7.16	137.07	7.48	80	0.057
Tail to Body Length Ratio	1.10	0.08	1.16	0.09	90	< 0.001
Tail Diameter (mm)	2.09	0.12	1.86	0.14	76	< 0.001
Mass (g)	11.24	1.90	10.02	1.80	62	< 0.001
Mass (g) without pregnant mice	10.77	1.56	9.70	1.44	51	< 0.001

**Table 3.** Comparison of categorical morphological characters between salt marsh harvest mouse (*Reithrodontomys raviventris raviventris*) and western harvest mouse (*R. megalotis*) in the South San Francisco Estuary.

Character	$\chi^2$	df	P
Tail Tip Shape	32.36	2	< 0.001
Ventral Tail Hair Color	140.15	2	< 0.001
Tail Pattern	112.6	2	< 0.001
Ventral Coloration	139.05	6	< 0.001

**Figure 4.** The frequency of harvest mice in each of the categories of Tail Tip Shape, Ventral Tail Hair Color, and Tail Pattern in the Central and South San Francisco Estuary. The significance of a  $\chi^2$  test is indicated. Grey = western harvest mouse (*Reithrodontomys megalotis*), Orange = salt marsh harvest mouse (*R. raviventris raviventris*), \*\*\*\* = significant at  $P < 0.0001$

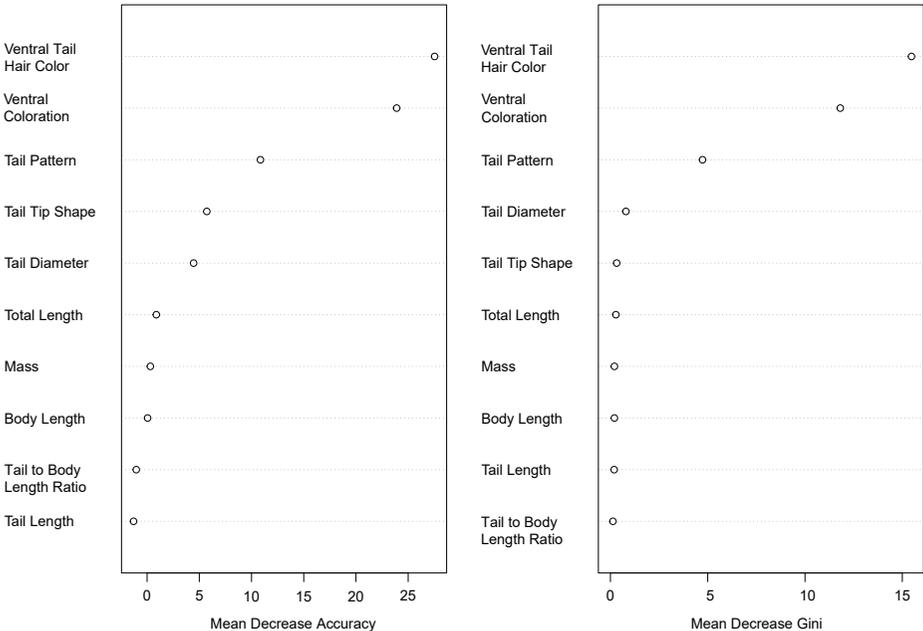


**Figure 5.** The frequency of Ventral Coloration categories in harvest mice from Central and South San Francisco Estuary. Orange = salt marsh harvest mouse (*R. raviventris raviventris*), Grey = western harvest mouse (*R. megalotis*). The significance of a  $\chi^2$  is indicated. \*\*\*\* = significant at  $P < 0.0001$ .

**Multivariable Analysis**

On the training dataset the model of 500 decision trees achieved a 97.8% accuracy rate (90 of 92 mice correctly assigned). When we applied the model to the test dataset (i.e., mice excluded from model training) it achieved a similarly high accuracy rate with 97.3% (31 of 32 mice) correctly assigned. We then assessed which variables were most important for classification (Fig. 6). For mean decrease in accuracy, the greatest drop in correct classification occurred when Ventral Tail Hair Color and Ventral Coloration were excluded. Excluding Tail Pattern, Tail Tip Shape, and Tail Diameter also resulted in some loss of accuracy. Exclusion of the remaining five characters (Total Length, Mass, Body Length, Tail to Body Length Ratio, and Tail Length) did not have a substantial impact on the model accuracy, indicating that these characters were not important for classification of harvest mouse taxa in the range of southern SMHM. The mean decrease in Gini largely identified the same most important characters, however Tail Diameter was identified as more informative than Tail Tip Shape. The same following characters were largely identified as uninformative: Total Length, Mass, Body Length, Tail to Body Length Ratio, Tail Length, and Tail Tip Shape.

**Morphological Characteristic Importance**

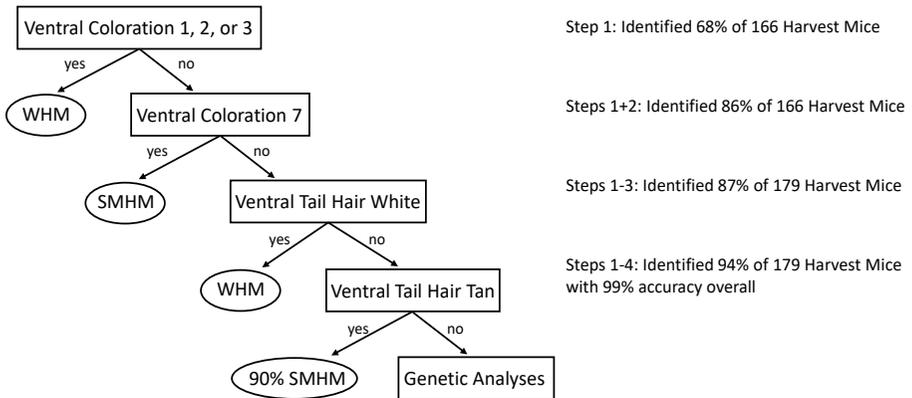


**Figure 6.** Morphological character importance for the differentiation of southern salt marsh harvest mouse (*Reithrodontomys raviventris raviventris*), and western harvest mouse (*R. megalotis*) in the Central and South San Francisco Estuary. This analysis is based on results of a random forest analysis. Here ‘Mean Decrease’ indicates the reduction of resolving power if a character is not used for classification.

## Decision Tree

We used the two most informative characters to design a decision tree for the identification of both species in the field (Fig. 7). Ventral Coloration was the primary character used because it was highly ranked in the multivariable analysis, it had well defined categories (Fig. 2), and on its own it was diagnostic for 86% of harvest mice sampled. We used Ventral Tail Hair Color as the secondary character because it was also highly ranked in the multivariate analyses, and white Ventral Tail Hair Color was diagnostic for WHM. The majority of SMHM had tan Ventral Tail Hair Color and overall it was 90% accurate for the identification of SMHM. Applying the decision tree to our dataset correctly identified 94% (169 of 179) of harvest mice to species, while the remaining 6% were unassigned.

For comparison, application of the tail character scoring method of Shellhammer (1984) correctly identified 79% of 146 individuals to species. The multiple linear regression method developed on a population of the northern subspecies (Sustaita et al. 2018) correctly identified 51% of 155 individuals to species.



**Figure 7.** Decision tree for the identification of salt marsh harvest mouse (SMHM; *Reithrodontomys raviventris raviventris*) and western harvest mouse (WHM; *R. megalotis*) in the Central and South San Francisco Estuary. The tree uses just two morphological characters to assign 94% of mice to species with 99% accuracy. Harvest mice that are not assigned to species based on these characters should be identified using genetic analyses. This decision tree was validated only for the populations in this study. Application to other populations is not appropriate unless validated.

## DISCUSSION

We identified a suite of morphological characters that can be used to reliably distinguish SMHM and WHM in the southern San Francisco Estuary. Multivariable analyses identified Ventral Tail Hair Color and Ventral Coloration as the most important characters, followed by Tail Pattern, Tail Tip Shape, and Tail Diameter. Single-variable analyses broadly agreed and identified the three color characters as the most differentiated between species, followed by Tail Diameter, then Tail Tip Shape.

Species-specific morphological categories were identified in Ventral Coloration and Ventral Tail Hair Color, thus providing diagnostic characters for the identification of harvest mouse species in the field. SMHM and WHM exhibited Ventral Coloration on opposite ends of the spectrum. All mice with the three lightest categories of Ventral Coloration (1, 2, and 3) were WHM, while all mice with the darkest Ventral Coloration (category 7) were SMHM, which together identified 86% of mice to species. Similarly, all mice with white Ventral Tail Hair Color were WHM, which was diagnostic for 67% of western harvest mice, while 94% of SMHM had tan Ventral Tail Hair Color. However, a small proportion of WHM were also identified with a tan tail, thus making tan tail 90% accurate for the identification of SMHM. Tail Pattern was also a very useful character; only a single SMHM out of 72 total harvest mice (1.4%) was identified with a bicolored tail. Among the continuous variables, Tail Diameter was the most differentiated between species. However, tail diameter is one of the most difficult dimensions to measure accurately in the field (Sustaita et al. 2018).

Ventral Coloration and Ventral Tail Hair Color identified the majority of harvest mice to species and therefore were used to design a decision tree for the dual identification of both harvest mouse species in the field. This tree identified 94% (169 of 179) of harvest mice with an overall accuracy of 99%. This is a substantial improvement over other methods for the morphological differentiation of the species in the Southern San Francisco Estuary. It is also simpler, being based on two characters that are easier to assess, as opposed to four or more.

Additional characters could be used for further classification to species of the remaining mice. However, each of the subsequent assignments would be probabilistic because of overlap in measurements or categories between species. This would result in a tentative assignment for the remaining 6% of harvest mice in the dataset. For this reason, we recommend that genetic analyses be used for species-identification of unresolved individuals.

The decision tree was validated only for adult individuals in the populations in this study. The northern SMHM subspecies and the sympatric WHM are known to display different morphological relationships than the southern populations studied here (Fisler 1965; Shellhammer 1984). Therefore, application to the northern SMHM subspecies is not appropriate. Moreover, we were unable to include SMHM specimens from the northern portion of the southern SMHM subspecies' range. Therefore, if putative SMHM are discovered in the northern portion of the southern subspecies range, genetic analyses should be used to classify them unless and until the decision tree is validated on those populations. Additionally, it would be useful to assess how well our approach works on non-adults.

Our findings were consistent with previous analysis of Ventral Coloration in San Francisco Estuary harvest mice (Fisler 1965), suggesting that the differences observed are longstanding characters of the populations. Stability of this character over time strengthens its use as the primary means for differentiating between harvest mouse species in the southern San Francisco Estuary. Fisler (1965) did not identify any WHM with red bellies (Category 7), but he did identify WHM with category 6 bellies on the Marin coast, which is consistent with our results. Shellhammer (1984) stated that all SMHM had red bellies (categories 5, 6, and 7), while WHM fell into categories  $\leq 3$ , which is also close to our own findings. Shellhammer (1984) noted that color of the hair on the ventral portion of the tail and the tail diameter are easier to judge than the pattern of the tail and the tail tip. Thus, the characters we have selected for the decision tree were previously shown to be differentiated between species, and are among the easier ones to assign correctly.

## Geographic Range of the Subspecies

Despite trapping across the putative range of the subspecies, SMHM were only identified in the marshes at the southern end of the San Francisco Estuary. The range of the southern SMHM subspecies is considered to extend north to the Central San Francisco Estuary near Point Pinole (Contra Costa County) and Point San Pedro (Marin County) (Fisler 1965; Shellhammer 1989; Fig. 1). Although we trapped >60 harvest mice in the northern end of the range (at Bothin Marsh and Point Pinole Regional Shoreline), all of them were genetically identified as WHM, suggesting that SMHM populations at these sites may be much reduced or extirpated. The remaining marshes in the Central San Francisco Estuary tend to be relatively small and fragmented, thus making resident SMHM populations more susceptible to extirpation. A survey in 2014 by the U. S. Geological Service at one such location on Marin County coast (Corte Madera, south of Pt. San Pedro, within the San Francisco Bay) did not result in any SMHM captures. Additional surveys should focus on remaining marshes in the Central San Francisco Estuary to determine if SMHM are still present.

## Recommendations

Future surveys of SMHM in the central and southern San Francisco Estuary should assess the performance of the decision tree. The decision tree should also be validated for application to harvest mice from novel locations across the southern SMHM range. The latter point is especially important in the area just south of the putative subspecies dividing line, where no SMHM were identified in the current study. SMHM were considered present historically (Fisler 1965), yet their continued presence has not been confirmed using genetically verified animals. Similarly, the subspecific status of SMHM in the area has never been assessed using genetic analyses. Therefore, we do not know whether SMHM historically or currently present in the northern end of the southern range belong to the northern subspecies, the southern subspecies, represent an intermediate between the two, or are part of a distinct lineage. Therefore, we also do not know which field identification method is the most appropriate for species identification in those locations. Our analyses only included a single SMHM from the western shore of the south San Francisco Bay necessitating further testing and potential refinement for use on harvest mice on the western shoreline.

Assigning the color characters of mice to categories can be partly subjective. To minimize observer biases, we recommend using a card, with white and tan colors, for improved assignment of Ventral Tail Hair Color. Similarly, a card for Ventral Coloration would aid in the consistency of assignment, especially for mice with fully-belly coloration (categories, 1, 6, and 7). Further, the decision tree could be validated using museum specimens that have been genetically confirmed to species. However, an assessment would need to be made to determine how well the color characters are maintained in preserved skins. Additionally, because both Ventral Coloration and Ventral Tail Hair Color were recorded in earlier surveys; the decision tree could be applied to those records to reassess historical population trends. Information from the museum specimens and historical surveys would provide an updated assessment of the SMHM presence, range, abundance, and population trends.

We have developed a simple and accurate method for the field identification of harvest mice in the southern San Francisco Estuary. The previous method required a greater number of morphological measurements and still resulted in a substantial proportion of mice unassigned to species. Following years of uncertainty, our improved and simplified field identification method will aid conservation efforts and enhance recovery of the endangered SMHM.

## ACKNOWLEDGMENTS

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FULL RESEARCH ARTICLE

## **Conservation of endangered Tipton kangaroo rats (*Dipodomys nitratoides nitratoides*): status surveys, habitat suitability, and conservation recommendations**

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The Tipton kangaroo rat (*Dipodomys nitratoides nitratoides*; TKR) is listed as endangered both Federally and by the state of California due to profound habitat loss throughout its range in the southern San Joaquin Valley of California. Habitat loss is still occurring and critical needs for TKR include identifying occupied sites, quantifying optimal habitat conditions, and conserving habitat. Our objectives were to (1) conduct surveys to identify sites where TKR were extant, (2) assess habitat attributes on all survey sites, (3) generate a GIS-based model of TKR habitat suitability, (4) use the model to determine the quantity and quality of remaining TKR habitat, and (5) use these results to develop conservation recommendations. We surveyed for TKR on 44 sites by live-trapping and detected TKR on 15 sites. Sites with TKR tended to have larger alkali scalds and no obvious sign of past tilling compared to sites without TKR. Also, sites with TKR usually had relatively sparse ground cover and seepweed (*Suaeda nigra*) was present. The non-protected Heermann's kangaroo rat (*Dipodomys heermanni*), a larger competitor, was either absent or present in relatively low numbers at sites with TKR, and when present its abundance was inversely related to that of TKR. Based on our habitat suitability modeling, an estimated 30,000 ha of moderately high or high quality TKR habitat and 60,000 ha of lower or medium quality habitat remain. However, habitat is still being lost and conversion of at least one survey site with TKR occurred during this project. Recommendations for TKR conservation are to (1) conduct additional TKR surveys on unsurveyed but suitable sites, (2) conserve suitable habitat on unprotected lands, (3) manage vegeta-

tion on occupied sites if necessary, (4) restore disturbed lands to increase suitability for TKR, and (5) research methods and conduct translocations of TKR to unoccupied sites with suitable habitat.

**Key words:** conservation, *Dipodomys nitratoides nitratoides*, endangered, habitat suitability, San Joaquin Valley, status survey, Tipton kangaroo rat

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The Tipton kangaroo rat (*Dipodomys nitratoides nitratoides*: TKR) is one of three subspecies of the San Joaquin kangaroo rat that is endemic to the San Joaquin Valley in central California (Best 1991; USFWS 1998). TKR once were widely distributed on the valley floor from about the Kings River in Kings County south to the southern end of the valley in Kern County (Fig. 1). They occur in arid scrub habitats on the valley floor, but much of this habitat has been converted to agricultural, urban, and industrial uses (USFWS 1998). By 1985, only an estimated 3.7% of historical habitat remained, and many of these lands consisted of small, isolated fragments of varying quality (Williams and Germano 1992). None of the remaining occupied habitat patches likely exceed 2,000 ha in size (USFWS 2010). Due to this profound habitat loss, fragmentation, and degradation, TKR were Federally listed as Endangered in 1988 and California-listed as Endangered in 1989 (USFWS 1998).

As of the early 2000s, TKR were known to persist at a number of locations (USFWS 2010). However, habitat loss is still occurring throughout the range of TKR and this continuing loss threatens to extirpate existing populations and could even preclude recovery. A critical conservation need is to locate remaining populations and also to identify suitable habitat so that efforts can be optimized for conserving and recovering of TKR.

Specific habitat attribute preferences for TKR are poorly quantified. According to the recovery plan that includes TKR (USFWS 1998), they are limited to arid-land communities with level or nearly level terrain. Furthermore, the plan states that shrubs typically present include spiny saltbush (*Atriplex spinifera*), desert saltbush (*Atriplex polycarpa*), arrowscale (*Atriplex phyllostegia*), quailbush (*Atriplex lentiformis*), iodine bush (*Allenrolfea occidentalis*), pale-leaf goldenbush (*Isocoma acradenia*), and honey mesquite (*Prosopis glandulosa*). Seepweed (*Suaeda nigra* [= *moquinii*]) is described as a "conspicuous semiwoody species" in areas with TKR. Shrub cover typically is sparse to moderate in areas with high TKR density. Because flat terrain on the valley floor is subject to flooding, some microtopography is considered important as it provides refugia during flood events. Finally, higher densities of TKR tend to occur on soils with higher salinity (USFWS 1998).

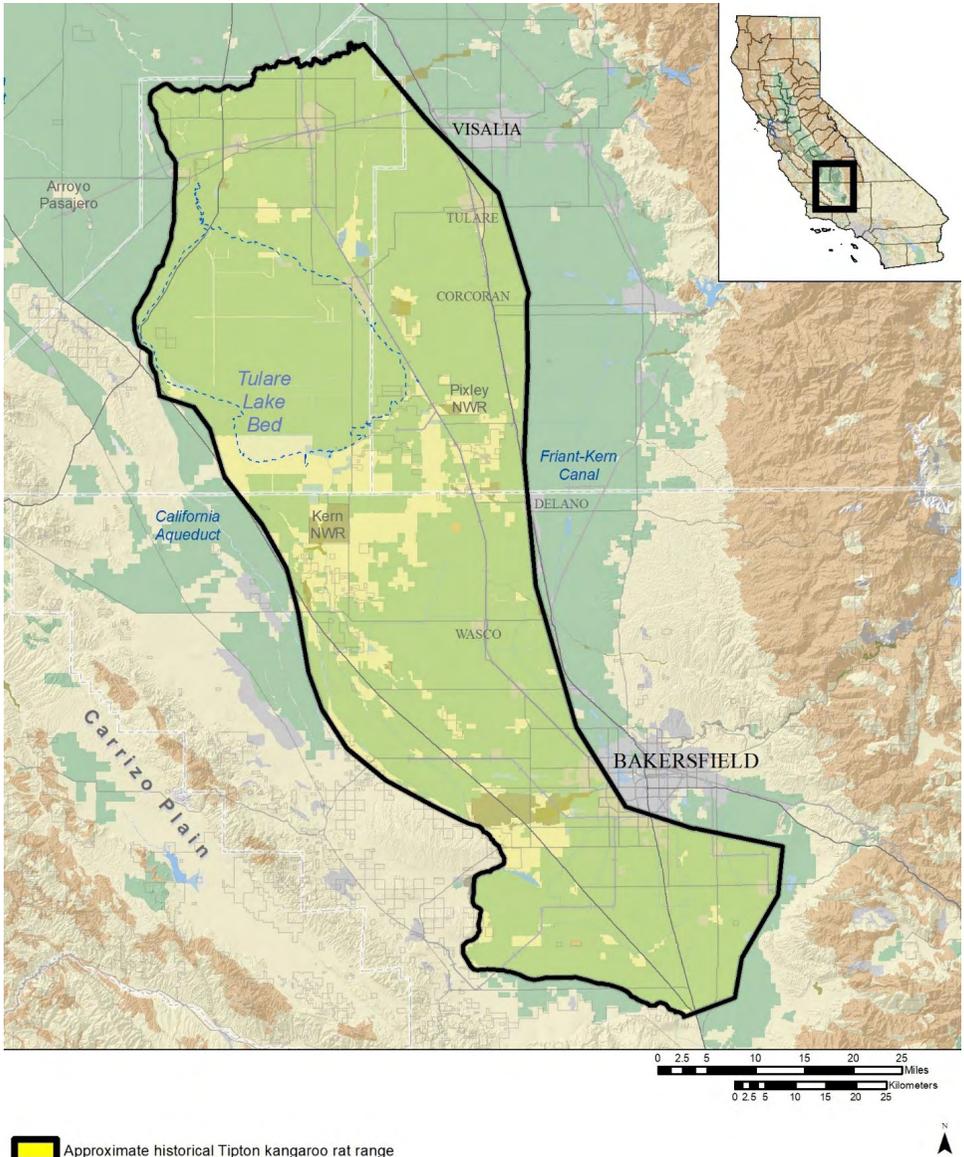
Optimal habitat attributes for TKR have not been determined. Spatially-explicit habitat suitability modeling is a powerful tool for determining suitable habitat attributes and identifying specific lands that possess those attributes. Such lands then can be targeted for protection, and conservation actions such as surveys, monitoring, and reintroductions can be considered as appropriate.

The goal of this project was to generate information and tools that will significantly enhance TKR conservation and recovery efforts. Specific objectives were to (1) conduct surveys throughout the range to identify sites where TKR were extant, (2) assess habitat attributes on all survey sites, (3) use the attribute data from sites with and without TKR to generate a GIS-based model of TKR habitat suitability, (4) extend the model across the TKR range to determine the quantity and quality of remaining habitat, and (5) use the findings from the above tasks to develop conservation recommendations.

## METHODS

### Study Area

The study area for this project was the historic range of TKR (Fig. 1). This area is within the region known as the San Joaquin Desert (Germano et al. 2011). The regional climate is Mediterranean in nature, and is characterized by hot, dry summers, and cool, wet winters with frequent fog. Mean maximum and minimum temperatures are 35°C and 18°C



**Figure 1.** Historic range of the Tipton kangaroo rat in the San Joaquin Valley, California, USA.

in summer, and 17°C and 5°C in winter. Annual precipitation averages about 15 cm and occurs primarily as rain falling between October and April (NOAA 2002).

Most of the region within the TKR range is largely flat valley bottom land with elevations generally around 100 m. Vegetation is characterized by desert scrub habitat on the upland sites and alkali sink habitat on the valley floor. Historically, there were riparian corridors along rivers and creeks that carried runoff water from the Sierra Nevada into the valley. This water collected in shallow lakes that were surrounded by seasonal wetlands (Griggs et al. 1992). Most of the riparian and wetland habitats have been drained and large proportions of the desert scrub and alkali sink habitats also have disappeared due to conversion of natural lands to agricultural, industrial, and urban uses (USFWS 1998; Kelly et al. 2005).

## TKR Surveys

To identify locations for TKR surveys, we conducted an initial habitat suitability analysis using existing information. We consulted with colleagues who had conducted small mammal surveys in the southern San Joaquin Valley to identify sites where TKR had been detected. Some of these sites were long-term monitoring plots; these sites were particularly valuable because TKR were both present and persistent over time (i.e., >20 years). We qualitatively categorized sites based on TKR abundance and persistence. High quality sites were those where multiple TKR were captured during individual trapping sessions and where TKR were consistently present based on annual monitoring or repeated surveys. “Medium” quality sites were those where only one or two TKR were captured during a given trapping session or where TKR were only intermittently detected based on annual monitoring or repeated surveys. Low quality sites were those where surveys or annual monitoring was conducted but no TKR had been detected.

Based on the criteria above, we identified 8 high quality sites, 8 medium quality sites, and 8 low quality sites. We then assessed two habitat attributes, land use and the amount of bare ground, on each of the sites using remotely sensed data layers. We used current GIS land use layers to identify lands that had not been converted to agricultural, urban, or industrial uses (USGS 2007; CDOC 2012; CDWR 2012). On undeveloped lands of 4 ha (10 acre) or more, we estimated the amount of peak growing season bare ground cover using the Web-Enabled Landsat Data, *Peak growing season Bare Ground cover per 30m pixel* dataset (USGS 2013). Based on this analysis, low quality areas had <29% bare ground, medium quality areas had 29-42% bare ground, and high quality areas had 42-60% bare ground. Areas with >60% bare ground appeared to be highly disturbed by oil development or disking and were not considered to be TKR habitat. These results were applied across the TKR range to produce a preliminary map of habitat suitability. Using this map, we selected sites within high and medium suitability areas to survey for TKR, assuming that we would have the highest probability of finding new populations on these sites.

The specific sites we chose to survey were those for which access was granted by the landowner and where no recent surveys (past 5 years) had been conducted. On these sites, we surveyed for TKR by live-trapping. On most sites, two lines of 15 or 20 traps each were set (the number of traps depended upon the amount of potential habitat on a given site). The lines generally meandered so that traps could be set close to areas with kangaroo rat activity (e.g., active burrows, fresh scats, dust baths). We used Sherman aluminum box traps (7.6 x 9.5 x 30.5 cm; H. B. Sherman Traps Inc., Tallahassee, FL) modified to prevent injury to the long tails of kangaroo rats. Traps were spaced 10–15 m apart, opened around sunset,

baited with white millet bird seed, and provisioned with a paper towel for bedding material. Traps were checked the next morning around sunrise. Captured animals were identified to species, age and sex were recorded, and then animals were marked on their ventral side with a non-toxic felt-tipped marker to identify recaptures. A site was considered to be occupied by TKR if any TKR were captured during the trapping session.

We trapped on most sites for just two nights. Prior experience by our team as well as that of colleagues indicated that this would be sufficient to detect TKR. In 79 trapping sessions on 5 long-term monitoring grids with high quality habitat in the Semitropic Ridge area in northern Kern County, TKR were detected in 100% of the sessions after just one night of trapping (G. Warrick, Center for Natural Lands Management [CNLM], personal communication). Even on one grid with lower quality habitat, TKR were detected in 75% of 12 sessions after one night of trapping and in 92% of the sessions after two nights. Similarly, in 32 trapping sessions on 4 long-term monitoring grids on the Coles Levee Ecosystem Preserve in western Kern County, TKR were detected in 84% of the sessions after one night of trapping and in 97% of the sessions after two nights (J. Jones, South Valley Biology, personal communication). Thus, when present, detection rates for TKR tend to be high in just one or two nights of trapping, even in lower quality habitat where TKR density may be lower.

### **Habitat Attributes**

At each site that we surveyed for TKR, a suite of habitat attributes was characterized and recorded (Table 1). We recorded information on alkali scalds (which are common features in alkali sink habitat), shrubs, ground cover, anthropogenic disturbances, microtopography, and distance to active agriculture. Much of this information was qualitative so that the survey sites could be characterized quickly (ca. 15 min). For shrubs, iodine bush and seepweed are commonly associated with TKR habitat (USFWS 1998) and so the presence of these species was of particular interest. For categorical variables, frequencies were compared between sites with and without TKR using contingency table analyses with a Yate's correction for continuity applied to 2x2 analyses.

To further explore variables that might affect TKR presence and abundance, we compared the frequency of the presence of Heermann's kangaroo rats (*D. heermanni*; HKR) between sites with and without TKR using 2x2 contingency table analyses with a Yate's correction for continuity. We also compared the mean number of HKR captured per 100 trapnights between sites with and without TKR using a paired *t*-test. Finally, for sites where TKR were present, we used linear regression analysis to examine the relationship between TKR and HKR abundance (number per 100 trapnights). Because of the presence of a number of zero values for HKR abundance, a square root transformation was applied to the data to correct normality prior to conducting the regression analysis (Zar 1984). Statistical tests were conducted using Excel (Microsoft Excel v. 2010) or Social Science Statistics (2020). *P*-values  $\leq 0.05$  were considered significant.

### **Habitat Suitability Modeling**

We used information on land condition and habitat attributes from the surveys (Table 1, see RESULTS) to produce a model and map of TKR habitat suitability. In particular, information on land use, disturbances, ecological community, and ground cover were incorporated into the model. We used GIS land use layers (USGA 2007; CDOC 2012; CDWR 2012) to identify undeveloped lands. Lands developed for agricultural, urban, or

**Table 1.** Habitat attributes assessed on sites surveyed for Tipton kangaroo rats in the southern San Joaquin Valley, California, USA.

Attribute	Measure
Alkali scalds	Present/absent
Average size of scalds if present	Large: > 36 m <sup>2</sup> Medium: 6-36 m <sup>2</sup> Small: < 6 m <sup>2</sup>
Shrubs	Present/absent
Average shrub density if present	Dense: < 2 m apart Medium: 2-10 m apart Sparse: > 10 m apart
Shrub species	List of species present
Average ground cover density	Dense: < 10% bare ground Medium: 10-29% bare ground Sparse: > 29% bare ground
Ground cover species	List of species with > 10% cover
Anthropogenic disturbances	List all present (e.g., OHV, tilling, trash dumping, excavations, etc.)
Microtopography	Generally flat Undulations < 25 cm tall Mounds or ridges > 25 cm tall
Distance to active agriculture	Measured in meters on Google Earth

industrial uses were considered non-habitat for TKR and were not included in the analysis. All undeveloped lands were assigned a habitat suitability rank from 1 to 4 with 1 being the highest quality habitat and 4 being the lowest. Among undisturbed lands, we used the layer described previously (USGS 2013) to estimate mean percentage of bare ground. Map units with a mean percentage of bare ground >29% were assigned to Rank 1 if the vegetation community was classified as alkali sink, or to Rank 2 if the vegetation community was classified as non-alkali sink rangelands. Undisturbed map units with a mean percentage of bare ground <29% were assigned to Rank 3. Lands with evidence of recent disturbance, such as disking or flooding, were assigned to Rank 4.

## RESULTS

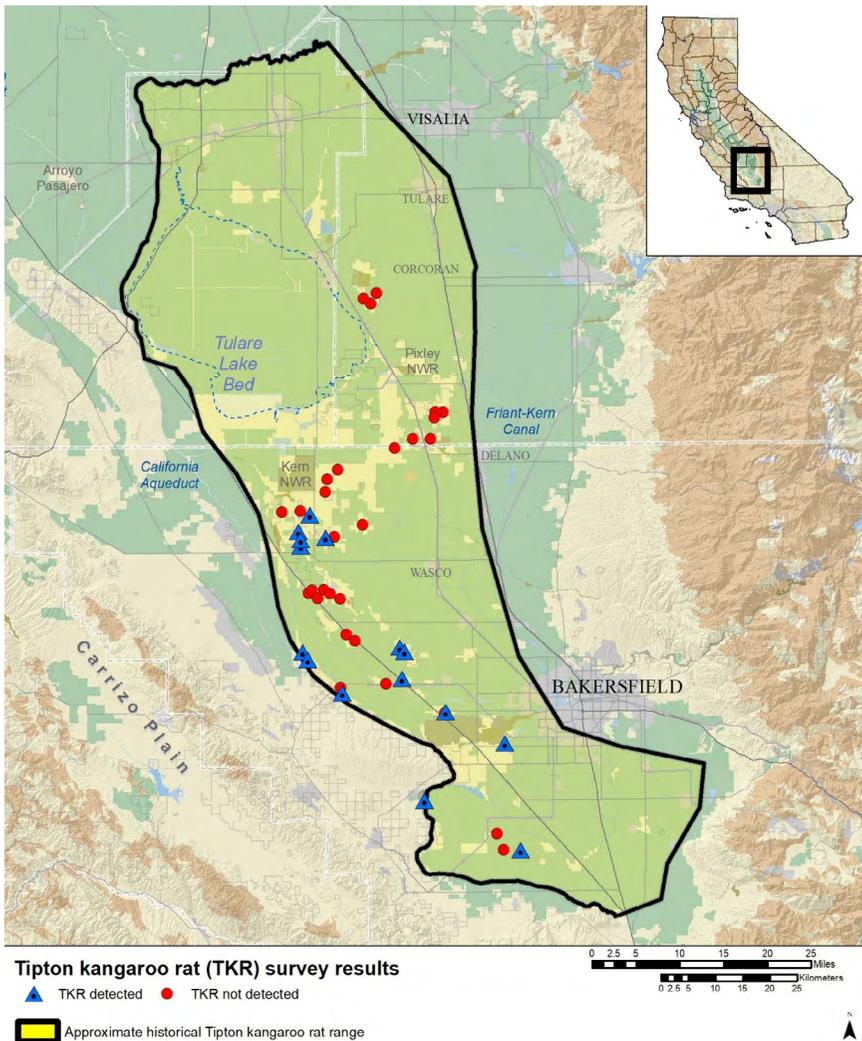
### TKR Surveys

We conducted surveys on 44 sites (Fig. 2). Most of the surveys were conducted during October 2013-May 2014. Information from four additional survey efforts conducted in November 2012, October 2014, and March 2015 also were included in our analyses. Of these 44 surveys, 32 were on California Department of Fish and Wildlife (CDFW) lands, 5 were on private lands, and 7 were on federal conservation lands (Pixley National Wildlife Refuge, U.S. Bureau of Land Management). TKR were captured on 15 sites, and were not

detected on 29 sites (Fig. 2). TKR had not been previously known to occur on 8 of the 15 sites where they were detected.

### Habitat Attributes

Habitat attribute data were collected at all sites surveyed for TKR (Table 2). Significant differences between sites with and without TKR were not detected for most attributes. However, sites with TKR tended to have larger sized scalds (Table 2). Obvious signs of past tilling were present on a greater proportion of sites without TKR (75.9%) compared to sites with TKR (7.7%; Table 2).



**Figure 2.** Sites ( $n = 44$ ) surveyed for Tipton kangaroo rats in the southern San Joaquin Valley, California, USA.

HKR were present on a significantly lower proportion ( $\chi^2_1 = 4.32, p = 0.04$ ) of sites with TKR (53.3%) compared to sites without TKR (82.8%). The mean ( $\pm$  SE) number of HKR captured per 100 trap-nights was significantly lower ( $t_{40} = -2.73, p = < 0.01$ ) on sites with TKR ( $1.8 \pm 0.6$ ) compared to sites without TKR ( $4.2 \pm 0.7$ ). On sites with TKR, the number of TKR captured per 100 trap-nights was negatively related ( $F_{1,13} = 6.10, p = 0.03, r^2 = 0.32$ ) to the number of HKR captured per 100 trap-nights.

### Habitat Suitability Modeling

Based on the TKR habitat suitability modeling, we identified approximately 30,000 ha (Table 3, Fig. 3) that were high or moderately high quality (Rank 1 or 2) habitat. We identified an additional 20,000 ha of medium quality (Rank 3) and about 40,000 ha of low quality (Rank 4) habitat.

## DISCUSSION

### TKR Occurrence and Distribution

The TKR surveys we conducted had some inherent limitations. Most of the surveys (93%) were only conducted for a maximum two nights, although for reasons stated in the methods, we felt that this usually was sufficient for detecting TKR. In addition to a limited number of trap nights at each site, we did not survey most sites in their entirety. A number of the sites were quite large (several hundred hectares) and we typically selected areas to trap where the habitat seemed to be in good condition and particularly where kangaroo rat activity was present. However, TKR could have been present in portions of sites that we did not trap. Also, most of our surveys were conducted in 2013 and 2014 when precipitation was below average and regional kangaroo rat populations generally were declining (G. Warrick, CNLM, unpublished data; E. Tennant, CDFW, unpublished data). Thus, TKR may have been present on some sites we surveyed, but in low density or patchy distributions, both of which would inhibit detection. Finally, we were not able to survey in many locations with potential TKR habitat because the sites were private lands and access was not granted.

For the reasons given above, our survey results should not be considered definitive, although when combined with trapping results by others, the cumulative findings provide an informative assessment of the current distribution of sites where TKR are extant. We detected TKR at 15 sites (see Fig. 2). We also examined results of TKR trapping survey and monitoring efforts conducted during the past 20 years. These results were provided by colleagues (G. Warrick, CNLM; J. Jones, South Valley Biology; D. Germano, California State University-Bakersfield; C. Uptain, QuadKnopf) and also by the USFWS permit office in Sacramento, CA, and they yielded additional sites where TKR have been detected. At some sites, natural habitat is no longer present based on Google Earth imagery. Disregarding these sites, another 51 sites were identified where TKR presumably still occur. These detections in combination with those from our surveys resulted in 66 sites (Fig. 4) where TKR were potentially extant as of 2017.

An important caveat is that the 66 sites do not equate to populations of TKR. A number of these sites were separated by  $< 1$  km of contiguous habitat. In some cases, different portions of the same site were surveyed but at different times. In other cases, multiple monitoring grids were located within a population area, such as occurred in the Coles Levee Ecosystem Preserve (9 grids) and Semitropic Ridge area (8 grids). Consequently, even though 66 sites

**Table 2.** Habitat attributes on sites with and without Tipton kangaroo rat detections during surveys conducted in the southern San Joaquin Valley, California, USA.

Attribute	Sites w/ TKR (n = 15)	Sites w/o TKR (n = 29)	Statistical tests
Scalds present	Yes: 12 (80.0%) No: 3 (20.0%)	Yes: 25 (86.2%) No: 4 (13.8%)	$\chi^2 = 0.28$ , 1 df $p = 0.59$
Scald size	Large: 9 (75.0%) Medium: 1 (8.3%) Small: 2 (16.7%)	Large: 8 (32.0%) Medium: 13 (52.0%) Small: 6 (24.0%)	$\chi^2 = 7.72$ , 1 df $p = 0.02$
Shrubs present	Yes: 15 (100%) No: 0 (0.0%)	Yes: 29 (100%) No: 0 (0.0%)	-
Shrub density	Dense: 1 (6.7%) Medium: 10 (66.6%) Sparse: 4 (26.7%)	Dense: 3 (10.3%) Medium: 15 (51.7%) Sparse: 11 (38.0%)	$\chi^2 = 0.90$ , 2 df $p = 0.64$
Iodine bush present	Yes: 6 (40.0%) No: 9 (60.0%)	Yes: 8 (27.6%) No: 21 (72.4%)	$\chi^2 = 0.70$ , 1 df $p = 0.40$
Sinkweed present	Yes: 11 (73.3%) No: 4 (26.7%)	Yes: 21 (72.4%) No: 8 (27.6%)	$\chi^2 < 0.01$ , 1 df $p = 0.95$
Ground cover density	Dense: 1 (6.7%) Medium: 2 (13.3%) Sparse: 12 (80.0%)	Dense: 3 (10.3%) Medium: 11 (38.0%) Sparse: 15 (51.7%)	$\chi^2 = 3.46$ , 2 df $p = 0.18$
Presently grazed	Yes: 8 (53.3%) No: 7 (46.7%)	Yes: 22 (75.9%) No: 7 (24.1%)	$\chi^2 = 2.31$ , 1 df $p = 0.13$
Previous tilling	Yes: 1 (7.7%) No: 14 (92.3%)	Yes: 22 (75.9%) No: 7 (24.1%)	$\chi^2 = 18.97$ , 1 df $p < 0.01$
Microtopography	Flat: 5 (33.4%) $\leq 30$ cm: 8 (53.4%) $> 30$ cm: 2 (13.3%)	Flat: 3 (10.3%) $\leq 30$ cm: 18 (62.1%) $> 30$ cm: 8 (27.6%)	$\chi^2 = 3.88$ , 2 df $p = 0.14$
Mean distance to agriculture	0.77 $\pm$ 0.07 km	0.61 $\pm$ 0.07 km	$t = -0.88$ , 31 df $p = 0.19$

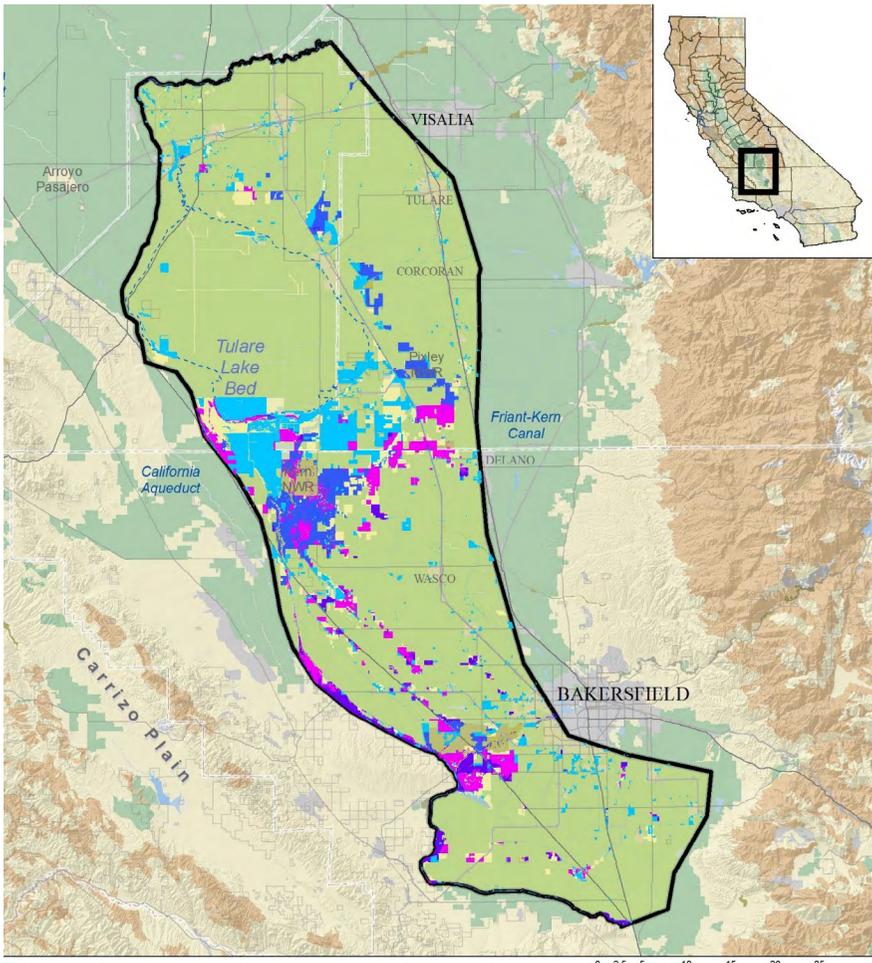
with TKR were identified, the number of actual populations clearly is <66, depending upon how “populations” are delineated.

Sites with TKR were distributed throughout the historic range of TKR (Fig. 4). More sites were extant on the western side of the San Joaquin Valley because a larger proportion of the habitat on the eastern and central portions of the valley has been converted to agricultural and other incompatible uses or not managed appropriately for TKR. Furthermore, TKR habitat is still being converted. As mentioned previously, several sites where TKR were detected in the past 20 years no longer have natural habitat, and one of our survey sites was disked in preparation for development within three months after we completed our survey. Consequently, the number of extant TKR populations continues to decline.

The distribution of sites with TKR has advantages and disadvantages for conservation. An advantage is that the sites were widely dispersed. This reduces the risk to the overall TKR

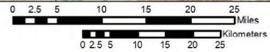
**Table 3.** Amount of remaining habitat by suitability rank for the Tipton kangaroo rat in the southern San Joaquin Valley, California, USA. Other habitats include saltbush scrub and grassland. Disturbed habitat was defined as previously tilled.

Land use	Disturbance	% Barren	Vegetation	Habitat rank	Area (ha)
Rangeland	Undisturbed	> 29%	Alkali sink	1	21,267 (24%)
	Undisturbed	> 29%	Other habitat	2	8,446 (9%)
	Undisturbed	≤ 29%	Any habitat	3	20,592 (23%)
	Disturbed	Variable	Any habitat	4	39,621 (44%)
<b>Total</b>					<b>89,926</b>



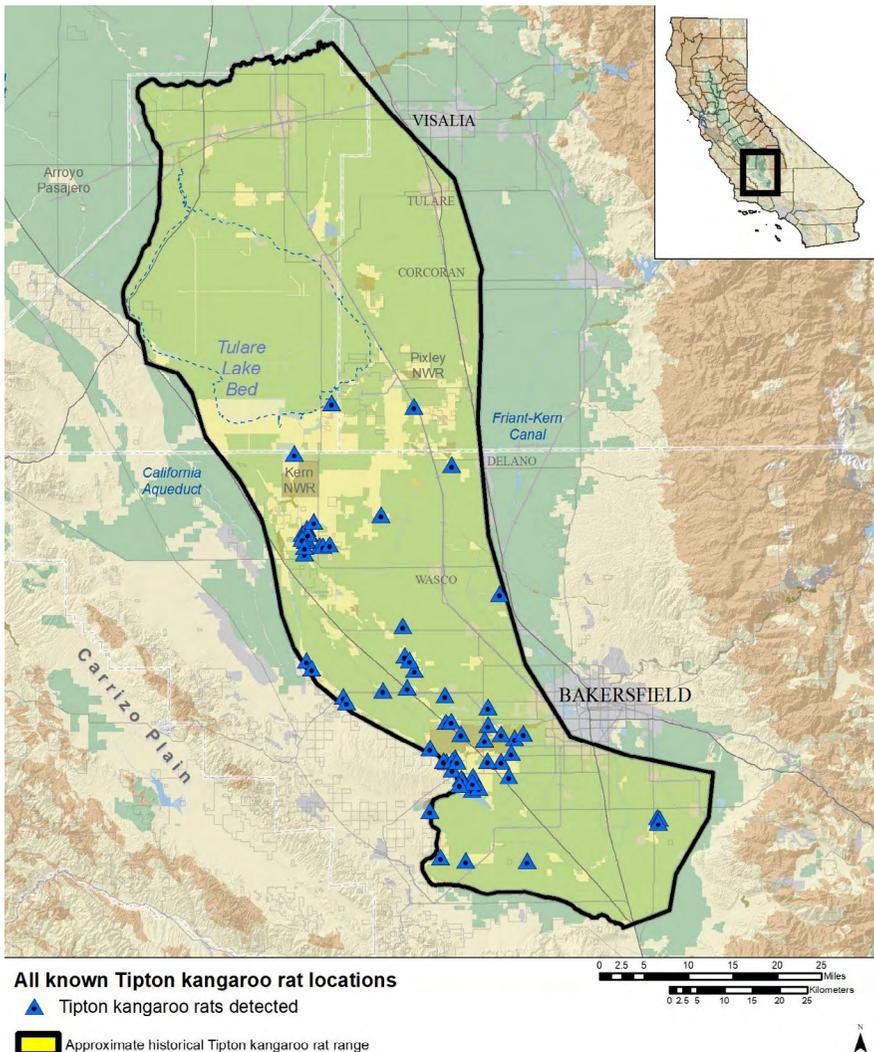
**Final suitability model**

- Habitat rank 1 (highest suitability)
- Habitat rank 2
- Habitat rank 3
- Habitat rank 4
- Approximate historical Tipton kangaroo rat range



**Figure 3.** Results of habitat suitability modeling analysis for the Tipton kangaroo rat.

population from localized catastrophic events such as flooding, fires, or disease. Flooding in the 1990s may have been responsible for the extirpation of the TKR population at Pixley National Wildlife Refuge (California State University-Stanislaus, Endangered Species Recovery Program, unpublished data) and significant reduction of the population at Allensworth Ecological Reserve (CDFW, unpublished data). The dispersed sites also increase the probability that when such catastrophic events occur, some populations will not be affected and could serve as source populations for reintroduction efforts. A disadvantage of the widely distributed sites is that in the event of local extirpation resulting from catastrophic or stochastic events, recolonization is highly unlikely without anthropogenic assistance.



**Figure 4.** Total sites ( $n = 66$ ) from previous efforts and from this study where Tipton kangaroo rats were detected and were assumed to be extant as of 2017 in the southern San Joaquin Valley, California, USA.

## Habitat Attributes

Based on the habitat description for TKR from the recovery plan (USFWS 1998) that was provided in the Introduction, as well as our previous survey experiences along with that of other researchers, we had specifically targeted sites with alkali sink habitat (e.g., alkaline playas with seepweed or iodine bush present) for surveys, but we surveyed sites with other habitat conditions as well. Generally, sites where TKR were detected usually had good quality alkali sink habitat, consistent with the habitat description in the recovery plan (USFWS 1998). The larger alkali scalds present on sites with TKR likely were indicators of good quality alkali sink habitat. The scalds themselves are not likely used extensively by TKR. We rarely found burrows in the scalds and there was little or no vegetation to provide a food source or cover.

A significant finding was that TKR rarely were found on sites with evidence of past tilling. Tilling and associated crop production likely result in the collapse of burrows and possibly direct mortality of TKR, as well as the removal of native vegetation, compaction of soil, and a reduction in microtopography. Thus, actively farmed land is unsuitable for TKR but once tilling and farming are discontinued, then TKR potentially can recolonize a site, particularly if there is adjacent occupied habitat. Recolonization of former agricultural lands has been observed among other kangaroo rat species (e.g., giant kangaroo rats [*D. ingens*]; U.S. Bureau of Land Management 2010) and at least one of our surveyed sites with TKR had evidence of past tilling. Habitat restoration might accelerate the recovery of previously farmed lands to suitable habitat for TKR.

Some survey sites, particularly in the northern portion of the range, had dense ground cover largely consisting of non-native grasses (e.g., red brome [*Bromus madritensis*], ripgut brome [*Bromus diandrus*]). TKR have previously been documented on some of these sites (e.g., Allensworth Ecological Reserve, Naval Air Station-Lemoore), but were not detected during our surveys. Most of the sites where we detected TKR had sparse ground cover. Dense ground cover renders habitat less suitable for TKR as it inhibits movements and increases predation risk (Williams and Germano 1992; Germano et al. 2001). Vegetation management may be necessary to enhance suitability on sites with dense ground cover. Such management is more likely to be necessary in the northern portion of TKR range where precipitation tends to be higher due to a north-south precipitation gradient in the San Joaquin Valley (Germano et al. 2011). Livestock grazing would be the most practical and effective strategy to reduce ground cover to more suitable levels for TKR (Williams and Germano 1992; Germano et al. 2001, 2011).

Our assessment of habitat attributes was coarse-scale. The assessments were rapid and qualitative, and they characterized entire sites. Thus, if suitable TKR habitat is defined by more subtle differences among attributes, we were less likely to detect them. Also, other factors that we did not assess (e.g., soil characteristics, flooding frequency, predator abundance) might influence the presence of TKR. Finally, past events also might determine whether TKR are present at a given site regardless of habitat suitability. Several of the sites we surveyed appeared to have suitable habitat but TKR were not detected. Many of the remaining parcels of TKR habitat are relatively small and also are isolated due to habitat fragmentation. Past events, such as flooding or rodenticide use, could have extirpated TKR from a site. Flooding was previously mentioned as the likely cause of TKR extirpation at Pixley National Wildlife Refuge and Allensworth Ecological Reserve, and also likely caused the extirpation of closely related Fresno kangaroo rats (*D. n. exilis*) from the Alkali Sink

Ecological Reserve (Williams and Germano 1992). Lack of connectivity of many sites to other occupied habitat would preclude recolonization following extirpation events. Thus, some sites with suitable habitat may not be currently occupied by TKR. Such sites may be good candidates for reintroductions of TKR.

Another important habitat attribute is the presence of competitors. Competition between larger HKR (56-74 g; Jameson and Peeters 1988) and smaller TKR (35-38 g; USFWS 1998) has long been suspected (Williams and Germano 1992; USFWS 1998), but evidence for such competition has been limited. Tennant and Germano (2013) documented a 500% increase in TKR on a plot from which HKR had been removed whereas no increase in TKR was observed on an associated control plot. Our results provided further evidence for competitive interactions between HKR and TKR. At sites with TKR, HKR were more likely to not be detected. HKR abundance was lower on average on sites with TKR, and HKR and TKR abundance were inversely related. These results suggest that HKR engage in interference competition with TKR. However, we also cannot dismiss an alternative hypothesis that habitat preferences of the two species are sufficiently dissimilar such that attributes more optimal for TKR are less optimal for HKR, and that this might be the reason at least in part for the inversely related abundance. Regardless of whether it is competition or habitat attributes, sites where HKR are abundant seem to be less suitable for TKR.

### **Habitat Suitability Modeling**

We used the best available information on TKR occurrence and habitat attributes in developing our habitat suitability model. However, we caution that as with any suitability model, the results do not guarantee that TKR are present on a parcel. Instead, modeling results should be viewed as an estimate of the potential for TKR to occur on given lands; higher suitability rankings indicate a higher probability of TKR occurrence. Surveys are the only sure way to determine whether TKR are present on a parcel.

Our model results indicated that approximately 90,000 ha of habitat of any suitability remained throughout the range of TKR. Of that 90,000 ha, about 40,000 ha are highly degraded (e.g., recent farmland) and/or fragmented and are considered low quality (Rank 4). Around 20,000 ha have dense herbaceous ground cover or lack scalds and are considered medium quality habitat (Rank 3). The remaining 30,000 ha are what we considered moderately high to high quality habitat (Rank 1 or 2) consisting of alkali sink habitat that was less-disturbed, less fragmented, and less-densely vegetated. The 5-year review conducted for TKR indicated that 24,270 ha of habitat remained as of 1985, although the methods for this estimation are not provided (USFWS 2010). Our analysis indicates that more habitat may remain, but probably not in sufficient quantity to assure the continued existence of TKR in perpetuity.

Some of the remaining good quality habitat occurs in relatively large patches (Fig. 3). Such areas include the Coles Levee Ecosystem Preserve region, Semitropic Ridge region, and Lokern region (east of the California Aqueduct) in Kern County. Other large blocks of habitat are present farther to the northeast near the Kern-Tulare County line and in southern Tulare County. Most of these northeastern lands are owned by either CDFW (e.g., Allensworth Ecological Reserve) or USFWS (e.g., Pixley National Wildlife Refuge). However, TKR were detected on few of these lands during recent surveys. Many of these more northern sites have dense ground cover, consisting largely of non-native grasses. These sites will require active vegetation management to improve or maintain suitability. Some of

these northern sites also have chronic flooding issues related to diversions of natural water flows, and this issue will need to be resolved as well.

Fortunately, many of the remaining lands with highly suitable habitat are conserved and owned/managed by conservation organizations (e.g., CDFW, USFWS, CNLM). Large blocks of highly suitable habitat on private lands occur in the Goose Lake region in Kern County and just south of the Tulare Lakebed on the Tulare-Kern County boundary (Fig. 3). These areas should be targeted for habitat protection. Additionally, lands with lower quality habitat that link patches with higher quality habitat also should be targeted for conservation. Due to their small size, TKR have limited capacity to cross large stretches of unsuitable habitat (e.g., active agricultural lands, industrial developments, urban areas) and habitat linkages would facilitate recolonizations, as well as demographic and genetic exchange.

## Conclusions and Recommendations

Based on our surveys and information provided by colleagues, TKR were still present at many locations throughout their historic range (Fig. 4). Some of these locations comprised relatively large blocks of habitat whereas other locations consisted of relatively small parcels without connections to other occupied habitat. Continuing loss of natural habitat, some of which was observed during this study, is reducing the number of sites with TKR as well as further isolating populations through habitat fragmentation. Small, isolated populations are more vulnerable to extirpation via stochastic demographic, environmental, or catastrophic processes (Frankham et al. 2017). Thus, goals for TKR conservation should include conserving as much of the remaining higher quality habitat as possible, expanding buffers around occupied habitat, and increasing connectivity between habitat patches.

We offer the following recommendations based on our results:

1. Conduct additional surveys for TKR, particularly on lands that have not been surveyed in the past 20 years.
2. Conserve unprotected lands where TKR have been detected or that have high quality habitat based on suitability modeling.
3. Manage vegetation if necessary (e.g., grazing) on lands with extant or recent TKR populations to improve or maintain suitability.
4. Restore previously disturbed lands to render them suitable for TKR.
5. Translocate TKR from appropriate source populations to restored habitat or unoccupied conserved parcels with suitable habitat. Opportunities for the latter currently exist. However, additional research on effective translocation strategies is necessary first as past TKR translocations have had poor success (Germano 2001, 2010; Germano et al. 2013; Tennant et al. 2013).

## ACKNOWLEDGMENTS

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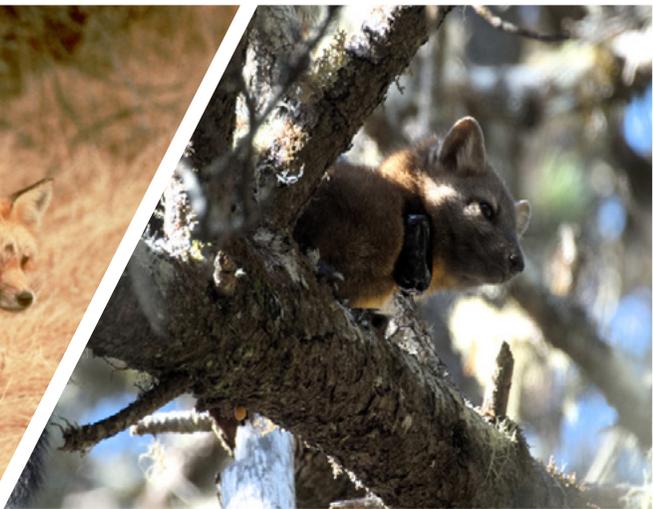
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# Carnivores & Ungulates



# Carnivores & Ungulates

Photos, previous page:

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1. Island fox (*Urocyon littoralis*). Photo Credit: Chuck Graham, U.S. Fish & Wildlife Service, Pacific Southwest Research Station, CC BY 2.0
2. Sierra Nevada bighorn sheep (*Ovis canadensis sierrae*). Photo Credit: Visit Mammoth
3. Pacific fisher (*Pekania pennanti*). Photo Credit: U.S. Forest Service
4. San Joaquin kit fox (*Vulpes macrotis mutica*). Photo Credit: Greg Schechter, CC BY 2.0
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6. Humboldt marten (*Martes caurina humboldtensis*). Photo Credit: Oregon State University, CC BY-SA 2.0

FULL RESEARCH ARTICLE

## Ecological variation among island foxes relative to reproductive events

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Ecological attributes of a species can vary as resource requirements and social interactions change in response to the annual reproductive cycle. We examined variation in home range size, home range overlap, activity (2005–2006), and food item selection (2006–2007) of island foxes (*Urocyon littoralis*) on San Nicolas Island relative to reproduction-related events. Home ranges, particularly for males, were larger during the mating period compared with the post-mating, pup-rearing, and non-reproduction periods. Home range overlap with non-mate neighbors also increased during the mating period. The greater home range size and overlap during the mating season is consistent with foxes, particularly males, traveling into the ranges of neighboring pairs in an attempt to secure extra-pair copulations. Daily activity patterns did not vary among the reproductive periods. Use of vertebrate prey items increased during the period when adults would have been provisioning weaning young. These items (e.g., mice, birds, lizards) are protein-rich and easier to transport compared with smaller items (e.g., fruits, snails, insects) that also are commonly consumed by island foxes. Variation in ecological attributes among island foxes across the different seasons defined by reproductive events likely represents efforts to maximize mating opportunities, particularly among adult males, and to secure optimal resources for provisioning growing young. These patterns are consistent with those observed among other small canid species.

**Key words:** activity, Channel Islands, food habits, home range, island fox, reproductive cycle, San Nicolas Island, *Urocyon littoralis*

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Ecological attributes of a species can vary temporally as resource requirements and social interactions change in response to the annual reproductive cycle. This is particularly pronounced in mammals, where finding and defending mates in addition to increased energetic demands associated with bearing and rearing young can influence use of space and resources (Gittleman and Thompson 1988; Oftedal and Gittleman 1989). However, the capacity for species to alter ecological patterns may be reduced in situations where space or resources are limited, such as in insular habitats (Whittaker and Fernández-Palacios 2007).

We examined variation in ecological attributes of San Nicolas island foxes (*Urocyon littoralis dickeyi*) relative to reproduction-related events. Island foxes are dwarf descendants of the mainland gray fox (*U. cinereoargenteus*) and earlier evidence based on genetics suggested that the two species diverged about 16,000 to 20,000 years ago (Gilbert et al. 1990; Wayne et al. 1991; Goldstein et al. 1999). However, more recent evidence suggests that mainland gray foxes initially colonized the northern islands about 9,200–7,100 years ago, probably by rafting or human introduction, followed by human translocation from the northern to the southern islands, and these foxes then underwent rapid evolution and speciation resulting in the six subspecies recognized currently (Rick et al. 2009; Hofman et al. 2015, 2016). These six subspecies occur on the six largest Channel Islands. All six are listed as California Threatened (CDFG 1987) because of small population sizes, and one population (Santa Catalina Island) is also listed as Threatened by the U.S. Fish and Wildlife Service (USFWS 2016) because of the high risk of disease introduction (Coonan et al. 2013). Enhanced understanding of the ecology and biology of island foxes could facilitate conservation efforts.

Island fox densities typically are higher than those of mainland gray foxes due to a lack of predators and severe limits on dispersal associated with the limited space of an island (Roemer et al. 2001). Furthermore, this insular situation results in limited resources compared to mainland areas with similar habitat conditions. Thus, these pressures might restrict or even suppress changes in ecological attributes associated with reproductive activities. San Nicolas Island is the second smallest of the islands that support a fox population, but population density commonly is higher than that on any of the other islands with foxes (Coonan et al. 2010). This high density further enhances resource and social pressures on foxes. Our objectives were to determine whether home range size, home range overlap, activity, and food item selection of island foxes varied with reproductive cycle chronology and the reproductive status of individual foxes. We compared our results to those available from other islands as well as to those for other small canids in non-insular situations.

## METHODS

### Study Area

Our study was conducted on San Nicolas Island (SNI), California (33°14'23"N, 119°27'29"W). SNI comprises 58 km<sup>2</sup> and is located in the Pacific Ocean ca. 100 km off the coast of southern California (Fig. 1). The island largely consists of an elevated sandstone plateau with steep, eroded slopes leading to the shoreline (Schoenherr et al. 1999).



**Figure 1.** San Nicolas Island and its location relative to the other Channel Islands off the coast of southern California, USA.

Maximum elevation is 277 m. Climate on the island is relatively arid with annual precipitation averaging 200 mm. Much of the island is sparsely vegetated from a combination of aridity and continued effects of past overgrazing by domestic sheep (sheep were present from the 1800s to the 1940s; U.S. Navy 2005). Primary vegetation communities are mixed coastal scrub and grasslands dominated by non-native Eurasian annual species. Non-native grassland and barren or sparsely vegetated areas (resulting from severe erosion) comprise approximately 36% of the land cover on the island; coastal scrub covers an additional 42% but much of this community is degraded by encroachment of non-native species (Junak 2008). Dominant plants include coastal goldenbush (*Isocoma menziesii*), giant coreopsis (*Leptosyne gigantea*), bush lupine (*Lupinus albifrons*), coyote brush (*Baccharis pilularis*), and non-native grasses such as slender wild oats (*Avena barbata*), ripgut brome (*Bromus diandrus*), and foxtail barley (*Hordeum murinum*).

SNI is managed by the U.S. Navy which conducts missile testing and other military support activities (USFWS 2009). The island is closed to the public; access is limited to Navy personnel, federal civil servants, and contractors. Large portions of the island are regularly closed for military operations and to protect sensitive environmental and cultural sites. Consequently, we collected data primarily in the eastern third of the island where restrictions were less frequent.

### Live-trapping and Study Animals

Island foxes were trapped and handled following protocols approved by the Institutional Animal Care and Use Committee at San Francisco State University. We trapped and radio-collared 18 island foxes: six adult females, eight adult males, and four juvenile males. We used single-door, wire-mesh box traps with dimensions of 66 x 23 x 23 cm (Tomahawk

Live Trap Co., Tomahawk, WI). We attempted to capture all foxes present in our study area. Traps were placed along roads, fox trails, and transects traversing the plateau. A rubber hose was attached to the inside of each trap as a chew bar to prevent potential injuries to the teeth of foxes caused by biting the trap. We covered the top and sides of all traps with burlap and heavy vegetation for protection from sun, wind, and dew exposure, and dry grass was placed inside the traps for bedding. We baited traps daily with wet cat food, opened at sunset, checked at sunrise, and closed during the day. Trapping began on 6 November 2005 and ended on 7 January 2006 when only recaptures were caught for several consecutive days.

For captured foxes, we observed and recorded the general health, mass, age, sex, and reproductive condition. Age was determined by an age-class system based on molar wear that was originally used for mainland gray foxes (Wood 1958), and adapted for island foxes. We attached a radiocollar (Model 1930 with mortality sensor by Advanced Telemetry Systems, Isanti, MN) to each fox, and the fox was immediately released at the capture site.

### Tracking

We tracked collared foxes from 10 December 2005–1 April 2006 (reproduction) and from 15 June–12 July 2006 (non-reproduction). We located signals from collared foxes with a hand-held H type antenna (Telonics, Inc., Mesa, AZ) and a R-1000 telemetry receiver (Communications Specialists, Inc., Orange, CA). We recorded the UTM coordinates for each monitoring location, and then the compass bearing for the direction of the strongest signal from each fox being monitored. We then moved rapidly to a second monitoring location and repeated the process. Each night, we monitored one or two of the collared females (focal females) and any other foxes in their vicinity. Monitoring began at sunset and we attempted to collect locations on each monitored fox at about 20-min intervals for a period of approximately 2 h per focal female. Females were designated as focal at intervals of  $\geq 3$  nights. We also collected one afternoon location for each fox at least twice per week.

Recommendations for achieving independence of locations require that the animal can theoretically traverse its home range in the time between recorded locations (Swihart and Slade 1985). Island fox home ranges are relatively small (see Results), and each fox in our study demonstrated an ability to move the length of its range between telemetry fixes. Also, we used the minimum convex polygon (MCP) method to estimate home ranges, and non-statistical home range methods like the MCP are less sensitive to non-independence among locations compared with statistical home range methods (e.g., kernel density), provided the temporal distribution and sample size of locations are sufficient to capture full home range use by animals (Swihart and Slade 1985).

### Activity

We recorded activity patterns of collared island foxes during four time periods: sunrise, afternoon, sunset, and night. We defined sunrise as 1.5 h before to 1.5 h after sunrise, and sunset similarly was defined as 1.5 h before to 1.5 h after sunset. Fluctuations in signal strength indicated when a fox was active. Over the course of a 2-min time period, if the signal strength was consistent, we recorded the fox as “inactive”, and if the signal strength fluctuated the fox was recorded as “active.” Activity was recorded for each collared fox a minimum of twice per week during each of the four time periods. For each fox and reproductive period, we determined the proportion of active detections.

## Food Item Selection

We did not collect island fox scats during the telemetry field work, but we did collect scats during comparable months the next year. We collected scats from December 2006 to July 2007, mostly along roads where foxes commonly scent mark. Scat samples were collected into paper bags and allowed to air-dry. We then carefully separated the contents of each scat, and identified individual food items within the samples to the lowest taxonomic level possible. We identified mammalian remains based on bone and dental fragments and guard hair characteristics. Bird identification was based on feather and foot characteristics, and we identified insects from exoskeleton characteristics. Fruits were identified based on seed and exocarp characteristics. Items were identified using guides (e.g., Moore et al. 1974, Glass 1981, Roest 1986, Young and Young 1992) or by comparison with reference collections. We were primarily interested in the temporal pattern of use of vertebrates by the foxes as these are the items that are used to provision pups post-nursing.

## Data Analyses

We defined three periods during island fox reproduction: mating, post-mating, and pup-rearing. Mating period data were gathered during 15 December–31 January. Island foxes have a gestation period of 50–53 days with parturition reported in late April or early May on Santa Cruz Island (Laughrin 1977). The mating period on San Miguel Island, another of the northern islands, is in February, also indicating that the peak of parturition would occur in April (Ralls et al. 2013). However, females in our study bore litters from early to mid-March, which indicates they were all pregnant in February and that the mating period occurs earlier on SNI, which is one of the southern islands. Therefore, we defined the post-mating period as 1–29 February and the pup-rearing period as 1 March–31 May, when parturition occurred and foxes nursed pups and subsequently provisioned them with solid food during weaning. For comparison, we also collected data during 15 June–12 July, defined as the non-reproduction period.

We used location data to analyze changes in home range size and overlap over the three reproductive periods and the non-reproductive period. We entered monitoring location coordinates and signal bearings into the program LOAS (Ecological Software Solutions, LLC, Hegymagas, Hungary) and used these data to calculate triangulated fox locations. These locations were then entered into the program BIOTAS (Ecological Software Solutions, LLC, Hegymagas, Hungary) to calculate home range size and overlap. We used the 95% MCP method to delineate home ranges because it provided a better representation of home range configuration compared to the commonly used Kernel method. The Kernel method tended to artificially divide many fox home ranges into segments consisting of ridges or plateau areas because we had difficulty obtaining locations when foxes were in the intervening deep canyons. The MCP method better captured the actual extent of home ranges and is considered a more conservative estimate of home range size (Harris et al. 1990). The minimum number of locations used to calculate a home range was 20, and the overall average was 47 locations per home range. Juveniles were excluded from home range analyses because of an insufficient number of locations. The mean number of locations used to estimate home ranges in each reproductive period was  $49.4 \pm 3.6$  for females and  $43.8 \pm 2.9$  for males. The 75% and 55% MCP ranges were calculated to identify areas of intense use, or “core areas”. Because the island fox home ranges were relatively small, the 75% MCP core areas and the 55% MCP core areas were similar and thus we used the former.

To assess whether social interactions between foxes varied with reproductive period, we calculated home range overlaps. For a given fox, overlap was calculated as the proportion of total home range or core area that was overlapped by another monitored fox; thus, these data were not completely independent as data from a given were used in the calculations of two or more overlap estimates. For each fox, we calculated overlap for three types of dyads: male-female mates, most overlapping non-mate neighbor, and most overlapping same sex neighbor. We determined mates based on observations of males being present at the dens of a particular female during pup-rearing. For the other two dyads, we determined which neighboring fox of the opposite sex overlapped the most and which neighboring fox of the same sex overlapped the most with the subject fox. Overlaps were calculated for each fox for each reproductive period, and for both 95% and 75% MCP ranges.

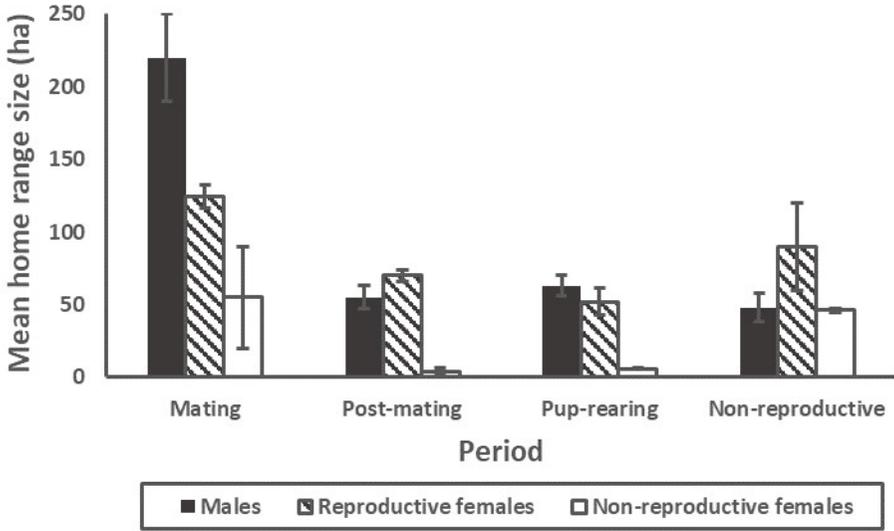
We conducted statistical analyses using SYSTAT 10.0 (SPSS Inc., Chicago). We compared mean home range size between sexes and reproductive periods with a two-way repeated measures analysis of variance. We used multivariate analysis of variance to compare mean proportional overlap of 95% and 75% ranges between sexes, dyads, and reproductive periods and to compare mean proportional activity between sexes, reproductive period, and daily period. Prior to analysis, we transformed overlap and activity proportions using an arcsine transformation (Zar 1984). Results are reported using the multivariate Wilks' lambda and post-hoc paired *t*-tests with Bonferroni corrections when applicable. All results are presented as mean  $\pm$  SE. Use of vertebrate food items was qualitatively compared between reproductive periods.

## RESULTS

### Home Range Size

Mean home range sizes of reproductive adult males and females were similar ( $F_{1,6} = 5.36$ ,  $P = 0.060$ ) although male ranges trended toward being larger than those of females during the mating period (Fig. 2). Home range size of reproductive foxes differed among reproductive periods ( $F_{3,4} = 13.48$ ,  $P = 0.015$ ). During the mating period mean home range size for both sexes was  $181.3 \pm 25.4$  ha and was significantly smaller in the post-mating ( $t_{3,7} = 4.33$ ,  $P = 0.021$ ), pup-rearing ( $t_{3,7} = 4.71$ ,  $P = 0.013$ ), and non-reproductive periods ( $t_{3,7} = 3.60$ ,  $P = 0.053$ ) averaging  $63.9 \pm 6.6$  ha,  $61.6 \pm 7.2$  ha, and  $63.5 \pm 15.8$  ha, respectively. The two non-reproductive females had very small home ranges throughout the study, especially in the post-mating ( $4.1 \pm 2.1$  ha) and pup-rearing ( $5.7 \pm 0.4$  ha) periods (Fig. 2).

*Home range overlap.*—Overlap of 95% MCP ranges among foxes was extensive (47–100%), even between non-mate neighbors. Consequently, there were no differences in amount of overlap between reproductive periods or fox dyads. However, the 75% MCP area overlaps provided more insights. Mean overlap varied among reproductive periods ( $F_{3,14} = 3.55$ ,  $P = 0.042$ ) and was significantly greater in the mating period than in the pupping period ( $t_{3,10} = 3.42$ ,  $P = 0.017$ ) but similar among all other periods (Table 1). Mean overlap varied among the three categories of fox dyads ( $F_{2,16} = 9.32$ ,  $P = 0.002$ ). It was highest for mated pairs and similar between nearest non-mate neighbors and nearest same sex neighbors (Table 1). The amount of overlap in each dyad category varied with reproductive period ( $F_{8,26} = 2.80$ ,  $P = 0.022$ ). Specifically, percentage overlap of mated pairs was larger than that of the nearest non-mate neighbor of opposite sexes and the nearest same sex neighbor in the post-mating ( $F_{2,16} = 4.61$ ,  $P = 0.026$ ), pup-rearing ( $F_{2,16} = 13.81$ ,  $P = 0.001$ ), and non-



**Figure 2.** Island fox home range sizes (mean  $\pm$  SE ha based on 95% MCP) of adult males ( $n = 5$ ), reproductive females ( $n = 3$ ), and non-reproductive females ( $n = 2$ ) in each reproductive period during December 2005–July 2006, San Nicolas Island, California, USA.

**Table 1.** Mean percentage ( $\pm$  SE) of overlap of 75% MCP ranges of island foxes by reproductive period for three dyad categories: (1) mate, (2) most overlapping non-mate neighbor, and (3) most overlapping same sex neighbor during December 2005–July 2006, San Nicolas Island, California, USA.

	Mates ( $n = 6$ )	Most overlapping non-mate neighbor ( $n = 8$ )	Most overlapping same sex neighbor ( $n = 8$ )	Average
Mating	75.5 $\pm$ 10.0	44.6 $\pm$ 10.2	53.9 $\pm$ 10.9	57.1 $\pm$ 6.4
Post-Mating	64.7 $\pm$ 7.5	33.4 $\pm$ 11.6	27.3 $\pm$ 6.5	40.6 $\pm$ 6.1
Pupping	70.2 $\pm$ 7.4	24.6 $\pm$ 8.6	24.1 $\pm$ 3.1	38.1 $\pm$ 6.1
Non-Reproductive	67.8 $\pm$ 9.4	39.0 $\pm$ 7.4	33.4 $\pm$ 6.4	45.7 $\pm$ 5.4
Average	69.5 $\pm$ 2.3	35.4 $\pm$ 4.3	34.7 $\pm$ 6.7	

reproductive ( $F_{2,16} = 5.33$ ,  $P = 0.017$ ) periods (Table 1). Home range overlap was similar for all fox dyad categories in the mating period ( $F_{2,16} = 2.26$ ,  $P = 0.137$ ).

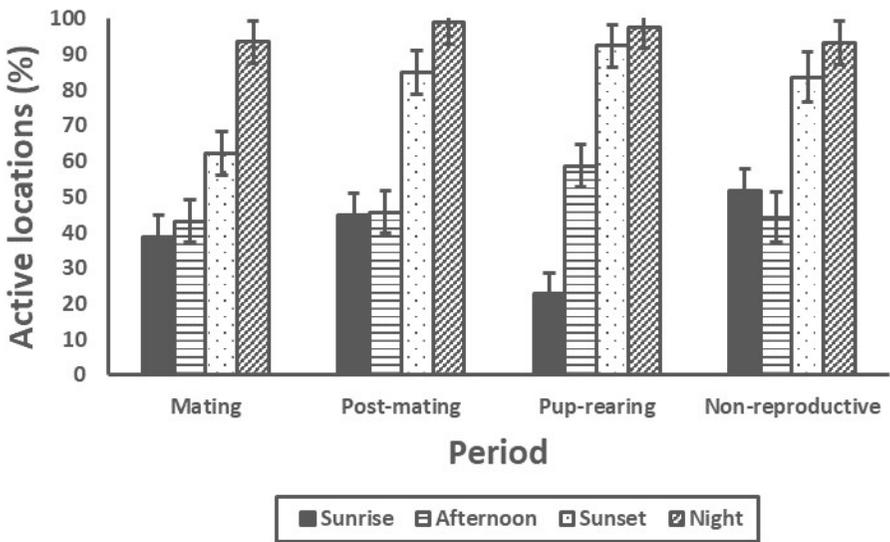
### Activity

The activity analysis was based on data from 14 foxes: 6 adult males, 5 adult females, and 3 juvenile males. There was no difference in the proportion of active locations for males and females ( $\chi_1^2 = 0.24$ ,  $P = 0.624$ ) with mean proportion of active locations estimated at 66.0  $\pm$  3.3% for males and 63.5  $\pm$  2.3% for females. Fox activity varied among the four daily periods ( $F_{3,42} = 94.73$ ,  $P < 0.001$ ). Sunrise and afternoon activity (39.6  $\pm$  2.7% and 47.9  $\pm$  2.7%, respectively) was consistently lower than sunset and night activity (80.8  $\pm$  2.7% and 95.9  $\pm$  2.8%, respectively). Activity increased from sunrise to sunset (sunrise

vs. sunset:  $t_{1,54} = 6.02, P < 0.001$ ; afternoon vs. sunset:  $t_{1,54} = 6.79, P < 0.001$ ). The highest activity occurred at night and was significantly greater than all other time periods (sunset vs. night:  $t_{1,54} = 4.02, P = 0.001$ ). Activity patterns did not vary with reproductive period. Mean proportion of active locations was  $59.4 \pm 2.7\%$  in the mating period,  $68.6 \pm 2.8\%$  in the post-mating period,  $67.9 \pm 2.8\%$  in the pup-rearing period, and  $68.3 \pm 2.73\%$  in the non-reproductive period. An interaction between reproductive period and daily period ( $F_{9,124} = 2.67, P = 0.007$ ) revealed that activity was lowest at sunrise in the pup-rearing period and lowest at sunset during the mating period (Fig. 3).

**Food Item Selection**

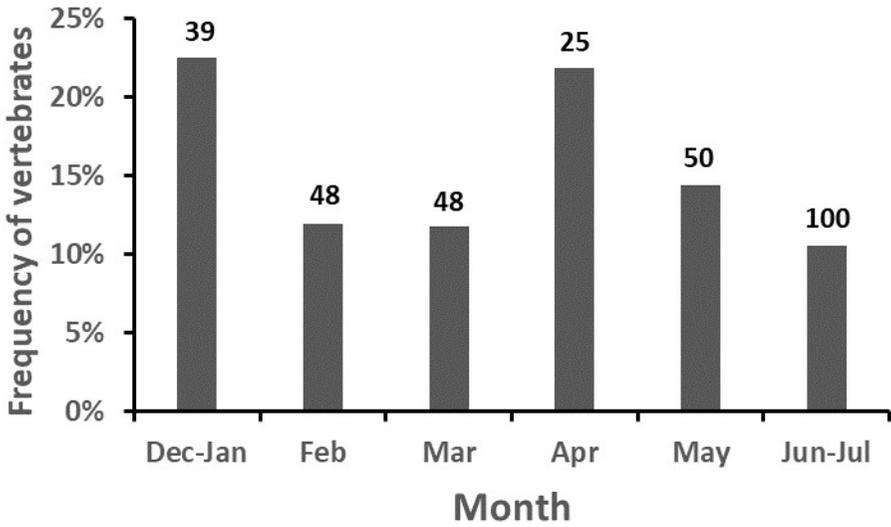
Vertebrate food items found in island fox scats consisted primarily of deer mice (*Peromyscus maniculatus*) with occasional lizards (side-blotched lizards [*Uta stansburiana*] or island night lizards [*Xantusia riversiana*]) and unidentified birds. Other food items consisted primarily of invertebrates (Jerusalem crickets [*Stenopelmatus* sp.], silk-spinning sand crickets [*Cnemotettix* sp.], ground crickets [*Gryllus* spp.], grasshoppers [Family Acrididae], ten-lined June beetles [*Polyphylla decemlineata*], June beetles [*Phyllophaga* spp.], darkling beetles [*Eleodes* spp.], beetle larvae, European earwigs [*Forficula auricularia*], European garden snails [*Helix aspersa*], sand crabs [*Emerita* spp.], and beach fleas [*Megalorchestia californiana*] and fruits (sea fig [*Carpobrotus* spp.], prickly pear cactus [*Opuntia* spp.], Australian saltbush [*Atriplex semibaccata*], Myoporum [*Myoporum laetum*], and red sand verbena [*Abronia maritima*]. Use of vertebrates was highest during December–January and during April–May, coinciding with mating and pup-rearing (Fig. 4).



**Figure 3.** Mean ± SE percent active locations of island foxes by daily period in each reproductive period during December 2005–July 2006, San Nicolas Island, California, USA.

## DISCUSSION

Variation in ecological attributes of island foxes reflected changing behavior patterns and resource use associated with reproduction. Other factors, such as seasonal variation in food resource availability, also can influence ecological attributes. However, the differences we observed among sexes and dyads suggested that social interactions relative to reproduc-



**Figure 4.** Frequency of occurrence of vertebrate food items in island fox scats during December 2006–July 2007, San Nicolas Island, California. Numbers above the bars are the samples sizes (scats).

tive events likely were a primary causal factor for the variation in attributes. Increased home range size during the mating period in both sexes likely can be attributed to incursions by foxes into neighboring home ranges seeking extra-pair copulations. The amount of time that mates spend together on San Miguel Island varies with the reproductive cycle, peaking during the mating season in February. Non-pair males and females interact more in January and February, the pre-mating and mating season on San Miguel Island, than in subsequent months (Ralls et al. 2013). Murdoch et al. (2008) reported that interactions between adult kit foxes from neighboring social groups, particularly non-pair males and females, increased sharply during the mating season and attributed this to extra-pair copulation attempts.

Mating with non-mates has been documented in island foxes (Roemer et al. 2001) and is common among other small canids such as arctic foxes (*Alopex lagopus*; Cameron et al. 2011), red foxes (*Vulpes vulpes*; Baker et al. 2004), swift foxes (*Vulpes velox*; Kitchen et al. 2006), and kit foxes (*Vulpes macrotis*; Murdoch et al. 2008; Ralls et al. 2001; Westall et al. 2019). Such extra-pair copulations may enhance the relative fitness of males by increasing their number of descendants. Several possible benefits to females include a reduced risk of mating with an infertile or closely related male and increased genetic diversity within litters (Clutton-Brock 2016). There are also potential costs to both sexes, such as the energetic costs of multiple matings and an increased risk of acquiring disease (Clutton-Brock 2016).

Both Laughrin (1977) and Fausett (1993) reported that male island fox home ranges expanded in fall and winter whereas those of females did not. Similar to our results, swift fox home ranges were found to be larger during the breeding season compared with other seasons (Hines 1980; Lebsack et al. 2012). Related, nightly movement distances, particularly for males, were reported to increase during the breeding season for both kit foxes (Zoellick et al. 1989, 2002) and swift foxes (Kitchen et al. 1999). However, in one extra-pair copulation observed in kit foxes, the female was outside of her home range (Murdoch et al. 2008).

Overlap of the 95% ranges of SNI foxes was extensive. This overlap and apparent reduction in territoriality could be a function of patchy or low resource availability, high population density, or a combination of the two. Higher fox densities were correlated with smaller home ranges on San Clemente Island (Sanchez and Hudgens 2015). Extensive overlap and loss of territoriality related to low resource density also has been reported in Darwin's foxes (*Pseudalopex fulvipes*; Jiménez 2007) and arctic foxes (*Alopex lagopus*; Angerbjörn et al. 1997, Eide et al. 2004), and commonly occurs in island species as a result of limited space (Stamps and Buechner 1985, Adler and Levins 1994). The number of foxes estimated on SNI in 2006 was approximately 542 (approximately 9.3 foxes/km<sup>2</sup>; Schmidt et al. 2007) and fox density on SNI typically is the highest among islands with foxes (Coonan et al. 2010). At high population densities, territory maintenance becomes costly because of the increased intra-specific competition (Stamps and Buechner 1985). For example, red fox home range overlap went from an average of 24% to 0% after an experimental reduction of the population density (Frey and Conover 2007).

Even the 75% ranges that more closely represent core areas overlapped considerably among SNI foxes in all periods and for all dyads. Not surprisingly, home range overlap was greatest between mates and less so between neighbors, regardless of sex. Among canids, mates share space and resources and would be expected to discourage overlap by non-mates who would compete for available resources (Kleiman 1977; Moehlman 1989; Ralls et al. 2007), and this pattern holds for island foxes (Roemer et al. 2001; Ralls et al. 2013). On Santa Cruz Island, Crooks and Van Vuren (1996) reported that home range overlaps were > 70% for mates and < 30% for non-mates, and Roemer et al. (2001) reported that mean overlap was 85% for mates and 11% for neighbors regardless of sex. These results are generally similar to the mean overlaps of approximately 70% between mates and 35% for non-mates that we observed on SNI. Significantly higher spatial overlap between mates compared with that of non-mates also has been observed among gray foxes (Chamberlain and Leopold 2000), kit foxes (Zoellick and Smith 1992; White and Ralls 1993; Zoellick et al. 2002) and swift foxes (Olson and Lindzey 2002; Schauster et al. 2002; Lebsack et al. 2012). Such overlap between mates is consistent with a monogamous mating system (Kleiman and Eisenberg 1973; Geffen and Macdonald 1992), which is the typical system among small canids (Kleiman 1977, 2011; Moehlman 1989).

The observed variation in home range overlap among reproductive periods was likely attributable to an effort by foxes to secure extra-pair copulations. Overlap was higher between mates compared with non-mates in all periods except mating. During mating, overlap increased between non-mates and did not differ from that of mates. Similarly, Lebsack et al. (2012) documented increased overlap between neighboring swift foxes during the breeding season and concluded that this overlap likely resulted from foxes attempting to secure extra-pair copulations. Zoellick and Smith (1992) concluded the same for kit foxes after not only finding a similar increase in overlap during the breeding season, but also finding males in dens with neighboring females.

SNI foxes exhibited considerable activity during all daily periods. Most foxes exhibited activity during the sunset and night periods. Additionally, about 40% of fox locations were active during the sunrise period and almost half of fox locations were active during the afternoon period. These patterns are similar to those reported for foxes on Santa Cruz Island (Hudgens and Garcelon 2011), and considerable diurnal activity also was reported for foxes on Santa Catalina Island (Swarts et al. 2009). Observed activity patterns on SNI were generally consistent across all reproductive periods indicating that activity was not influenced by reproductive events. The relatively high diurnal activity was possibly a function of a lack of predators, particularly avian predators, which would be active during the day. Indeed, a significant shift to nocturnal activity by foxes on Santa Cruz Island was observed after golden eagles (*Aquila chrysaetos*) became established and began preying on foxes (Swarts et al. 2009; Hudgens and Garcelon 2011). Closely related gray foxes are considered to be primarily nocturnal but can exhibit considerable diurnal activity (Yearsley and Samuel 1980; Haroldson and Fritzell 1984). Trapp and Hallberg (1975) also reported variation in diurnal activity among gray foxes and suggested this might represent behavioral plasticity facilitating exploitation of more diurnal prey species.

Island foxes consume a diversity of food items including vertebrates, invertebrates, fruits, and anthropogenic material (Cypher et al. 2014). In winter 2005–2006, 184.2 mm of precipitation was recorded on SNI, which is just under the 200 mm annual average. Thus, no food items likely were extraordinarily abundant or rare. Food item selection by SNI foxes probably reflected seasonal item availability as well as requirements for reproduction. Use of vertebrate prey items was higher in December–January (mating period), and this likely was a function of lower invertebrate and fruit abundance during winter. Use of vertebrates also increased in April and, to a lesser extent, May. Pups were born in early–mid March. Weaning usually begins at 3–4 weeks old and this process generally lasts for 4–8 weeks (Moore and Collins 1995). Thus, pup provisioning would have occurred during April and May, coinciding with the period of increased vertebrate use we observed.

Vertebrates tend to be preferred items for provisioning pups because they are relatively large, protein-rich items that are more easily transported to pups compared with smaller items like invertebrates. Likewise, although they have diverse diets as do island foxes, kit foxes primarily provision pups with kangaroo rats (*Dipodomys spp.*; B. Cypher, unpublished data), and coyotes (*Canis latrans*) in Illinois primarily provisioned pups with white-tailed deer fawns (*Odocoileus virginianus*; Cypher 1993). Island fox scat samples were collected as encountered, and therefore our sample likely included scats from both reproducing and non-reproducing individuals. Use of vertebrates in April and May might have been considerably higher if samples could have been collected only from reproducing foxes that were provisioning pups.

We conclude that variation in ecological attributes among island foxes across the different seasons defined by reproductive events likely represents efforts to maximize mating opportunities, and to secure resources optimal for provisioning growing young. These patterns are consistent with those observed among other small canid species. We also acknowledge that other factors can contribute to variation in the attributes, particularly temporal and spatial variation in food resource availability. All of the foxes monitored in our study were in the same general area on SNI, so spatial variation in resources probably was not a significant factor. Temporal variation in resource availability potentially could have occurred as the island fox reproduction period on SNI coincided with the wetter winter season and the non-

reproduction period coincided with the drier summer season. Future investigations could examine variation in ecological attributes of island foxes relative to spatial and temporal (e.g., seasonal, annual) variation in resource availability as well as reproductive events and social interactions.

### ACKNOWLEDGMENTS

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## FULL RESEARCH ARTICLE

**Artificial dens for the conservation of San Joaquin kit foxes**BRIAN L. CYPHER<sup>1\*</sup>, JAMES D. MURDOCH<sup>1,2</sup>, AND ALEX D. BROWN<sup>1</sup><sup>1</sup> *California State University-Stanislaus, Endangered Species Recovery Program, One University Circle, Turlock, CA 95382, USA*<sup>2</sup> *Wildlife and Fisheries Biology Program, Rubenstein School of Environment and Natural Resources, University of Vermont, Burlington, VT 05405 USA*\* *Corresponding Author: bcypher@esrp.csustan.edu*

San Joaquin kit foxes (*Vulpes macrotis mutica*) are federally endangered and California threatened, primarily due to profound habitat loss. Kit foxes are obligate den users and in some locations den availability may be limited due to natural or anthropogenic factors. We conducted a study during 2001–2004 to determine whether kit foxes would use artificial dens, and if so, whether they exhibited a preference for den designs or construction materials. We tested six different den designs, four different construction materials, and two different chamber types. We constructed 34 dens in 12 locations in Bakersfield, CA. We conducted 9,271 den checks and detected kit foxes or their sign on 1,198 of those checks. Kit foxes may not have found one of the locations, but kit foxes used (i.e., entered) 29 of the 31 dens at the other 11 locations. Kit foxes did not exhibit preferential use of any designs, materials, or chamber types. Internal conditions (i.e., temperature and relative humidity) within artificial dens can provide thermoregulatory and moisture conservation benefits to foxes, although these benefits were not as strong as those provided by natural dens. At least nine other species were documented using the artificial dens, including some that might compete with kit foxes. San Joaquin kit foxes readily used artificial dens and clearly such dens can be used to mitigate den losses or to enhance habitat for kit foxes. Due to lower cost and ease of installation, we recommend installing two-entrance dens constructed of high-density polyethylene plastic with an irrigation valve box for a subterranean chamber.

**Key words:** artificial dens, conservation, internal climate, mitigation relative humidity, San Joaquin kit fox, San Joaquin Valley, temperature, threatened, urban environment

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The San Joaquin kit fox (*Vulpes macrotis mutica*; SJKF) is endemic to the San Joaquin Desert in central California (U.S. Fish and Wildlife Service [USFWS] 1998; Germano et al. 2011). The SJKF once was widely distributed in arid shrubland and grassland habitats

in the San Joaquin Valley, Carrizo Plain, and Cuyama Valley with intermittent populations occurring in the Salinas Valley. Considerable habitat in this region has been converted to agricultural, urban, and industrial uses (Kelly et al. 2005; Cypher et al. 2013). Due to this profound habitat loss, the SJKF was listed as federally endangered in 1967 and California threatened in 1980 (USFWS 1998).

A significant attribute of SJKF ecology is their obligate use of subterranean dens (Grinnell et al. 1937). Use of dens by most other North American canids is limited to the period of parturition and early young rearing. However, kit foxes, along with closely related swift foxes, (*V. velox*) are unique in using dens daily throughout the year (Cypher 2003). Dens are used not only for rearing young but also for diurnal resting, predator avoidance, thermoregulation, and water conservation (Koopman et al. 1998). Consequently, kit foxes annually use multiple dens, which are dispersed throughout each individual's home range. At Elk Hills in western Kern County, kit foxes used an average of 11.8 dens per year with a maximum of 16 dens (Koopman et al. 1998). At Camp Roberts in northern San Luis Obispo County, the average number of dens used annually by each individual SJKF over 3 years ranged from 11.4 to 15.5 dens with a maximum of 49 dens used by one fox in one year (Reese et al. 1992). Thus, dens are a critical resource for kit foxes and den availability can affect kit fox occupancy and persistence in a given area.

Past and continuing habitat fragmentation and degradation within the range of the SJKF can result in lower densities of foxes or even intermittent occupation of impacted areas (USFWS 1998). Without routine maintenance by foxes, dens deteriorate and eventually disappear with lack of use, and therefore infrequent use of an area by kit foxes can result in low den availability. An absence or scarcity of dens can inhibit use of an area by kit foxes, thus further limiting the abundance and distribution of the SJKF. Similarly, lands that may be "retired" from other uses (e.g., agriculture) and restored as habitat would also be lacking in dens, which could inhibit colonization by kit foxes. Although kit foxes are able to dig new dens, the creation of a network of dens required to successfully occupy an area may take months or even years.

Artificial dens are a potential solution to a dearth of natural kit fox dens. Artificial burrows have been constructed and successfully used by a number of species including burrowing owls (*Athene cunicularia*; Smith and Belthoff 2001), Chatham petrels (*Pterodroma axillaries*; Sullivan et al. 2000), desert tortoises (*Gopherus agassizii*; Bulova 1993), and eastern woodrats (*Neotoma floridana*; Horne et al. 1998). Artificial dens also have occasionally been constructed within the range of the SJKF to mitigate the destruction of natural dens or to enhance habitat (Harrison et al. 2011b). However, use of artificial dens by kit foxes and preference by foxes for particular designs or den materials have not been assessed.

During 2001–2004, we investigated use of artificial dens by SJKF in the city of Bakersfield. Our goal was to determine whether artificial dens might constitute a useful conservation tool for SJKF. The specific objectives of our investigation were to (1) determine whether kit foxes would use artificial dens, (2) determine whether kit foxes exhibited any preference among den designs and construction materials, (3) determine for what purposes kit foxes used artificial dens (e.g., resting, pup-rearing), and (4) compare internal climate conditions (temperature and relative humidity) between natural dens and artificial den designs.

## METHODS

### Study Area

We assessed use of artificial dens by SJKF at sites in the city of Bakersfield, California. Bakersfield is located in Kern County in the southern San Joaquin Valley, and is bounded by occupied SJKF habitat to the northeast and southwest (Cypher et al. 2013). The current human population of Bakersfield is ca. 390,000. Average elevation is 124 m, with little topographic variation. Climate is characterized by hot, dry summers and cool winters with infrequent precipitation in the form of rain. Average temperatures range from 13.7 C and 3.9 C in December and 36.2 C and 21.4 C in July. Mean annual precipitation is 164 mm (NOAA 2020).

A robust SJKF population occurs in the urban environment of Bakersfield (Cypher 2010; Cypher and Van Horn Job 2012). Kit fox numbers have been estimated at over 100 animals and possibly up to 400. Demographically, the population exhibits high survival and reproduction. Ecologically, the foxes forage for both natural and anthropogenic foods and use a variety of urban habitat types (Cypher and Warrick 1993; Cypher 2010; Deatherage et al. in press). They establish earthen dens in undeveloped lots, school campuses, golf courses, canal banks, drainage basins, and railroad and power line rights-of way. Kit foxes also have been found denning in culverts, pipes, rubble piles, and under buildings (Frost 2005; Cypher 2010).

We chose this study area because SJKF were abundant (Cypher 2003), thereby increasing the potential to observe use of artificial dens by kit foxes. Also, natural dens were abundant in the study area (Frost 2005; Bjurlin et al. 2005; CSU-Stanislaus Endangered Species Recovery Program unpublished data). Thus, we assumed that kit foxes would only use artificial dens by choice and any preferences by foxes for particular designs or materials would be easier to detect.

### Den Designs, Materials, and Installation

We used six different designs of artificial dens: two surface den designs, two designs of subterranean dens without chambers, and two designs of subterranean dens with chambers. The two surface den designs consisted of one straight pipe either 3 m long or 6.1 m long. The pipes were laid on the ground surface and covered with ca. 0.5 m of dirt to provide some thermal insulation (Fig. 1 and 2). Both ends of each pipe were left open to provide two entrances into the den. The longer design would allow foxes to be farther away from the entrances and might give the foxes a greater sense of security. Therefore, they might be more inclined to use this longer design compared to the shorter design.

The second two designs were subterranean dens without chambers. One design consisted of a 3-m length of pipe buried underground at approximately a 30-degree angle (Fig. 3). The upper end of the pipe was exposed thus providing an entrance. The lower end, although buried, was not capped thereby providing an opportunity for foxes to excavate farther and expand the den if they desired. The other non-chambered subterranean design consisted of a "U" shaped configuration (Fig. 4). A 1-m length of pipe was buried approximately 0.5 m underground. Each end of that pipe connected at a 45-degree angle to 1.5-m pipes that extended to the surface thereby providing two entrances to the den.

The last two designs were subterranean dens with chambers. One design consisted of a 1.5-m length of pipe with one end exposed and the other end buried. The buried end was



**Figure 1.** A 3-m long concrete surface den for San Joaquin kit foxes before and after being covered with soil in Bakersfield, CA.



**Figure 2.** A 6.1-m long metal surface den being used by San Joaquin kit foxes in Bakersfield, CA.



**Figure 3.** A PVC one-entrance subterranean den being installed for San Joaquin kit foxes in Bakersfield, CA.



**Figure 4.** A HDPE two-entrance subterranean den being installed for San Joaquin kit foxes in Bakersfield, CA.

connected to a subterranean chamber using either a 45-degree or a 90-degree elbow joint (Fig. 5). The other design was similar, but it had a second entrance pipe and elbow joint leading into the opposite side of the chamber (Fig. 6). The bottoms of the chambers were buried approximately 1–1.5 m deep, and the bottoms were left open thereby allowing foxes to excavate further and expand the dens if desired.

Pipes used to construct artificial dens consisted of four materials (see Figs. 1–7): Schedule 40 polyvinylchloride (PVC), corrugated galvanized aluminum (metal), double-walled high-density polyethylene plastic (HDPE), and cement. The cement pipes were 25 cm (10 in) in diameter while all other types of pipes were 20 cm (8 in) in diameter. Strips 10 cm in width were cut out of the bottom of HDPE and PVC pipes to enhance traction for foxes and water drainage. Elbow joints consisted of PVC or HDPE with a 45-degree bend, or galvanized aluminum with a 90-degree bend. All pipe-pipe and pipe-elbow connections were covered with an approximately 60 x 20-cm strip of plastic (ca. 3-mm thick carpet runner) to exclude dirt from entering through the connection.

Two types of structures were used for artificial den chambers. One chamber type consisted of a hard plastic box used commercially to cover underground valves that are part of landscape irrigation systems (Fig. 5). The box measured 61 x 51 x 51 cm. The second chamber type consisted of a small-sized igloo style doghouse or “dogloo” (Fig. 6). The dogloos were made of hard plastic, measured approximately 61 cm tall, and had a diameter of approximately 76 cm at their base. The entrance pipes were connected to the chambers through holes cut in the sides of the chambers. All den materials were purchased or ordered through local businesses in Bakersfield.



**Figure 5.** A HDPE one-entrance chambered den with an irrigation box chamber being installed for San Joaquin kit foxes in Bakersfield, CA.



**Figure 6.** A HDPE two-entrance chambered den with a dogloo chamber being installed for San Joaquin kit foxes in Bakersfield, CA.

We chose locations in which to establish artificial dens based on several criteria. We knew of areas frequented by kit foxes based on telemetry data, sign (e.g., dens, tracks, scats), and opportunistic observations of foxes (Frost 2005; Bjurlin et al. 2005; CSU-Stanislaus Endangered Species Recovery Program, unpublished data). Such areas were targeted because establishing artificial dens in these areas would increase the potential for discovery by foxes. We also chose locations that we would be able to freely access to conduct monitoring. Finally, dens were only installed on sites where we secured permission from landowners. The final sites chosen included the tops of banks surrounding municipal sumps (storm water drainage basins), canal rights-of-way, golf courses, and a field within a natural area on a university campus. All of these sites were within fences, which reduced human access, but that were permeable to kit foxes.

At each location, a “complex” of three dens was installed (Fig. 7). Each complex included a surface den, a subterranean den, and a chambered subterranean den. Our intent was to provide foxes with a choice of designs. However, no surface dens were installed at two golf course locations, per the landowner’s request. Within a given complex, the dens were constructed of different materials to provide foxes with a choice of materials. Most of the artificial dens were installed using hand tools. However, for complexes located in five sumps owned by the city of Bakersfield, a backhoe and crew were provided to excavate holes for the dens although the dens still were buried by hand. After installation, 2-3 shovels of dirt were tossed down each den entrance to provide a more natural feeling floor.



**Figure 7.** An artificial den complex being installed for San Joaquin kit foxes in Bakersfield, CA. A concrete two-entrance non-chambered subsurface den is being installed on the upper slope and a metal two-entrance chambered den with a dogloo chamber is being installed on the lower slope. A surface den also was installed on the flat ground above the other dens.

## Monitoring Den Use

To determine whether kit foxes were using the dens, we used sifted soft dirt or diatomaceous earth to create a track station approximately 0.5 m<sup>2</sup> in area in front of each den entrance. We also extended this track station into the den entrance to help determine whether foxes were actually entering the dens. Our goal was to visit each den complex every 2-3 days to assess use by kit foxes. Use by other species also was recorded. Track station data were supplemented with other information such as observations of kit foxes (or other species) entering or exiting dens during monitoring. Also, kit foxes with radio-collars were being monitored in the study area concurrently for another study, and collared foxes occasionally were tracked to the artificial dens. Finally, in spring 2004, trail cameras (Cuddeback Trail Cameras; Non Typical, Green Bay, WI) were used to determine whether pups were present at dens exhibiting possible pup sign (e.g., small tracks and scats, digging, prey remains), and this information also was used to supplement the track station data.

Kit fox detection rates were calculated for each den by dividing the number of kit fox detections by the number of times a den was monitored. Prior to statistical analysis, these rates were transformed using an arcsin transformation to normalize data (Zar 1984). Mean transformed rates were then compared among den categories (surface, subterranean, and chambered) and among materials (cement, metal, PVC, and HDPE) using a one-way analysis of variance and *F*-test. Mean rates also were compared between the two surface den types, the two subterranean den types, the two chambered den types, and the two chamber types using *t*-tests.

## Den Climate Measurements

We measured the temperature (°C) and relative humidity (%) of natural and artificial dens in Bakersfield with a HOBO Micro Station 4-channel data logger (Onset Computer Corporation, Pocasset, MA) outfitted with 3-m and 20-m thermistor temperature/relative humidity probes. The two probes provided near-identical temperature (within 0.1 °C) and humidity (within 1%) readings. Measurements were conducted during two periods in 2003: 5–20 August (summer) and 8–28 December (winter). To standardize for variation due to time of day, we collected measurements from dens only during 1200–1500h.

We had identified natural dens from a concurrent investigation in which radio-collared kit foxes were being tracked to dens several times per week. From these known natural dens, we selected dens that were in the vicinity of artificial den complexes and that had been used by a radio-collared fox within the previous four months. The selection of dens included both single-entrance and multiple-entrance dens.

We inspected all dens, both natural and artificial, using a burrow probe to ensure that each den was not occupied by foxes or other species prior to taking measurements. Animals in the dens likely would elevate temperature and humidity thereby confounding our measurements. Also, we wanted to avoid harassing animals when we inserted the probes. We deployed the 20-m probe into dens using a small, remote controlled tractor outfitted with a miniature infrared camera. This was a “home-made” system constructed by a colleague. For chambered dens, we situated the probe in the middle of the chamber. For subterranean dens, we situated the probe at the furthest below ground point in the tunnel for the one-entrance dens, and in the middle of the underground 1-m section for the two-entrance dens. For surface dens, we positioned the probe at the midpoint of the pipe. For natural dens, we measured

conditions at a point between 2 to 4 m into the den, which was similar to the distance at which measurements were collected in the artificial dens. For all dens assessed, we recorded ambient temperature and relative humidity measurements using the 3-m probe positioned just within the den entrance (usually about 0.5 m in) so that it was not in direct sunlight.

After positioning both probes, we waited 10 min before recording measurements to allow each probe to fully equilibrate to the surrounding conditions (as per manufacturer recommendations). After the 10-min equilibration period, the probes recorded temperature and relative humidity every 10 sec for 5 min into the data logger. The logger simultaneously logged den conditions and ambient conditions. The final values recorded by the logger represented a mean of the measurements recorded during the 5-min monitoring period.

For both the summer and winter periods, we used *t*-tests to compare mean temperature and mean relative humidity between: natural and artificial dens; both den types and ambient conditions; one-entrance and two-entrance natural dens; surface and subterranean artificial dens; one-entrance and two-entrance subterranean artificial dens; and chamber types. For both the summer and winter periods, we used a one-way analysis of variance to compare mean temperature and mean relative humidity among the subterranean artificial dens constructed from the four different materials.

All statistical tests were conducted in SPSS Statistics (ver. 27; IBM Corporation, Armonk, NY). We used  $\alpha = 0.05$  for all tests.

## RESULTS

Artificial den complexes were installed at 11 locations during July-September 2001 (Table 1). An additional complex (Sump 56) was installed in July 2002. A total of 34 dens were installed among the 12 complexes (Table 1). From July 2001 to June 2004, 9,271 den monitoring checks were conducted and kit foxes were detected on 1,198 of those checks. Eleven of the 12 complexes were used by kit foxes. No visits by kit foxes to the Calloway complex were ever detected. Kit foxes likely were not present in this area during the study based on field observations, surveys, and trapping efforts related to the radio-collar study. We excluded the dens in this complex from the analyses of kit fox use of designs and materials. Kit foxes used 29 of the remaining 31 dens (Table 1). Kit foxes were detected at 9 dens within 1–3 days following installation and at 14 dens within 7 days of installation (Table 1). First detections at the remaining dens ranged from 11–922 days.

Kit foxes used dens of all designs and materials. Mean kit fox detection rates (Table 2) did not differ statistically among the three den-type categories ( $F_{2,28} = 1.025, p = 0.372$ ) or among the four pipe materials ( $F_{3,27} = 0.730, p = 0.543$ ). Mean detection rates also were not different between the two surface den designs ( $t_7 = -0.764, p = 0.470$ ), the two subterranean den designs ( $t_9 = -0.578, p = 0.577$ ), the two chambered den designs ( $t_9 = -0.167, p = 0.871$ ), or the two chamber types ( $t_9 = -1.144, p = 0.282$ ). Kit fox family groups (i.e., adults and pups) were confirmed using den complexes on five occasions. Family groups used the dens in the Sump 143, Sump 125, and 7 Oaks East complexes in spring 2003, and the dens in the Sump 125 and City canal complexes in spring 2004.

Other species also were detected using the dens. These other species included feral cats (*Felis catus*; 34 dens, 12 complexes), striped skunks (*Mephitis mephitis*; 17 dens, 7 complexes), red foxes (*V. vulpes*; 5 dens, 3 complexes), raccoons (*Procyon lotor*; 3 dens, 1 complex), opossums (*Didelphis virginiana*; 16 dens, 8 complexes), California ground squirrels (*Otospermophilus beechyi*; 25 dens, 10 complexes), desert cottontails (*Sylvilagus*

**Table 1.** Artificial dens installed in Bakersfield, CA and use by San Joaquin kit foxes (KF), July 2001–June 2004.

Complex	Site	Installed	Design	Materials	Den checks	KF detections	KF detection rate	Days to first KF visit
FACT	Field	7/17/01	Surface - 3 m	Cement	306	30	0.098	1
FACT	Field	7/17/01	Subterranean - 1 entrance	Metal	306	24	0.078	1
FACT	Field	7/17/01	Chambered - 1 entrance	HDPE w/box	308	26	0.084	1
Brimhall	Canal	7/25/01	Surface - 3 m	Metal	295	0	0	-
Brimhall	Canal	7/25/01	Subterranean - 1 entrance	PVC	295	1	0.003	922
Brimhall	Canal	7/25/01	Chambered - 2 entrance	HDPE w/dogloo	295	1	0.003	922
Riverlakes	Canal	7/26/01	Surface - 3 m	PVC	295	1	0.003	557
Riverlakes	Canal	7/25/01	Subterranean - 2 entrance	HDPE	296	1	0.003	557
Riverlakes	Canal	7/25/01	Chambered - 1 entrance	Metal w/box	296	0	0	-
Calloway	Canal	7/31/01	Surface - 3 m	HDPE	241	0	0	-
Calloway	Canal	7/31/01	Subterranean - 1 entrance	Metal	241	0	0	-
Calloway	Canal	7/31/01	Chambered - 2 entrance	PVC w/box	241	0	0	-
Sump 143	Sump	8/3/01	Surface - 6.1 m	Cement	291	41	0.141	7
Sump 143	Sump	8/3/01	Subterranean - 2 entrance	Metal	291	96	0.330	3
Sump 143	Sump	8/3/01	Chambered - 1 entrance	PVC w/box	291	104	0.357	2
7 Oaks East	Golf course	8/7/01	Subterranean - 1 entrance	PVC	263	23	0.087	65
7 Oaks East	Golf course	8/7/01	Chambered - 2 entrance	HDPE w/box	263	40	0.152	65
7 Oaks West	Golf course	8/9/01	Subterranean - 2 entrance	HDPE	263	21	0.080	63
7 Oaks West	Golf course	8/9/01	Chambered - 1 entrance	PVC w/dogloo	263	70	0.266	63

Table 1. continued.

Complex	Site	Installed	Design	Materials	Den checks	KF detections	KF detection rate	Days to first KF visit
City canal	Canal	8/14/01	Surface - 6.1 m	Metal	286	10	0.035	654
City canal	Canal	8/14/01	Subterranean - 1 entrance	Cement	286	38	0.133	13
City canal	Canal	8/14/01	Chambered - 2 entrance	PVC w/box	286	29	0.101	7
Sump 125	Sump	8/15/01	Surface - 6.1 m	HDPE	286	90	0.315	2
Sump 125	Sump	8/15/01	Subterranean - 2 entrance	PVC	286	153	0.535	2
Sump 125	Sump	8/15/01	Chambered - 2 entrance	Cement w/dogloo	286	146	0.510	2
Sump 51	Sump	8/22/01	Surface - 6.1 m	PVC	282	15	0.053	5
Sump 51	Sump	8/22/01	Subterranean - 2 entrance	Cement	282	43	0.152	5
Sump 51	Sump	8/22/01	Chambered - 2 entrance	Metal w/dogloo	282	50	0.177	5
Sump 1	Sump	9/10/01	Surface - 6.1 m	HDPE	271	1	0.004	36
Sump 1	Sump	9/10/01	Subterranean - 2 entrance	PVC	271	7	0.026	11
Sump 1	Sump	9/10/01	Chambered - 1 entrance	Cement w/box	271	13	0.048	2
Sump 56	Sump	7/24/02	Surface - 3 m	Cement	184	26	0.141	31
Sump 56	Sump	7/24/02	Subterranean - 1 entrance	HDPE	186	45	0.242	47
Sump 56	Sump	7/24/02	Chambered - 1 entrance	PVC w/dogloo	186	53	0.285	47

**Table 2.** Mean detection rates for San Joaquin kit foxes at artificial dens by den design and materials, Bakersfield, CA, July 2001–June 2004.

Den group	n	Mean (SE) detection rate
Design:		
Surface – 3 m	4	0.061 (0.035)
Surface – 6.1 m	5	0.109 (0.056)
Subterranean – 1 entrance	5	0.109 (0.039)
Subterranean – 2 entrance	6	0.188 (0.084)
Chambered – 1 entrance	6	0.173 (0.060)
Chambered – 2 entrance	5	0.189 (0.086)
Design category:		
Surface	9	0.088 (0.034)
Subterranean	11	0.152 (0.049)
Chambered	11	0.181 (0.048)
Material:		
Cement	7	0.175 (0.058)
Metal	6	0.103 (0.053)
PVC	10	0.172 (0.057)
HDPE	8	0.110 (0.042)
Chamber:		
Box	6	0.124 (0.051)
Dogloo	5	0.248 (0.082)

*audubonii*; 15 dens, 7 complexes), burrowing owls (*Athene cunicularia*; 8 dens, 3 complexes), and side-blotched lizards (*Uta stansburiana*; 10 dens, 6 complexes).

Statistical results for comparisons of internal den conditions (temperature, relative humidity) are presented in Table 3. In summer, mean temperature in natural dens and artificial dens was cooler than mean ambient temperature, and mean relative humidity was higher in both types of dens compared to ambient humidity. In winter, mean temperature and mean relative humidity were similar to mean ambient values. Mean temperature in natural dens was lower in summer and higher in winter compared to artificial dens. Mean relative humidity was higher in natural dens in summer compared to artificial dens, but did not differ between natural and artificial dens in winter. Mean temperature and mean relative humidity were not different between one-entrance and two-entrance natural dens in either summer or winter. Mean temperature did not differ between one-entrance and two-entrance artificial dens in either summer or winter, but mean relative humidity was higher in one-entrance dens in both seasons. Among artificial dens in summer, mean temperature was higher and mean relative humidity was lower in surface dens compared to subterranean dens, but neither temperature or humidity were different between surface and subterranean dens in winter. Finally, for subterranean artificial dens, mean temperature and mean relative humidity did not differ among materials (i.e., metal, PVC, concrete, and HDPE) or between chamber types.

**Table 3.** Comparisons of mean ( $\pm$ SE) temperature and relative humidity among ambient conditions, natural San Joaquin kit fox dens, artificial dens, den attributes, and den materials, Bakersfield, CA, July 2001–June 2004. *P*-values in bold are significant at  $\alpha = 0.05$ .

Comparison	Temperature ( $^{\circ}$ C)		Relative humidity (%)	
	Summer	Winter	Summer	Winter
Natural dens	31.2 $\pm$ 0.8	16.2 $\pm$ 0.7	49.2 $\pm$ 5.4	70.2 $\pm$ 5.6
Ambient	39.3 $\pm$ 1.6	15.7 $\pm$ 1.4	17.8 $\pm$ 1.4	59.7 $\pm$ 4.1
	$t_{16} = -4.48$ $p < 0.001$	$t_{16} = 0.09$ $p = 0.763$	$t_{16} = 5.62$ $p < 0.001$	$t_{16} = 2.29$ $p = 0.149$
Artificial dens	35.8 $\pm$ 0.5	14.0 $\pm$ 0.4	31.7 $\pm$ 2.8	63.6 $\pm$ 2.5
Ambient	40.3 $\pm$ 0.5	14.1 $\pm$ 0.7	22.1 $\pm$ 1.9	60.9 $\pm$ 1.9
	$t_{64} = -6.48$ $p < 0.001$	$t_{58} = 0.01$ $p = 0.964$	$t_{64} = 2.85$ $p = 0.006$	$t_{58} = 0.73$ $p = 0.398$
Natural dens	31.2 $\pm$ 0.8	16.7 $\pm$ 0.7	49.2 $\pm$ 5.4	70.2 $\pm$ 5.6
Artificial dens	35.8 $\pm$ 0.5	14.0 $\pm$ 0.4	31.7 $\pm$ 2.8	63.6 $\pm$ 2.5
	$t_{40} = 20.39$ $p < 0.001$	$t_{37} = 6.96$ $p = 0.012$	$t_{40} = 8.51$ $p = 0.006$	$t_{37} = 1.43$ $p = 0.239$
1-entrance natural	30.9 $\pm$ 1.8	15.0 $\pm$ 1.1	57.6 $\pm$ 7.4	64.4 $\pm$ 6.6
2-entrance natural	31.4 $\pm$ 0.7	17.1 $\pm$ 0.9	42.5 $\pm$ 6.9	74.8 $\pm$ 8.6
	$t_7 = 0.10$ $p = 0.765$	$t_7 = 2.20$ $p = 0.182$	$t_7 = 2.21$ $p = 0.181$	$t_7 = 0.86$ $p = 0.385$
1-entrance artificial <sup>a</sup>	35.0 $\pm$ 0.8	14.0 $\pm$ 0.6	44.0 $\pm$ 6.0	74.5 $\pm$ 5.5
2-entrance artificial <sup>a</sup>	35.2 $\pm$ 0.7	14.0 $\pm$ 0.7	28.1 $\pm$ 3.1	58.7 $\pm$ 2.5
	$t_{21} = 0.03$ $p = 0.875$	$t_{19} = 0.01$ $p = 0.935$	$t_{21} = 5.94$ $p = 0.024$	$t_{19} = 7.23$ $p = 0.015$
Surface artificial	37.3 $\pm$ 0.9	14.1 $\pm$ 0.7	22.3 $\pm$ 1.8	57.6 $\pm$ 1.8
Subterranean artificial	35.1 $\pm$ 0.5	14.0 $\pm$ 0.5	35.7 $\pm$ 3.6	66.2 $\pm$ 3.4
	$t_{31} = 5.30$ $p = 0.028$	$t_{28} = 0.15$ $p = 0.904$	$t_{31} = 5.62$ $p = 0.024$	$t_{28} = 2.61$ $p = 0.117$
Metal pipe	35.8 $\pm$ 1.0	15.3 $\pm$ 0.9	27.6 $\pm$ 2.5	55.5 $\pm$ 4.4
PVC pipe	34.9 $\pm$ 1.2	13.8 $\pm$ 0.8	45.1 $\pm$ 8.8	71.0 $\pm$ 6.5
Concrete pipe	34.6 $\pm$ 0.4	15.0 $\pm$ 0.9	37.9 $\pm$ 3.2	65.6 $\pm$ 8.5
HDPE pipe	35.2 $\pm$ 1.0	13.0 $\pm$ 0.9	28.5 $\pm$ 4.1	66.2 $\pm$ 5.9
	$F_{3,19} = 0.15$ $p = 0.927$	$F_{3,17} = 1.23$ $p = 0.331$	$F_{3,19} = 1.63$ $p = 0.216$	$F_{3,17} = 0.68$ $p = 0.577$
Box chamber	34.8 $\pm$ 1.1	13.5 $\pm$ 0.8	41.6 $\pm$ 4.6	71.4 $\pm$ 6.0
Dogloo Chamber	35.2 $\pm$ 1.1	14.4 $\pm$ 1.0	35.6 $\pm$ 8.8	63.1 $\pm$ 7.4
	$t_9 = 0.06$ $p = 0.819$	$t_8 = 0.48$ $p = 0.508$	$t_9 = 0.33$ $p = 0.582$	$t_8 = 0.78$ $p = 0.404$

<sup>a</sup> Subsurface artificial dens

## DISCUSSION

In our study, SJKF used artificial dens of six different designs that were constructed of four different materials. However, some caveats are warranted regarding the rates of detection of kit foxes at the dens. First, once foxes encountered a den complex, they appeared to use all of the dens in that complex. We suspect that this was a function of the close proximity of the dens in a complex, and this increased use likely obscured any preferences the kit foxes might have exhibited for designs or materials. This effect was exacerbated when a family group was present. Trail cameras set at dens to confirm the presence of family groups captured images of pups chasing each other in and out of multiple dens in a complex during their play bouts. Another issue is that we did not have enough dens to assess any interaction effects between den designs and materials. This also likely obscured preferences for particular designs or materials. Finally, detection rates should be considered minimums because sometimes the track stations were disturbed rendering detections difficult. Rain or wind or irrigation systems (e.g., sprinklers on the golf course) sometimes affected the stations, as did heavy traffic by animals (kit foxes and other species) in and out of the dens.

SJKF apparently readily used the artificial dens (Fig. 8). A number were used within a day or two after installation and some were used extensively (e.g., kit foxes were detected in over 50% of the den checks for two of the dens in the Sump 125 complex; Table 1). Kit foxes used the dens as they would natural dens. Foxes were observed to run into the dens at the approach of potential threats (e.g., people, dogs). Radio-collared foxes were tracked to the dens during the day indicating that foxes also were using the dens for daytime resting and also probably to avoid hot daytime temperatures. Although we were not able to determine whether any kit foxes gave birth to young in the dens, we did confirm that foxes used the dens for pup-rearing based on the presence of family groups at some of the complexes in the spring. The complexes may have provided a reasonable approximation of the large, multi-entrance natal dens that kit foxes commonly use when rearing young (Egoscue 1962; Berry et al. 1987; Spiegel et al. 1996).

Per the caveat above, our assessment of preferences by kit foxes for particular den designs was likely confounded, and consequently detection rates of the different designs were not statistically different. However, based on anecdotal evidence, chambered dens may have been used more extensively than the other designs. Although foxes were detected at all designs, fox activity based on the number of tracks entering and exiting dens seemed greater at chambered dens. Prey remains were more common outside of these dens as well, suggesting more frequent use. Also, other signs such as the appearance of new entrances excavated by the foxes typically were observed at chambered dens. Greater use of chambered dens, particularly by family groups, would not be unexpected as these dens were larger and could accommodate more foxes.

Similar to the den design analysis, our assessment of preferences by kit foxes for particular materials was likely confounded, and consequently detection rates of the different designs were not statistically different. That said, kit foxes have been documented denning in cement culverts and in both metal and PVC pipes (Berry et al. 1987; Bjurlin et al. 2005; Cypher 2010), so their use of a variety of materials was not unexpected. Interestingly, of the 31 dens in the 11 complexes in the study, the only two dens where kit fox use was never detected were both metal dens.

Assuming that kit foxes do not exhibit a preference for materials, then other factors might be considered in the installation of artificial dens. The metal and cement pipes were



**Figure 8.** San Joaquin kit foxes using artificial dens in Bakersfield, CA. Top: Adult fox entering a concrete surface den. Bottom: Two pups outside of a HDPE two-entrance chambered den.

more difficult to work with as both materials are heavy and could not be modified in the field (e.g., cut in any way). We noticed that on sunny days, the exposed portions of the metal dens could get quite hot and this heat may have been conducted farther down into the dens. The PVC and HDPE pipes were relatively easy to modify by cutting with almost any type of saw (e.g., hack saw, PVC saw, wood saws). The PVC and HDPE pipes were smooth on the inside and therefore potentially slippery. However, we were able to cut 10-cm wide strips out of the bottoms of these pipes so that foxes would have contact with dirt and therefore better traction. Removing the strips also provided drainage as well as opportunities for foxes to create new tunnels or chambers within the den. Single-walled HDPE pipes would be an even better choice as they are flexible and the inside surface is corrugated thus providing better traction. The foxes also exhibited no preference for chambers. The irrigation boxes were more readily available and easier to cut to create entrance holes for the pipes.

Regarding costs, in 2001, the cost per foot was \$12.55 for 10-in (25-cm) concrete pipe, \$9.89 for 8-in (20-cm) galvanized aluminum pipe, \$5.56 for 8-in HDPE pipe, and \$4.50 for 8" Schedule 40 PVC pipe. The cost for the chambers was \$26.49 for the irrigation box and \$52.99 for the dogloo-style doghouse. Thus, the HDPE and PVC pipes and irrigation box also would be better materials to use based on cost. Installation costs obviously will vary depending upon the labor pool used (e.g., construction company versus volunteers). However, installation of surface dens requires less excavation compared to the installation of subterranean dens, and therefore labor costs associated with installing surface dens will be lower.

The range occupied by the San Joaquin kit fox is very warm and arid. Two of the primary reasons that kit foxes use dens are to avoid temperature extremes, particularly during the heat of summer, and to conserve body moisture (Koopman et al. 1998). The ability of natural dens to provide these benefits was confirmed in that compared to ambient conditions outside of dens, internal temperatures were cooler in summer and more humid during both summer and winter. These results are consistent with those of Loredó et al. (2020) who compared ambient and internal conditions for 92 kit fox dens (44 in summer and 48 in winter) to assess potential survival times for mange mites (*Sarcoptes scabiei*).

Although not to the same degree as natural dens, the artificial dens in our study also were cooler and more humid in summer, and therefore provided thermoregulatory and moisture conserving benefits to foxes during this warm, dry season. These benefits are likely less critical during winters, which are relatively mild and moist within the range of the SJKF. Cowan et al. (2020) found that artificial dens created for northern quolls (*Dasyurus hallucatus*) in a semi-arid region of western Australia also had internal climatic conditions similar to those of natural dens. The soil of natural dens has greater moisture-holding capacity compared to the more impermeable materials we used to construct artificial dens, and this likely accounted for the more favorable conditions inside natural dens. Unsurprisingly, subterranean dens provided more favorable temperature and humidity conditions compared to surface dens with one-entrance dens having higher humidity than two-entrance dens. Surface dens only had a relatively thin cover of insulating soil and all had two entrances. Two-entrance dens have greater potential for flow-through air movement that can bring in external air and this can cause internal temperature and humidity to be more similar to ambient conditions. The materials used to construct the dens all produced similar internal conditions.

The artificial dens we installed were used by a number of other species. The benefits and detriments of this result likely vary with perspective and also with the particular species. Many biologists as well as members of the public might find use of the dens by other species desirable because it enhances biodiversity in the urban environment or they simply like

seeing more wildlife. However, use by other species also might be viewed as undesirable. Species such as red foxes, striped skunks, raccoons, and California ground squirrels occasionally create nuisance issues. Species such as skunks and raccoons might even be viewed as threats due to noxious odors and the potential for rabies. Feral cats used all 34 dens (Fig. 9). Their presence and any actions that facilitate their presence can elicit strong reactions from people, both positive and negative (Lord 2008; Loyd and Miller 2010; Crowley et al. 2020). Finally, the presence of some species in the dens also may be detrimental for kit foxes. Kit foxes can be competitively excluded by red foxes, raccoons, skunks (Fig. 9), and even feral cats (Harrison et al. 2011a). Thus, the kit foxes may not be able to use the dens (or the areas around them) when they are occupied by these other species. Also, use of the dens by other species can expose kit foxes to greater risk of disease.

Burrowing owls used a number of artificial dens. Burrowing owls are a California Species of Special Concern (CDFW 2008). They are regularly observed in Bakersfield (Wingert 2012) and frequently observed using kit fox dens and California ground squirrel burrows. Similar to kit foxes, burrowing owls are burrow obligates (Gervais et al. 2008) and also will use man-made structures including artificial burrows (Smith and Belthoff 2001). Thus, although installed for kit foxes, artificial dens also could contribute to the conservation of burrowing owls in Bakersfield by providing additional shelter.

In conclusion, kit foxes appear to readily use artificial dens and installation of such dens may constitute a useful conservation strategy, particularly in areas where natural dens may be uncommon or absent. Such areas might include lands that had been disturbed for other uses (e.g., agriculture) but that are being restored back to habitat. Artificial dens also can be used to mitigate for loss of natural dens due to focal disturbances, such as road or well pad construction. We recommend installing chambered dens with two entrances as these larger dens have the greatest utility to kit foxes (e.g., escape cover, daytime resting, thermoregulation, moisture conservation, and rearing young). For materials, we recommend single-wall HDPE for the entrances and an irrigation box for the chamber (Fig. 10). These materials are easy to work with and relatively inexpensive. In areas where habitat is being restored or where predation risk might be high, a combination of chambered dens and surface dens might enhance kit fox occupancy and survival. The surface dens are easy to install and can provide additional escape cover. In northwest Texas, surface dens were installed for swift foxes at a density of 36/2.6 km<sup>2</sup> in three study areas, one of which was unoccupied by swift foxes (McGee et al. 2006). Swift fox survival was significantly higher on the treatment areas compared to nearby control areas, and swift foxes successfully colonized the previously unoccupied area where dens had been installed. Kit foxes likely would respond similarly.

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**Figure 9.** Feral cat (top) and striped skunk (bottom) using artificial dens installed for San Joaquin kit foxes in Bakersfield, CA.



**Figure 10.** Recommended artificial den design for San Joaquin kit foxes: single-walled HDPE two-entrance chambered den with an irrigation valve box for the chamber.

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## RESEARCH NOTE

## New detections extend the known range of the state-threatened Sierra Nevada red fox

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**Key words:** alpine, carnivore, California Endangered Species Act, noninvasive, remote cameras, Sierra Nevada, Sierra Nevada red fox, state-threatened, *Vulpes vulpes necator*

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The Sierra Nevada red fox (*Vulpes vulpes necator*; SNRF) is a subspecies of red fox native to the upper montane, subalpine, and alpine zones of the Sierra Nevada and Cascades in California and Oregon (Grinnell et al. 1937; Perrine et al. 2010; Sacks et al. 2010). Declines in the distribution and abundance of SNRF populations led to the designation of the subspecies as state-threatened in California in 1980 (Gould 1980). More recently, the remnant population in the Sierra Nevada was proposed for federal listing as an endangered Distinct Population Segment (USFWS 2020). Lack of certainty as to the distribution of remnant SNRF populations has hindered conservation efforts and population recovery. Using noninvasive survey techniques, we documented the southernmost SNRF detections in recent decades, greatly expanding the known contemporary range of this state-threatened subspecies (Perrine et al. 2010; Hatfield et al. 2020).

Although the subspecies existed historically as far south as the Mt. Whitney region (Grinnell et al. 1937), the verified contemporary distribution of SNRF in the Sierra Nevada is far more restricted. Prior to 2010, the last confirmed detection in the Sierra Nevada was a photograph taken in 1991 on the Inyo National Forest near Tioga Pass in Yosemite National Park (Perrine et al. 2010). Although there were several observations of apparent red foxes in the southern Sierra Nevada during the 1960s–1990s (Schempf and White 1977; Perrine et al. 2010), the last confirmed SNRF detections south of Yosemite National Park were reported by Grinnell et al. (1937). In 2010, a U.S. Forest Service survey documented SNRF near Sonora Pass, north of Yosemite National Park (Statham et al. 2012). Between 2010–2013, confirmed detections of red foxes in the Sierra Nevada were limited to an approximately 30-km stretch of the Sierra Crest immediately north of Yosemite National Park (Quinn et al. 2019).

The scarcity of contemporary SNRF detections may indicate local extirpations, but may also be due to insufficient survey effort. Because SNRF exist at very low densities in

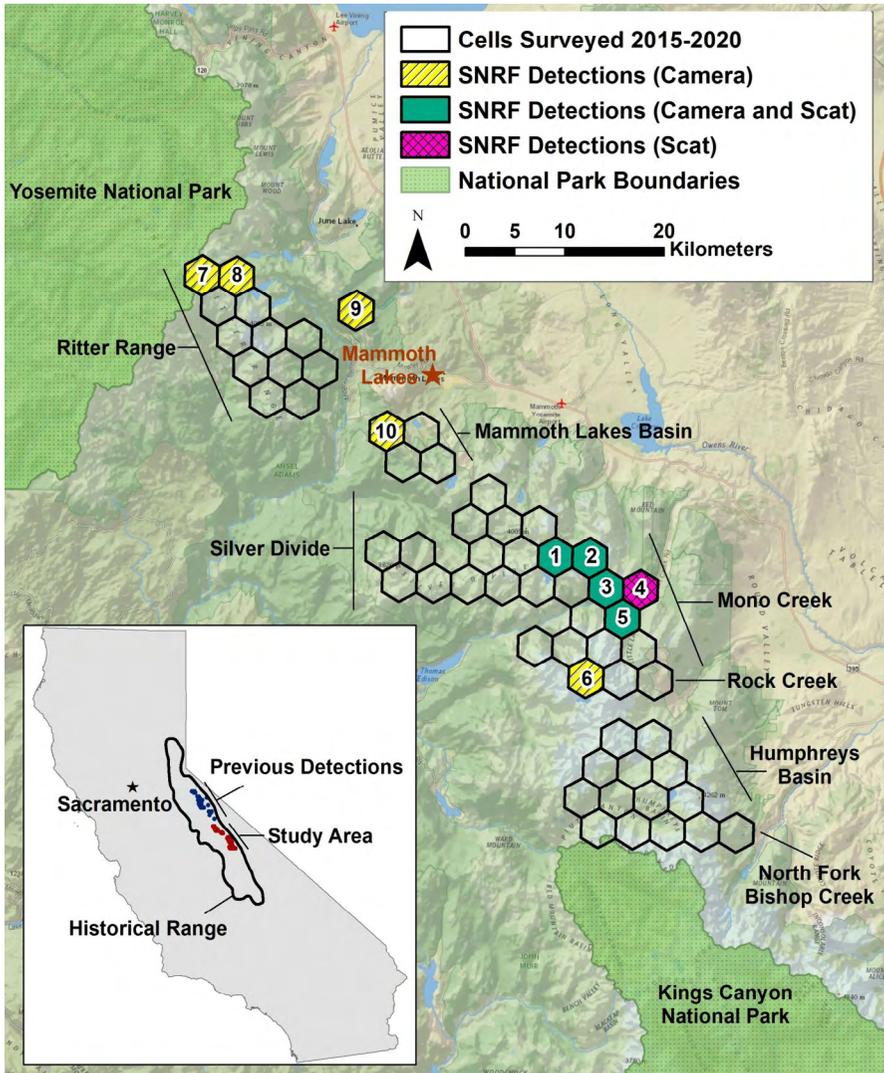
remote, high-elevation areas, documenting their distribution can be extremely challenging. Most previous systematic surveys targeted multiple species and were optimized for mustelids (e.g., Zielinski et al. 2005; Green 2006; IWS 2006; Perrine et al. 2010), which have different habitat requirements and behaviors than SNRF. Only a small number of previous surveys were conducted in the high-elevation habitat historically ascribed to SNRF, using methods with high probabilities of detection for canids (Statham et al. 2012; Stock and Eyes 2017; Quinn et al. 2019; CDFW, unpublished data).

In 2013, a noninvasive genetic study documented apparent SNRF population growth in the Sonora Pass area, immediately following immigration from a nearby population (Quinn et al. 2019). The observed increase in reproductive output and local abundance raised the possibility that SNRF could be expanding their distribution and suggested a need for systematic surveys throughout the historical range. In 2014, SNRF were documented in northern Yosemite National Park (Stock and Eyes 2017). In 2015, we began implementing noninvasive SNRF surveys at elevations above 2,700 m between the southern boundary of Yosemite National Park and the northern boundary of Kings Canyon National Park (Fig. 1). We used a combination of baited camera stations during the winter and scat surveys during the summer and fall to survey for SNRF.

Each fall, we selected a focal watershed containing potential SNRF habitat (Cleve et al. 2011). We applied a sampling frame of 10.4-km<sup>2</sup> hexagonal cells across the selected watershed, placing two remote cameras (Reconyx, Holmen, Wisconsin, USA; Stermer et al. 2015) per sampling cell with at least 1.6 km between cameras (e.g., Stermer et al. 2015). We attached cameras to trees or large boulders using bungee cord or parachute cord. We baited each station with a scent lure 5 m away from the camera to attract carnivores (Gusto, Minnesota Trapline Products, Pennock, MN, USA). Each annual survey consisted of 10 to 15 sampling cells containing 20–30 cameras, operational during October–June at minimum. Between 2015–2020, we deployed 114 survey cameras (average 18.4 per annual survey) which were operational for 23,262 total days (average 4,652.4 operational days per annual survey; Table 1). Because cameras would need to remain operational through winter, we prioritized camera placement on high-elevation passes and ridges that were exposed to prevailing winds and unlikely to become buried in snow. We suspected that these windswept, geographically constrained features might also function as travel corridors for wildlife. In addition to cameras deployed within a systematic survey framework, we maintained passive monitoring cameras year-round in locations where we detected SNRF or where camera placements were easily accessible to surveyors. We used similar methods to deploy passive monitoring and survey cameras, but passive cameras were not deployed according to a systematic framework.

We followed camera surveys with scat surveys during the summer and fall to collect fecal material for genetic analysis. Surveyors traversed ridges, passes, and trails in sampling cells where cameras had detected SNRF and collected all apparent mesocarnivore scats. The Mammalian Ecology and Conservation Unit at the University of California, Davis conducted genetic analysis of scat samples, using mitochondrial sequences to confirm species, and nuclear microsatellites and a sex marker to identify individuals (e.g., Quinn et al. 2019).

Between 2015–2020, we surveyed 58 sampling cells and detected SNRF in 10 cells (Fig. 1). We did not detect SNRF in the cells surveyed between 2015–2017 (North Fork of Bishop Creek, Rock Creek, Mammoth Lakes Basin, and Humphreys Basin), indicating either that SNRF were not present in the areas we surveyed during those years or simply that our



**Figure 1.** Locations of 10.4 km<sup>2</sup> sampling cells where high-elevation Sierra Nevada red fox (SNRF) remote camera surveys and scat detections occurred between 2015–2020. In inset map, “Previous Detections” are from Statham et al. 2012, Stock and Eyes 2017, Quinn et al. 2019, CDFW unpublished data, National Park Service unpublished data, and U.S. Forest Service unpublished data. “Historical Range” is from Grinnell et al. 1937.

surveys failed to detect SNRF. In 2018, we detected SNRF at six camera sites within the Mono Creek watershed, southeast of the town of Mammoth Lakes (Fig. 1, cells 1, 2, 3, 5, and 6). We collected five SNRF scats within this survey area (Fig. 1, cells 1–5), representing two females and one male. The male had been detected by scat at Sonora Pass in 2017 (Quinn et al. 2019), demonstrating a dispersal of more than 120 km within eight months (Fig. 2). We maintained passive monitoring cameras at or near locations where survey cameras had detected SNRF, and continued to detect SNRF in the Mono Creek watershed in 2019 and 2020. During the summer and fall of 2019, we collected five SNRF scats from the Mono

**Table 1.** Number of survey cameras, operational days, and Sierra Nevada red fox (SNRF) detections per study area and year for SNRF surveys between 2015–2020.

Study area	Year	Cameras	Operational Days	SNRF Detections
East Side <sup>a</sup>	2016	19	2834	0
Humphreys Basin	2017	24	4475	0
Mono Creek	2018	19	4546	13
Ritter Range	2019	26	5935	3
Silver Divide	2020	26	5472	0

<sup>a</sup> The East Side study area encompassed the Mammoth Lakes Basin, Rock Creek, and the North Fork of Bishop Creek.

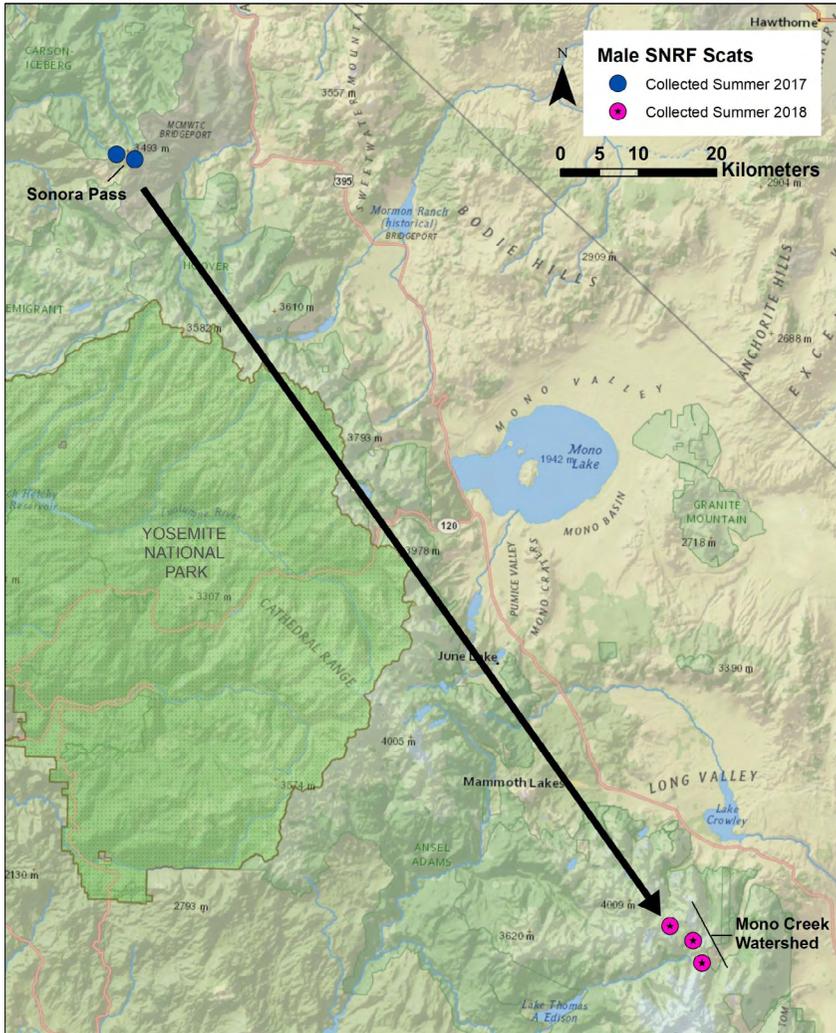
Creek watershed and one from an adjacent watershed (McGee Creek). Genetic analysis of the scats collected in 2019 documented the same male but only one of the two females that were first detected in 2018. No new individuals were identified.

In addition to the SNRF individuals detected in the Mono Creek watershed, we also detected SNRF in 2019 and 2020 on four remote cameras located north of the Mono Creek watershed and south of Yosemite National Park. Two of the detections were in the Ritter Range, a sub-range of the Sierra Nevada west of the town of Mammoth Lakes (Fig. 1, cells 7–8). The other two detections were on the Sierra Crest in the vicinity of Mammoth Lakes (Fig. 1, cells 9–10). Although we maintained passive monitoring cameras in these locations and conducted scat surveys nearby, we have not yet obtained genetic samples or had repeat photographic detections from these sites. Therefore, we cannot confirm the number of individuals detected or whether the detections represent dispersers or resident SNRF. A camera survey conducted in 2020 in the Silver Divide, adjacent to the Mono Creek watershed, did not detect SNRF. During the winter of 2020–2021, in collaboration with the National Park Service (NPS), we will be deploying a camera survey in the northern portion of Kings Canyon National Park, approximately 15 km south of our southernmost detection in the Mono Creek watershed.

Our findings suggest that SNRF are more broadly distributed in the Sierra Nevada than previously suspected, although they likely exist at low density within our study area. Recent detections of SNRF in the Sierra Nevada are relatively continuous along the Sierra Crest between Ebbetts Pass and Mono Pass, with no gaps in distribution >20 km (Quinn et al. 2019; Hatfield et al. 2020; CDFW, unpublished data, NPS, unpublished data). The movement of at least one individual from Sonora Pass to Mono Creek indicates some level of connectivity within the range. SNRF use the highest available elevations year-round, with detections ranging from 3,044 m to 3,738 m. Continuing surveys are crucial to determine the current distribution of SNRF in the Sierra Nevada and to inform conservation and recovery efforts for this state-threatened subspecies.

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**Figure 2.** Locations of scat detections of a single Sierra Nevada red fox (SNRF) male in summer 2017 near Sonora Pass and in summer 2018 in the Mono Creek watershed, demonstrating a dispersal of more than 120 km within eight months.

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FULL RESEARCH ARTICLE

## Predation impedes recovery of Sierra Nevada bighorn sheep

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Translocation of animals into formerly occupied habitat is a key element of the recovery plan for Sierra Nevada bighorn sheep (*Ovis canadensis sierrae*), which are state (California) and federally listed as endangered. However, implementing Sierra bighorn translocations is a significant conservation challenge because of the small size of the extant population and the limited number of herds available to donate translocation stock. One such herd, the Mt. Langley herd, recently became unusable as a translocation source following a substantial population decline. At the time of listing in 1999, predation by mountain lions (*Puma concolor*; hereafter lion) was considered a primary threat to Sierra bighorn, and since then lion predation may have continued to limit the ability of source herds to provide translocation stock. We evaluated the relationship between lion predation and ewe survival rates within three source herds of the Southern Recovery Unit, compared lion abundance and ewe survival among years of varying predation levels, provided a range of estimated times for the Mt. Langley herd to recover to its former status as a translocation source, and determined if the rates lions have been removed to mitigate Sierra bighorn predation exceeded sustainable harvest guidelines. We found compelling evidence that lion predation has impeded the recovery of Sierra bighorn by reducing survival rates of adult ewes (and consequently, population growth) and by preying upon individuals that could have otherwise been translocated. Ewe survival was poor during years of extreme predation but even during years of typical predation, survival rates were below a level needed to ensure population growth, indicating that years with

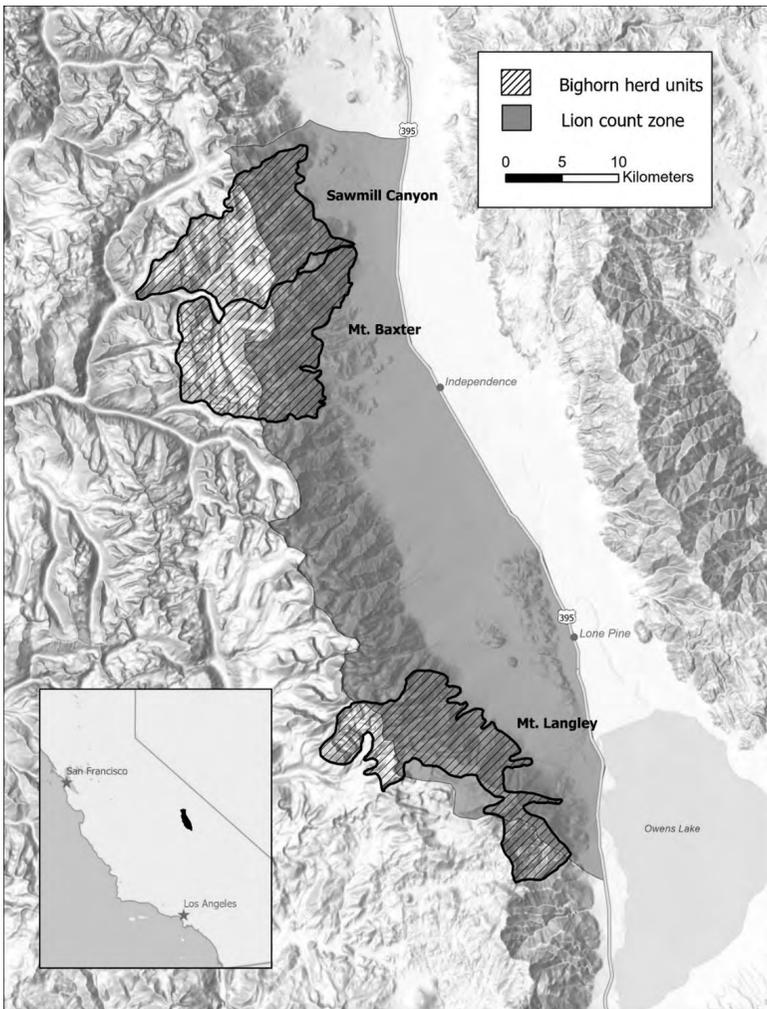
little or no lion predation may be necessary for the population to grow and meet recovery goals. Because the intensity of predation was related to lion abundance, monitoring lion populations could provide managers with advance warning of periods of extreme predation. We found that following a period of particularly extreme predation, the Mt. Langley herd decreased in abundance far below the threshold needed to be considered a source of translocation stock, resulting in the loss of approximately 25% of the recovery program's capacity for translocations. It is unclear how many years it will take for this herd to recover, but management actions to reduce lion predation are likely needed for this herd to grow to a size that can afford to donate individuals to translocation efforts in the near future, even when optimistic growth rates are assumed. We found that lion removal may also be needed to prevent predation from leading to Sierra bighorn population decline. Lion removal rates that have been implemented thus far are well below what would be needed to reduce the abundance the eastern Sierra lion population itself. We recommend continued monitoring of Sierra bighorn and sympatric lions and note that lion removal may be required to facilitate bighorn recovery for the foreseeable future.

**Key words:** additive mortality, endangered species, mountain lion, *Ovis canadensis sierrae*, predation, *Puma concolor*, removal, survival, translocation

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Sierra Nevada bighorn sheep (*Ovis canadensis sierrae*; hereafter Sierra bighorn) are a distinct subspecies of bighorn sheep (Wehausen and Ramey 2000; Wehausen et al. 2005; Buchalski et al. 2016) that once ranged throughout much of California's Sierra Nevada, from Olancha Peak in the south to Sonora Pass in the north (Wehausen and Jones 2014). They disappeared from most of their native range following the appearance of Europeans, primarily from diseases of introduced domestic livestock, and continued to decline through most of the 20<sup>th</sup> century (Wehausen et al 1987; Wehausen et al. 2011). By the early 1970s, when Sierra bighorn were included in the first list of species compiled under the California Endangered Species Act (Leach et al. 1974), they persisted in only three subpopulations (i.e., herds) near the community of Independence in the southern Owens Valley: Sawmill Canyon, Mt. Baxter, and Mt. Williamson (Wehausen and Jones 2014). Restoration actions during 1978-1979 re-established four additional herds in historic habitat and increased the total population from 250 (Wehausen 1980) to about 300 by 1985 (USFWS 2007). That success was followed by a severe decline in abundance to just over 100 animals by 1995 (USFWS 2007) coincident with a steep increase in mountain lion (*Puma concolor*, hereafter, lion) activity and documented predation upon Sierra bighorn (Wehausen 1996; USFWS 2007). It became clear that Sierra bighorn were on a trajectory towards extinction, and they were listed as endangered by the California Fish and Game Commission and the U.S. Fish and Wildlife Service (USFWS) in 1999 (USFWS 1999). Lion predation was considered a primary threat to Sierra bighorn at the time of listing (USFWS 1999, 2007). The California Department of Fish and Wildlife (hereafter, the Department) has led recovery efforts for Sierra bighorn since that time, guided by the Recovery Plan for Sierra Nevada Bighorn Sheep (USFWS 2007).

The ultimate goal of recovery programs for endangered species is to ‘delist’ species after recovery goals are met. Recovery goals for Sierra bighorn specify abundance and distribution targets. Translocation has been the primary management tool to restore bighorn sheep to unoccupied habitat (Douglas and Leslie 1999) because of their slow rate of natural colonization (Geist 1971). Both an early conservation plan (Sierra Nevada Bighorn Sheep Interagency Advisory Group 1984) and the Recovery Plan for Sierra Nevada Bighorn Sheep (USFWS 2007) recognized the need to develop additional large herds capable of serving as sources of translocation stock, beyond the native geographically overlapping Sawmill Canyon and Mt. Baxter herds that had been used as the source populations for early translocations in the 1970s and 1980s (Fig. 1). That need was met in 2004 when Wheeler Ridge (initiated in 1979) became the first reintroduced herd used as a source for translocation. Translocation stock from the Mt. Langley (initiated in 1980) were used for the first time in 2008.



**Figure 1.** Study area in the eastern Sierra Nevada, California in which Sierra Nevada bighorn sheep and lions were monitored, 1999–2019.

Translocation of Sierra bighorn is a significant conservation challenge however, because few individuals are available in a given year. First, because Sierra bighorn are a distinct subspecies, there is no alternative translocation source. Second, the Department has established a conservative translocation policy to minimize risk of over harvest (Few et al. 2015), which recommends herds contain least 40 yearling and adult females (hereafter, ewes) before they are eligible as a source of translocation stock, and only 4 herds have reached this threshold. Third, the maximum abundance a single source herd has reached is only 63 ewes, meaning that the number of recruits produced annually remains small. Fourth, as of 2017 the Mt. Langley herd—arguably the most productive Sierra bighorn herd since the recovery program began—can no longer be used as a source of translocation stock following a substantial population decline from 49 ewes in 2016 to 19 ewes in 2019, which is hypothesized to have been caused primarily by a dramatic increase in lion predation during the winter of 2016–2017.

Lion predation is a common cause of mortality in ungulates and often occurs at relatively low and constant rates over time, with minimal impact on prey population dynamics (Lauré et al. 2006; Forrester and Wittmer 2013), but in small populations of bighorn sheep, impacts can be pronounced (reviewed in Rominger 2018). Irruptions in lion predation rates, particularly on small and/or endangered prey populations, can substantially exceed long-term averages in an apparently stochastic manner (Festa-Bianchet 2006) and may be the result of individual “specialist” predators whose dietary selection differs from the population mean (Ross et al. 1997; Logan and Sweanor 2001; Festa-Bianchet 2006; Elbroch and Wittmer 2013; Wittmer et al. 2014). If patterns of lion predation can be predicted, it may facilitate more proactive management of predation risk for Sierra bighorn, thus enhancing their recovery.

The U.S. Fish and Wildlife Service has emphasized the need to attempt to reach recovery goals “as quickly as possible” (USFWS 2007). While there has been some success in using translocation to restore extirpated Sierra bighorn herds, such as the re-establishment of the Wheeler Ridge and Mt. Langley herds, recovery goals for Sierra bighorn will likely be reached most rapidly and reliably through considerably more translocations. Maintaining and increasing the abundance of source herds is a necessary prerequisite for these translocations to occur. One way to grow herds and increase translocation stock is to increase survival rates of ewes. While Sierra bighorn die from a variety of causes, most are not amenable to management action that could mitigate them (e.g., deaths from avalanches associated with severe winters). However, lion predation may be reduced through removal of lions that prey upon Sierra bighorn (e.g., Goldstein and Rominger 2012).

Removal of individual predators that negatively impact populations of prey species can be controversial. Objections to removal arise from questions about its efficacy in some circumstances (e.g., Mosnier et al. 2008; Hurley et al. 2011 but see also Boertje et al. 1996; Lewis et al. 2017) or ethics related to animal rights philosophy in others (e.g., Muth and Jamison 2000; Perry and Perry 2007). The latter reason is particularly relevant to Sierra bighorn because, in California, lions are “specially protected”, meaning that sport harvest is not permitted (Fish & G. Code § 4800–4809). While this designation is not based on biological information regarding population abundance or trend (Dellinger and Torres 2020), objections to the removal of lions are frequent and often gain significant media attention, both within California (e.g., Los Angeles Time Editorial Board 2020) and nationally (Vera 2020). Objections such as these underscore the need for decision-makers to have objective scientific evaluation of the efficacy of predator management to aid the recovery of Sierra

bighorn. Following the listing of Sierra bighorn as an endangered species, the California Legislature recognized the need for predation management and in 1999 amended the Fish & Game Code to authorize the removal of lions that threatened populations of bighorn sheep in California that were threatened, endangered, candidate, or fully protected (Fish & G. Code § 4801).

In this paper, we evaluate the role of lion predation in reducing the availability of translocation stock needed for Sierra bighorn recovery efforts. Our first objective was to test the hypothesis that lion predation impeded the ability of Sierra bighorn herds used as sources of translocation stock to produce surplus animals for translocation during 1999–2019. If lion predation impeded the ability of Sierra bighorn herds to produce surplus animals for translocation, we predicted that lion predation would be a primary cause of mortality and ewe survival rates would be largely a function of lion predation rates. Our second objective was to (1) compare lion abundance and ewe survival and predation rates among years of varying predation levels and (2) test the hypothesis that predation severity was stochastic and unpredictable. If lion predation was stochastic, we predicted there would be no association among different levels of lion predation and different levels of lion abundance. Our third objective was to provide a range of estimated times it could take for the Mt. Langley herd to recover to 40 ewes and thus become a viable source of translocation stock again. Our final objective was to determine if the rates lions have been removed to mitigate Sierra bighorn predation exceeded sustainable harvest guidelines, which could indicate a negative impact to the eastern Sierra lion population.

## METHODS

### Study Area

We conducted this study within and adjacent to three Sierra bighorn herds that have provided translocation stock for the recovery program: the geographically overlapping Sawmill Canyon and Mt. Baxter herds (collectively referred to as the Sawmill-Baxter Complex) and the Mt. Langley herd (Fig. 1). The Sawmill Canyon and Mt. Baxter herds are two of the three herds to be continuously occupied by Sierra bighorn since European settlement and the Mt. Langley herd was established via reintroduction using individuals from the Sawmill-Baxter Complex in 1980, after having been extirpated decades prior. We chose these herds for analysis because each experienced lion predation substantial enough to warrant management intervention (i.e., removal of lions that were known or suspected to have preyed upon Sierra bighorn) and continuous data on lion abundance within and adjacent to the herds was available during our study period (1999–2019). We did not include a fourth source herd, Wheeler Ridge, in this analysis because documented lion predation was heavily biased to rams (California Department of Fish and Wildlife, unpublished data), and therefore of less consequence to the herd's productivity, and because data on lion abundance there was less complete after 2010.

### Sierra Bighorn Monitoring

We began radio-collaring Sierra bighorn in 2002 within the Sawmill-Baxter Complex and in 2003 within the Mt. Langley herd. During 2009–2019, 2000–2019, and 1999–2019 for Sawmill Canyon, Mt. Baxter, and Mt. Langley, respectively, we obtained annual estimates of Sierra bighorn ewe abundance using both minimum counts and mark-resight estimates.

Years were categorized as biological years (1 May–30 April) unless otherwise stated. Counts of Sierra bighorn within herds that numbered up to ~30 ewes were generally assumed to be relatively complete (i.e., censuses) and because typically ~30% of the ewes within a herd were collared at any time, the likelihood of missing large groups of individuals was minimal (Stephenson et al. 2012). We derived mark-resight estimates using the Bowden estimator (Bowden and Kufeld 1995).

Mortalities of uncollared Sierra bighorn were identified via (1) investigation of sites where VHF signals or GPS data indicated radio-collared lions were present for  $\geq 2$  consecutive nights, (2) tracking uncollared lions to cache sites, or (3) incidentally to other field work. Mortalities of radio-collared Sierra bighorn were identified via mortality signals from radio-collared individuals. We classified causes of death as lion predation, accident (i.e., injury due to rockfall, avalanche, or other non-predation injury), starvation, natural causes (i.e., old-age, unknown but not predation), other predator (i.e., bobcat [*Lynx rufus*] or coyote [*Canis latrans*]), and unknown. Two or more lines of physical evidence (i.e., neck hemorrhaging, lion tracks and/or scat, drag marks, cached remains, plucked hair, clipped ribs, intact rumen) were required to classify mortalities as caused by lion predation.

### Lion Population Monitoring

During 1999–2011 and 2016–2019, we (1) captured, collared, and monitored individual lions residing near Sierra bighorn; (2) determined the minimum number of independent-age (i.e., >18 months old, approximately) lions occurring each year (1 July–30 June) within a “count zone” encompassing the winter range of each Sierra bighorn herd (Fig. 1); (3) hazed or harassed lions to protect sheep when feasible; and (4) lethally removed lions when deemed necessary to protect Sierra bighorn. During all years, annual lion counts were determined by summing the number of marked individuals, unmarked individuals reported dead (e.g., vehicle collisions, depredation killing), and uniquely identifiable unmarked individuals documented via detection at radio-collared Sierra bighorn mortalities as well as through extensive track and trail camera surveys. We used methods adapted from McBride et al (2008) and further described in Davis et al. (2012) to distinguish unmarked individual lions from each other and avoid double-counting, considering detections of unmarked individuals to be distinct if they occurred >9.6 km apart for females and >16.1 km for males within a 24-h period. When track observations were used to distinguish between unmarked individuals, only tracks <24 hrs old were used. Track age was verified by wind, rain, or snow events, or evidence that tracks occurred the night prior to a survey, such as those occurring over vehicle tracks or dragged roads from the previous day. To avoid overestimating lion abundance, we did not reconstruct presumed incomplete counts by assuming that females initially captured when  $\geq 30$  months old were born within the study area (e.g., Logan and Sweanor 2001; Robinson et al. 2008). Individuals were only counted when there was direct physical evidence of their presence. This method is widely considered the most reliable way for monitoring lion abundance over time (Cougar Management Guidelines Working Group 2005).

Within the Mt. Langley herd, we had more fine-scale data available to index lion activity. Using the same method to develop annual lion counts, we developed minimum daily counts or “lion days per year” as the minimum number of lion-days annually within the Mt. Langley herd by summing the number of days each marked individual was detected (i.e., GPS or aerial VHF locations, or capture, photograph, visual, or track detections), the number of days each unmarked, uniquely identifiable individual was detected (where each

detection counted as 1 lion-day), and the number of days each individual was detected at a cached prey site, where a cache site counted as 3 lion-days, as a conservative estimate of handling time (Knopff et al. 2010). In all years except 2013-2015, we conducted extensive track and/or camera surveys to facilitate counting the number of independent-aged lions that used the Mt. Langley count zone and the Mt. Langley herd to ensure that few, if any, individuals remained undetected.

## Data Analysis

We fit time-to-event models to data from radio-marked Sierra bighorn ewes to estimate cause-specific hazard rates for each herd over the 52-week annual cycle. Before fitting models to data, we standardized the week number so that week 1 began on 1 May and week 52 ended on 30 April. The preceding year was then censored at week 52, and the first entry for the following year was set to week 1 (following Sandercock et al. 2011). We left-truncated data because individuals entered the at-risk group during different weeks (Pollock et al. 1989). We right-censored data in cases of collar failure, immigration or translocation of individuals between herds, or when individuals were still alive at the end of the study (i.e., 30 April 2019). In cases in which individuals were subsequently determined to be alive following a previous collar failure, we imputed their final fate based on the last date known alive, following DeCesare et al. (2015). Because of their geographic proximity and small sample sizes within herds, we pooled survival data from the Sawmill Canyon and Mt. Baxter herds (i.e., the Sawmill-Baxter Complex).

We estimated herd-specific annual ewe survival rates using the Kaplan–Meier staggered-entry estimator (Pollock et al. 1989) and cause-specific mortality using the competing-risks nonparametric cumulative incidence function estimator (NPCIFE) (Heisey and Patterson 2006). We implemented the NPCIFE following the methods used by Griffin et al. (2011), which allows for left-truncation and right censoring and partitioned mortality sources into the 5 aforementioned categories. In addition to the lion predation rate calculated via the CIF (hereafter CIFPR), we also calculated a lion predation rate based on simple ratios (hereafter the naïve predation rate, or NPR). For most years, we calculated the NPR by dividing the number of radio-collared ewes killed by lions by the number of radio-collared ewes present. For years prior to the initiation of radio-collaring, we calculated the NPR by dividing the number of uncollared lion-killed ewes detected by the estimate of ewe abundance (using data from Mt. Baxter during 2000-2003, when abundance for Sawmill Canyon was unavailable, to represent the Sawmill-Baxter Complex), which was justified on the basis that these 2 methods of calculating NPR were highly correlated (Pearson's correlation coefficient = 0.94,  $P < 0.001$ ). There were 2 years for the Mt. Langley herd in which we detected uncollared ewes killed by lions, but no collared ewes killed by lions (2009 and 2018); in these years we calculated NPR by dividing the number of lion-killed ewes by the estimate of ewe abundance. While the NPR suffers a shortcoming over the CIFPR in that it (1) does not account for the staggered nature of animal entry time into interval risk sets and (2) does not permit an estimate of variance (Heisey and Patterson 2006), the advantages of its use are (1) the number of years in which inferences about mortality rates can be made is greater, because it can be calculated even when radio-telemetry data are unavailable and (2) estimates of mortality rates can be made even when only 1 individual dies from a given cause and no individuals die from competing causes prior to that individual's death—in such cases the CIF is undefined.

To aid evaluation of our first objective, testing the hypothesis that lion predation negatively impacts Sierra bighorn herds used as source of translocation stock and impeded the ability of these herds to produce surplus individuals for translocation, we used linear regression to evaluate the relationship between the CIFPR and NPR with survival rates. This is a widely used method to detect when mortality agents have additive effects on survival (Bender and Rosas-Rosas 2006; Brody et al. 2013; Johnson et al. 2013; Murray et al. 2010; Wolfe et al. 2014). Predation was considered additive if the regression slope ( $\pm 95\%$  CI) of the arcsin-square root of predation and survival rates overlapped  $-1.0$ , fully compensatory if the slope =  $0.0$ , and intermediate slopes were considered partially compensatory (Murray et al. 2010).

For our second objective, to compare lion abundance and ewe survival between years of different predation severity levels, we categorized predation severity into 3 categories for each herd-year: no documented predation (i.e., no lion-killed ewes detected), typical predation (i.e., NPR less than the 90th percentile), and extreme predation (i.e., NPR was greater than or equal to the 90th percentile). To test the hypothesis that predation severity was related to lion abundance, we used a chi-squared test to compare the frequency of herd-years in which each level of predation severity (i.e., none, typical, extreme) occurred during years when 2–5 or 6–9 lions were counted.

For our third objective, to provide a range of estimated times it could take for the Mt. Langley herd to recover to 40 ewes and thus become a viable source for translocation stock, we calculated the time required by solving for  $t$  in the equation

$$N_t = N_0 e^{rt}$$

where  $N_t$  is the population size at time  $t$ ,  $N_0$  is the initial population size,  $e$  is the base of natural logarithms,  $r$  is instantaneous rate of population growth, and  $t$  is time. We evaluated return time using a range of growth rates observed during the past 20 years (German and Stephenson 2018; Johnson et al. 2010).

For our final objective, to evaluate how lion removal affected the dynamics of the eastern Sierra lion population, we quantified the fraction of suitable habitat for lions in the eastern Sierra population that was potentially impacted by Sierra bighorn recovery activities by dividing the area of occupied Sierra bighorn habitat, including habitat outside of the Sawmill-Baxter Complex and Mt. Langley herds (i.e., 2,004 km<sup>2</sup>, determined from herd boundaries used in the Recovery plan, (USFWS 2007), by the area of suitable lion habitat for the eastern Sierra population (i.e., 10,241 km<sup>2</sup>, from Dellinger et al. 2020). In addition, we estimated a conservative percentage of the eastern Sierra lion population removed annually by dividing the number of independent lions removed by the effective population size ( $N_e$ ) (i.e., 23, from Gustafson et al. 2019). To evaluate if removal rates exceed sustainable harvest guidelines, we compared the percentage of the eastern Sierra lion population removed annually to a guideline of 14% that has been used for sustainable harvest management in Washington (Wielgus et al. 2013; Beausoleil et al. 2021) but also thought to be widely applicable throughout the western U.S. (Beausoleil et al. 2013).

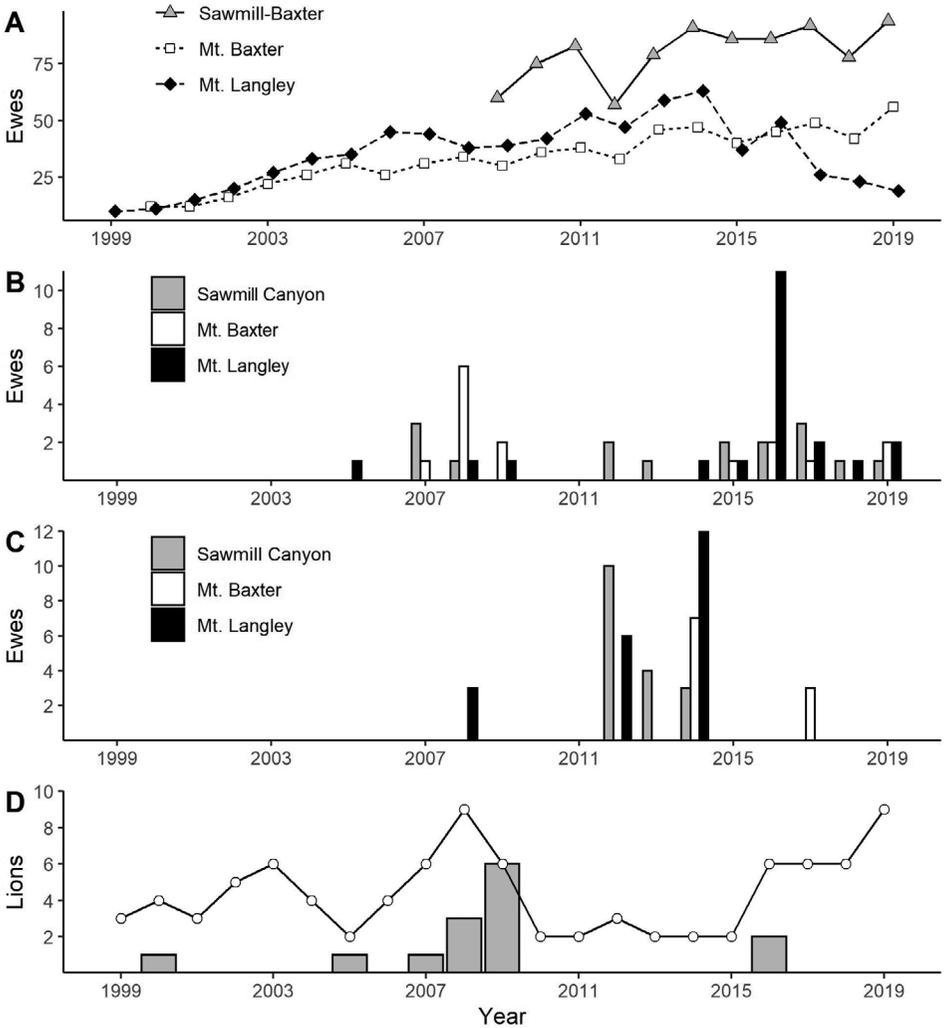
## RESULTS

We radio-monitored 158 Sierra bighorn ewes ( $n = 100$  in the Sawmill-Baxter Complex and 58 in the Mt. Langley herd) and 43 lions (18 female; 25 male) that used the count zone

encompassing these Sierra bighorn herds. These marked animals aided in assessing species demography and trends through time (Fig. 2), which are described below.

**1999–2005**

Lion counts averaged 3.9/yr and lion predation on Sierra bighorn was not detected until 2005. Two lions, both of which used habitat within the Sawmill-Baxter Complex and Mt. Langley herds and were either known or suspected of killing Sierra bighorn, were re-



**Figure 2.** (A) Sierra bighorn ewe abundance, (B) lion-killed ewes detected, both collared and uncollared within the Mt. Baxter, Sawmill Canyon, and Mt. Langley herds, (C) ewes translocated within the Mt. Baxter, Sawmill Canyon, and Mt. Langley herds, and (D) number of independent age lions (i.e.,  $\geq 18$  months old) counted (circles) and number of lions (i.e., any age) removed (bars) within the lion count zone adjacent to these herds during 1999–2019. Abundance of Sierra bighorn for the Sawmill Canyon herd was unavailable prior to 2009; subsequent to this year abundance for Sawmill Canyon and Mt. Baxter was combined (i.e., Sawmill-Baxter).

moved (0.3/yr). Both the Mt. Baxter and Mt. Langley herds experienced substantial growth, increasing from <12 ewes to >30 ewes, although some of the growth in the Mt. Baxter herd was the result of 5 ewes that were translocated into the it from the Wheeler Ridge herd in 2004. Presumably the Sawmill Canyon herd increased as well, but efficient surveys for this herd were not developed until 2009, and thus growth within the Sawmill Canyon herd prior to that year is assumed to correspond with growth of the Mt. Baxter herd. No translocations were conducted during this period because the herds were not yet of sufficient size.

### **2006–2009**

Lion counts steadily increased annually, reaching a peak of 9 in 2008. The increase in lion counts was associated with elevated predation upon Sierra bighorn, primarily within the Sawmill-Baxter Complex, that had not been documented up to that point, as well as decreases in growth from the previous period for both the Mt. Baxter and Mt. Langley herds. As in the previous period, we assume that growth rates within the Sawmill Canyon herd corresponded with that of the Mt. Baxter herd. In response to the increase in predation, 10 lions (i.e., most of the independent-aged lions within the count zone) were removed during 2007-2009 (3.3/yr), all but one of which were known to have preyed upon Sierra bighorn. In 2008, because the Mt. Langley herd had reached a sufficient number of ewes to permit translocation the year prior ( $n = 44$ ) and had had little previous documented lion predation, 3 ewes were translocated from this herd.

### **2010–2015**

The number of lions counted remained low during this 6-year period (range = 2–3), and lion predation remained relatively infrequent. As Sierra bighorn herds increased in abundance, we translocated 27 ewes from the Sawmill-Baxter Complex (17 ewes from Sawmill Canyon and 10 ewes from Mt. Baxter) and 18 ewes from Mt. Langley to aid in the re-establishment of herds within formerly occupied habitat and augment existing small herds (Table 1). All herds (Mt. Baxter, Mt. Langley, and Sawmill Canyon and Mt. Baxter combined [i.e., the Sawmill-Baxter Complex]) experienced modest growth during this period.

### **2016–2019**

Recolonization of lions occurred during these years and by the end of this period the number of lions counted recovered to the former peak of 9 that was observed during 2008. Elevated predation upon Sierra bighorn resumed during this period as well, most notably during 2016, when the NPR and CIFPR at Mt. Langley were 0.42 and 0.47, respectively. In response, 2 of the 7 lions known to be using the Mt. Langley winter range were removed; an adult male and an adult female with at least 3 subadults (all uncollared animals) were unable to be removed. Modest growth within the Sawmill-Baxter Complex continued and the Mt. Langley herd declined in abundance from 49 females in 2016 to 19 females in 2019. Given the amount of lion predation that occurred during this time and concern about a combination of lion predation and translocation resulting in excess losses to source herds, we conducted only a single translocation of 3 ewes from the Sawmill-Baxter Complex (Mt. Baxter) in 2017.

**Table 1.** Translocations of Sierra bighorn ewes from the Mt. Baxter, Sawmill Canyon, and Mt. Langley herds during 2012–2014.

Source Herd	Year	Recipient Herd	No. Ewes	Translocation purpose
Sawmill-Baxter Complex	2012	Olancha Peak	10	Reintroduction
	2013	Olancha Peak	4	Augmentation
	2014	Laurel Creek	7	Reintroduction
	2014	Mt. Gibbs	3	Augmentation
Mt. Langley	2008	Mt. Warren	3	Augmentation
	2012	Convict Creek	3	Augmentation
	2012	Mt. Gibbs	3	Augmentation
	2014	Cathedral	10	Reintroduction
	2014	Mt. Gibbs	2	Augmentation
Total			48	

### Cause-Specific Mortality and Survival

We calculated annual survival and cause-specific mortality rates for the Sawmill-Baxter Complex in 2004–2019 and Mt. Langley in 2003–2019 (Table 2). There was one year (2003) for the Mt. Langley herd in which only 3 ewes were at risk, otherwise the mean number of radiomarked ewes annually was 25.2 for the Sawmill-Baxter Complex (range = 9–44) and 14.8 for Mt. Langley (range = 8–23). Over 35% of each herd was radio-marked each year, on average (range = 11.1–57.9%). Across the Sawmill-Baxter Complex and Mt. Langley, the mean CIFPR was 0.06 (range = 0–0.47,  $n = 33$ ) and the mean NPR was 0.05 (range = 0–0.42,  $n = 42$ ). Herd-years in which the NPR was  $> 0.11$  were in the 90th percentile and considered to be years of extreme predation (Fig. 3). In 73.8% ( $n = 31$ ) of herd-years, the NPR was based solely on radiomarked individuals.

We investigated the mortalities of 78 radiomarked Sierra bighorn ewes ( $n = 47$  in the Sawmill-Baxter Complex and  $n = 31$  in the Mt. Langley herd) between 2003 and 2019. Lion predation was the dominant cause of mortality, accounting for 48.7% of all deaths investigated (38 of 78 deaths) and 70.1% of the deaths in which the cause could be determined (38 of 54 deaths; Fig. 4). Accidents (i.e., deaths from avalanches and rockfall, hypothermia, and physical injury) were the second-most common known cause of mortality. Sierra bighorn ewes were 3.3 times and 5.0 times more likely to die from lion predation than accidents in the Sawmill-Baxter Complex and Mt. Langley, respectively. We also documented lion predation of 14 uncollared ewes (Fig 2).

When annual survival rates from each herd were regressed against their respective annual predation rates, the relationship was highly correlated within both the Sawmill-Baxter Complex and Mt. Langley for each method of calculating predation rates (i.e., NPR and CIFPR; Fig. 5). Regression slopes for annual survival within the Sawmill-Baxter Complex for NPR (slope: -1.23 (-1.58, 0.88),  $t_{14} = 36.09$ ,  $P < 0.001$ ,  $R^2_{\text{adj}} = 0.76$ ) and CIFPR (slope: -1.05 (-1.39, 0.70),  $t_{14} = 33.73$ ,  $P < 0.001$ ,  $R^2_{\text{adj}} = 0.70$ ) and within Mt. Langley for NPR (slope: -0.88 (-1.28, 0.48),  $t_{15} = 26.0$ ,  $P < 0.001$ ,  $R^2_{\text{adj}} = 0.52$ ) and CIFPR (slope: -0.84 (-1.21, 0.47),  $t_{15} = 29.90$ ,  $P < 0.001$ ,  $R^2_{\text{adj}} = 0.54$ ) consistently indicated that lion predation was largely an additive source of mortality for Sierra bighorn within these herds.

**Table 2.** No. radiomarked Sierra bighorn ewes at risk of mortality and survival and cause-specific mortality rates ( $\pm$  standard error) in the Sawmill-Baxter Complex (SBC) and Mt. Langley herds, 2003–2019.

Year	Herd	NR <sup>a</sup>	Survival <sup>b</sup>	Cause-specific survival (Cumulative incidence function)						
				Lion predation	Accident	Natural causes	Other predator	Starvation	Unknown	
2003	Mt. Langley	3	1.0							
2004	Mt. Langley	8	1.0							
	SBC	9	1.0							
2005	Mt. Langley	9	0.88 $\pm$ 0.13	0.12 <sup>c</sup>						
	SBC	9	1.0							
2006	Mt. Langley	17	1.0							
	SBC	9	1.0							
2007	Mt. Langley	17	0.82 $\pm$ 0.12		0.06 <sup>e</sup>					0.13 $\pm$ 0.08
	SBC	13	0.71 $\pm$ 0.20	0.22 $\pm$ 0.10	0.07 $\pm$ 0.07					
2008	Mt. Langley	14	0.77 $\pm$ 0.15	0.08 $\pm$ 0.07						0.15 $\pm$ 0.07
	SBC	23	0.65 $\pm$ 0.15	0.17 $\pm$ 0.08	0.09 $\pm$ 0.07		0.04 $\pm$ 0.05			0.04 $\pm$ 0.05
2009	Mt. Langley	15	1.0							
	SBC	21	0.84 $\pm$ 0.10	0.12 $\pm$ 0.05						0.05 $\pm$ 0.05
2010	Mt. Langley	15	0.87 $\pm$ 0.10							0.13 $\pm$ 0.06
	SBC	17	0.88 $\pm$ 0.09							0.12 $\pm$ 0.06
2011	Mt. Langley	16	1.0							
	SBC	20	1.0							
2012	Mt. Langley	23	0.96 $\pm$ 0.04		0.04 <sup>e</sup>					
	SBC	33	0.94 $\pm$ -0.05	0.03 $\pm$ 0.04			0.03 <sup>c</sup>			0.07 <sup>c</sup>
2013	Mt. Langley	16	0.87 $\pm$ 0.1		0.07 $\pm$ 0.07					
	SBC	32	0.94 $\pm$ 0.05	0.03 <sup>c</sup>						0.03 $\pm$ 0.04

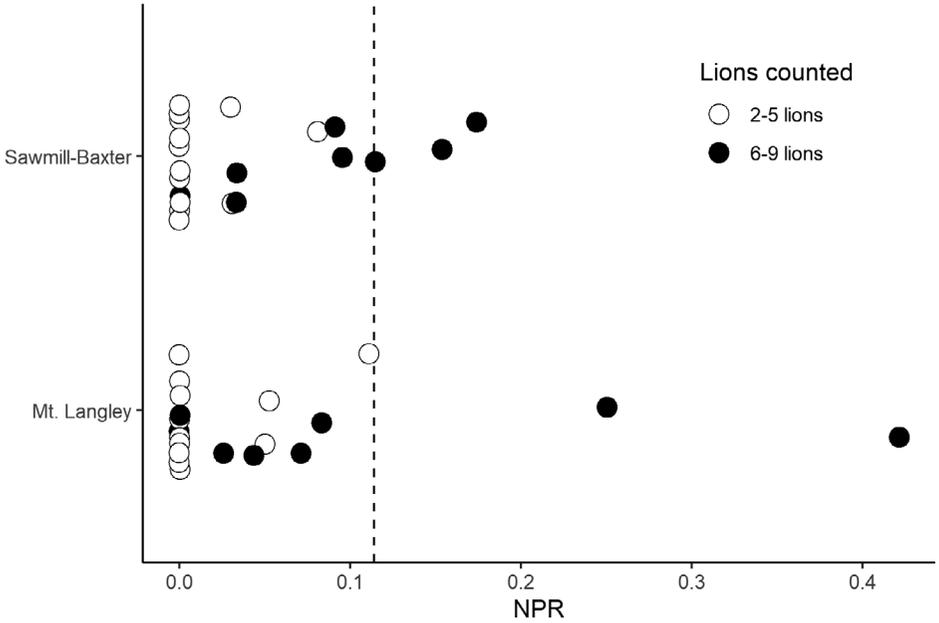
Table 2. continued

Year	Herd	NR <sup>a</sup>	Survival <sup>b</sup>	Cause-specific survival (Cumulative incidence function)						
				Lion predation	Accident	Natural causes	Other predator	Starvation	Unknown	
2014	Mt. Langley	19	0.94 ± 0.06	0.06 <sup>c</sup>						
	SBC	42	1.0							
2015	Mt. Langley	20	0.88 ± 0.09	0.07 <sup>c</sup>						0.05 ± 0.05
	SBC	37	0.86 ± 0.07	0.08 ± 0.04						0.05 ± 0.03
2016	Mt. Langley	19	0.42 ± 0.29	0.47 ± 0.11		0.12 ± 0.07				
	SBC	44	0.80 ± 0.08	0.10 ± 0.04	0.03 ± 0.03					0.06 ± 0.04
2017	Mt. Langley	12	0.72 ± 0.20	0.08 ± 0.08						0.20 ± 0.08
	SBC	35	0.83 ± 0.08	0.14 ± 0.06	0.03 ± 0.03					
2018	Mt. Langley	9	0.89 ± 0.12							0.11 <sup>c</sup>
	SBC	30	0.76 ± 0.10	0.03 ± -0.04	0.07 ± 0.05			0.03 ± -0.04		0.11 ± 0.04
2019	Mt. Langley	8	0.75 ± 0.20	0.25 ± -0.11						
	SBC	30	0.92 ± 0.06	0.04 ± -0.04						0.04 <sup>c</sup>

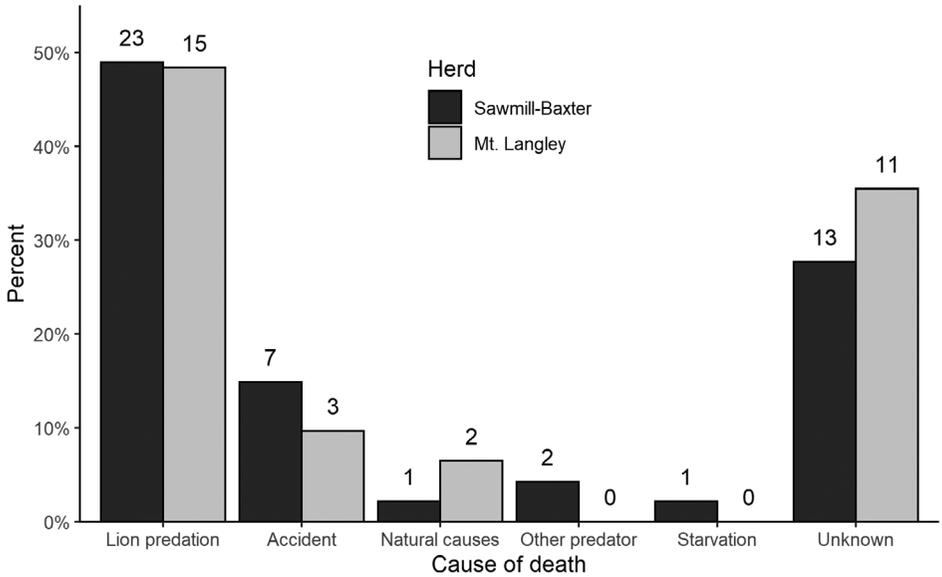
<sup>a</sup> No. of individuals at risk.

<sup>b</sup> Kaplan–Meier survival estimate.

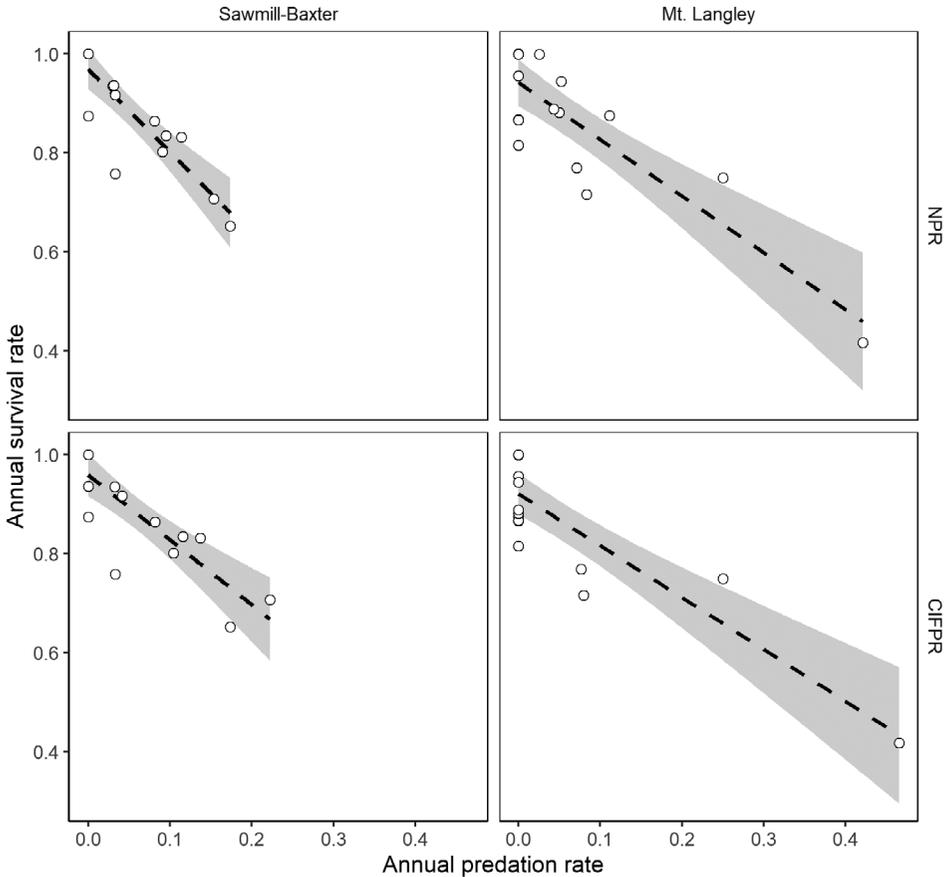
<sup>c</sup> Only 1 individual died from this mortality cause and no individuals died from competing causes prior to this individual's death. As a result, the CIF was undefined. As an approximation for the CIF, we report  $1 - (\text{Survival} + \text{the sum of the cause-specific mortalities})$ .



**Figure 3.** Distribution of annual naïve predation rate (NPR) within the Sawmill-Baxter Complex (2004–2019) and Mt. Langley (1999–2019) Sierra bighorn herds. Vertical dashed line represents the 90th percentile of the NPR.



**Figure 4.** Fates (%) of 78 radio-collared Sierra bighorn ewes within the Sawmill-Baxter Complex and Mt. Langley herd, 2003–2019. Numbers above bars are total deaths.

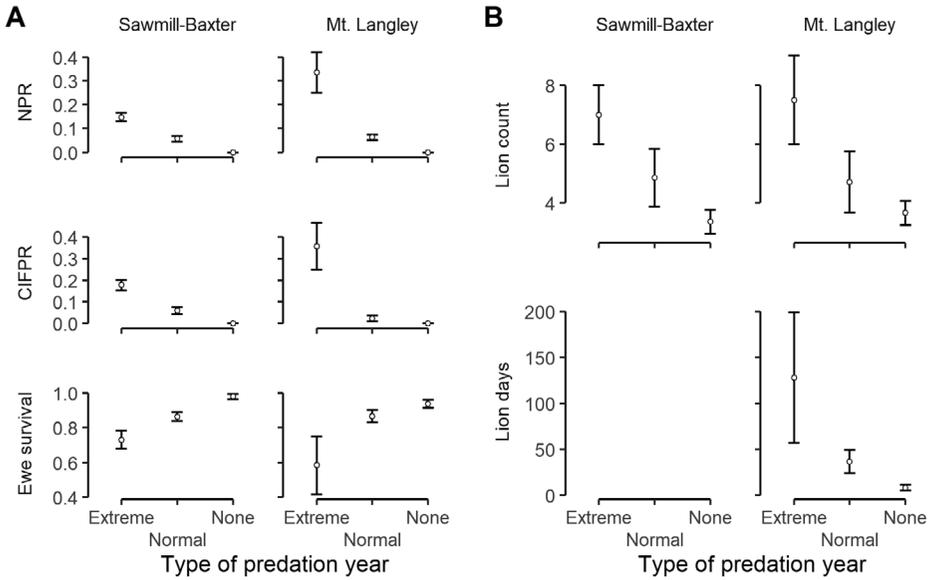


**Figure 5.** Relationship between annual naïve lion predation rates (NPR) and annual ewe survival rates (top 2 panels) and annual cumulative incidence function-derived lion predation rates (CIFPR) and annual ewe survival rates (bottom 2 panels) for the Sawmill-Baxter Complex and Mt. Langley Sierra bighorn herds, 2003–2019.

### Variation in Predation Intensity

In most years, lion predation was not detected within the Sawmill-Baxter Complex (52.4% of years,  $n = 11$ ) or Mt. Langley (57.1% of years,  $n = 12$ ). Extreme predation (i.e., when the NPR was  $\geq 0.11$ ) occurred in 14.3% of years in the Sawmill-Baxter Complex (during 2007, 2008, and 2017) and in 9.5% of years at Mt. Langley (during 2016 and 2019).

In the Sawmill-Baxter Complex, ewe survival during years of extreme predation ( $0.73 \pm 0.05$ ) was 17.8% less than during years of normal predation ( $0.86 \pm 0.03$ ) and 34.2% less than during years of no documented predation ( $0.98 \pm 0.02$ ; Fig. 6). The CIFPR during years of extreme predation ( $0.18 \pm 0.02$ ) was 3.0 times greater than during years of normal predation ( $0.06 \pm 0.02$ ) and NPR exhibited a similar pattern (Fig. 6). The lion count during years of extreme predation ( $7.0 \pm 1.0$ ) was 1.44 times greater than during years of normal predation ( $4.86 \pm \text{SE } 0.99$ ) and 2.08 times greater than during years of no documented predation ( $3.36 \pm 0.41$ ; Fig. 6).



**Figure 6.** Mean ( $\pm$ SE) naïve predation rate (NPR), cumulative incidence function predation rate (CIFPR) ewe survival, lion counts, and lion-days for the Sawmill-Baxter Complex and Mt. Langley Sierra bighorn herds during years of extreme (NPR greater than or equal to the 90th percentile), normal (NPR less than the 90th percentile), and no lion-predation. Lion-days were not evaluated for the Sawmill-Baxter Complex.

At Mt. Langley, average ewe survival during years of extreme predation ( $0.58 \pm 0.02$ ) was 50.0% less than during years of normal predation ( $0.87 \pm 0.04$ ) and 62.1% less than during years of no documented predation ( $0.94 \pm 0.02$ ; Fig. 6). The CIFPR during years of extreme predation ( $0.36 \pm 0.11$ ) was 18.0 times greater than during years of normal predation ( $0.02 \pm 0.01$ ) and NPR exhibited a similar pattern (Fig. 6). The lion count during years of extreme predation ( $7.50 \pm 1.50$ ) was 1.59 times greater than during years of normal predation ( $4.71 \pm 1.04$ ) and 2.04 times greater than during years of no documented predation ( $3.67 \pm 0.41$ ; Fig. 6). The number of lion-days detected during years of extreme predation ( $128.0 \pm 71.0$ ) was 3.49 times greater than during years of normal predation ( $36.71 \pm 12.61$ ) and 15.50 times greater than during years of no documented predation ( $8.25 \pm 2.95$ ).

The frequency in which the 3 levels of predation severity occurred varied over the 2 levels of lion abundance (i.e., 2–5 and 6–9 lions;  $\chi^2 = 16.4$ ,  $df = 2$ ,  $P < 0.001$ ). When 2–5 lions were counted, extreme predation did not occur at all and no documented predation occurred in 20 of 26 (76.9%) herd-years. In contrast, extreme predation occurred when 6–9 lions were counted in 5 of 16 (31.2%) of herd-years and no documented predation occurred in 3 of 16 (18.8%) herd-years. Typical predation was also more common when the lion count was 6–9 (50% of herd-years) versus 2–5 (23.0%; Fig. 3).

### Recovery Times for the Mt. Langley Herd

We estimated the time required for a herd of 19 females (i.e., the number present in 2019) to recover to 40 (i.e., the number required to be considered a source of translocation stock again) would be 37.2, 14.9, 7.4, and 3.7 years for growth rates ( $r$ ) of 0.02, 0.05, 0.1, and 0.2, respectively.

## Impacts of Lion Removal on the Lion Population

Based on estimates of the area of occupied Sierra bighorn habitat and suitable lion habitat for the eastern Sierra lion population (10,241 km<sup>2</sup>, from Dellinger et al. (2020), we estimated that 19.6% of the suitable habitat for the eastern Sierra lion population overlaps with occupied Sierra bighorn habitat and 3.8% overlaps with habitat occupied by the Sawmill-Baxter Complex and Mt. Langley herd, where both lion predation and lion removal has been most prominent relative to other Sierra bighorn herds. During 1999–2019, we removed 26 lions to protect Sierra bighorn (i.e., 11 independent and 3 dependent lions to protect Sierra bighorn in the Sawmill-Baxter Complex and Mt. Langley herds; 9 independent and 3 dependent lions to protect other herds). The number of independent lions removed annually averaged 0.95 (range = 0–5), corresponding with an estimated annual removal rate of 4.14% (range = 0–21.7%) of the eastern Sierra lion breeding population, based on the  $N_e$  estimate of 23 lions from Gustafson et al (2019).

## DISCUSSION

### Cause-Specific Mortality and Survival

Lion predation has been a management concern for the recovery of Sierra bighorn for decades and was a primary reason for listing Sierra bighorn as endangered (Wehausen 1996; USFWS 1999, 2007). We sought to evaluate the role of lion predation in the recovery of Sierra bighorn, including how lion predation may contribute to a shortage of translocation stock that is needed for the recovery effort. Our results indicated that lion predation limited the number of ewes available for translocation within the Sawmill-Baxter Complex and Mt. Langley herds during 1999–2019 by removing a substantial number of ewes in these source herds that could otherwise have served as translocation stock. Approximately half of all radiomarked ewe deaths (38 of 78), and potentially more given the high proportion of mortalities whose cause could not be identified, were the result of lion predation. Further, annual survival rates of ewes decreased substantially as lion predation rates increased, indicating that lion predation was primarily additive to other sources of mortality (e.g., Johnson et al. 2013; Bowyer et al. 2014). Because animals lost to predation are not available for translocation, which is a critical component of the recovery program, we conclude that lion predation has impeded recovery of Sierra bighorn throughout the recovery program's duration. Continued unmitigated predation at rates similar to observed 2016–2019 rates remain a substantial management concern, which if not addressed, could contribute to the extirpation of Sierra bighorn herds. Lion predation is an immediate threat to the viability of the depressed Mt. Langley herd.

Our findings are consistent with those from Johnson et al. (2013) that lion predation is an additive source of mortality within the Sawmill-Baxter Complex. However contrary to Johnson et al. (2013), we found lion predation was also an additive source of mortality within the Mt. Langley herd. This discrepancy is because our 17-year dataset included greater variation in survival and predation compared to the five-year dataset examined by Johnson et al. (2013). The contrasting results between these studies illustrate the importance of long-term monitoring, which can uncover patterns in data and important management implications that may be missed in shorter-term studies (Lovett et al. 2007).

Previous studies of Sierra bighorn provide additional evidence that supports our conclusion that lion predation was an additive cause of mortality, impeding the ability of source

herds to produce translocation stock. For example, population size was in none of the top models used by Conner et al. (2018) to explain variation in adult survival during 2002–2013, and most ewes during 2002–2018 had body fat levels well above the physiological minimum required for overwinter survival (Stephenson et al. 2020). Notably, out of 11 different herds examined, the Mt. Langley herd had some of the highest average body fat measurements recorded (Stephenson et al. 2020). If predation was primarily a compensatory cause of mortality (i.e., lions preyed upon individuals that likely would have died from another cause in the absence of predation), such findings would not be expected (Bowyer et al. 2014).

We calculated predation rates using two different methods, producing a NPR and CIFPR, which were remarkably similar to each other. However, while the methods of Heisey and Patterson (2006) for estimating CIFs are widely used (e.g., Murray et al. 2010; Griffin et al. 2011; Sandercock et al. 2011), in small populations such as ours, CIFs may have limited value if solely relied upon for inferring the importance of different mortality sources. For example, several cause-specific mortality rates in Table 2 were undefined because only 1 individual died from a given mortality cause during a year and no individuals died from competing causes prior to that individual's death. In addition, had we relied on the CIFPR alone, which uses only data from radio-marked animals, during 2009 and 2018 when ~40% of ewes in the Mt. Langley herd were radio marked and none died from lion predation, we would have inferred that lion predation was unimportant, even though we knew that at least 1 uncollared ewe was killed each year, representing 2.5% and 4.3% of the herd, respectively. These examples illustrate that in small, endangered populations like Sierra bighorn, where the death of even single individuals can be consequential, it may be useful to use simple ratio estimators for cause-specific mortality in addition to more statistically robust ones. These examples also illustrate the importance of maintaining radio-collars on as many lions as possible in Sierra bighorn habitat because predation can go unnoticed even with a substantial fraction of Sierra bighorn being collared. Maintaining radio-collars on lions would also aid in targeting management responses to only those that prey upon Sierra bighorn, which may help promote tolerance of lethal removal among stakeholders.

### **Variation in Predation Intensity**

When comparing Sierra bighorn ewe survival among years of extreme, typical, and no documented predation we found that during years of extreme predation ewe survival rates were poor, averaging 0.73 and 0.58 in the Sawmill-Baxter Complex and Mt. Langley herds respectively, values which resulted in population declines in those years. However, even during years of typical predation, average ewe survival rates in the Sawmill-Baxter Complex and Mt. Langley herd were 0.86 and 0.87 respectively, which are near the threshold needed for population stability, assuming recruitment rates remain within their historic range of variability (Few et al. 2015), but below a value needed to ensure population growth and ultimately recovery and delisting. Thus, typical predation, if not punctuated by years without predation could, especially in combination with other mortality sources, prevent population growth (e.g., Harris et al. 2009). Because other causes of mortality are relatively rare compared to lion predation (Fig. 4) and are not as amenable to management intervention as lion predation, there are few options for improving Sierra bighorn survival outside of lion removal. During this study, all lion removal was lethal, and it is likely that lethal removal will need to be an option in the future if Sierra bighorn survival rates are to be improved. However, it may be possible in limited circumstances to experiment with non-lethal removal

as well (i.e., translocating lions to areas away from Sierra bighorn), which could be viewed as more favorable by some stakeholders. There are numerous factors to be considered with translocation that are not present with lethal removal, including (1) the logistical difficulty in carrying large animals from remote, roadless areas to transport vehicles, which involves measurable risks for both project personnel and animals involved, (2) extended times (perhaps many hours) that animals must be under anesthesia, (3) nonestablishment, resulting in movements that bring animals into human-wildlife conflict or homing that brings them back into contact with Sierra bighorn, and (4) animal welfare concerns associated with intraspecific strife at release sites, disorientation at release sites that inhibits the ability to locate prey and/or shelter, or increased exposure to hazards, such as highways (e.g., see Craven et al. 1998). There has been very limited research on lion translocation to date (i.e., Ruth et al. 1998 is the only study of which we are aware that has tracked the fates of translocated lions) and thus, there is more speculation about potential outcomes than available data. Nonetheless, translocation of lions has been used successfully for conservation purposes, such as improving the genetics of the endangered Florida panther (*Puma concolor coryi*; van de Kerk et al. 2019). Given that several lion populations in southern CA are of conservation concern and have been petitioned for listing under the CA Endangered Species Act (Center for Biological Diversity and the Mountain Lion Foundation 2019) because of similar issues experienced by Florida panthers (Gustafson et al. 2017; Benson et al. 2019; Gustafson et al. 2019), translocation of lions to southern CA populations, as suggested by Benson et al. (2019), from the eastern Sierra Nevada may be an option that could benefit 2 at risk species (Sierra bighorn and southern CA lions) simultaneously.

Episodes of unusually high predation rates have been reported for Sierra bighorn previously, including in the Mt. Warren herd in the early 1980s (Chow 1992) and in the Sawmill-Baxter Complex in the 1990s (Wehausen 1996). Such periods of apparently stochastic changes in the frequency of lion predation upon rare prey have been documented elsewhere as well, such as bighorn sheep in Alberta (Festa-Bianchet et al. 2006) and endangered huemul (*Hippocamelus bisulcus*) in Patagonia (Elbroch and Wittmer 2013; Wittmer et al. 2014). The leading hypothesis to explain such irruptions in predation is that they are caused by stochastic changes in the composition of a lion population (e.g., death or immigration), combined with variation in dietary choices between individual lions—factors that are independent of lion abundance (Ross et al. 1997; Logan and Sweaner 2001). Based on our findings that severe predation did not occur when lion counts were relatively low (2–5 lions) but did occur in 50% of the years when lion counts were high (6–9 lions), and that on average our two indices of lion abundance (i.e., counts) and activity (i.e., lion-days) were progressively greater in years of no documented predation, typical predation, and severe predation respectively (Fig. 5), we do not consider lion predation on Sierra bighorn to be primarily a stochastic phenomenon. Rather, episodes of extreme predation are somewhat predictable, and provided that monitoring of lion abundance continues, advance warning of extreme predation years can be available. However, because we did not evaluate lion diet selection or estimate prey availability, we cannot exclude the possibility that specialist lions were present in some years. Increased predation in association with increases in lion abundance could be explained by the increased odds of lions encountering relatively rare Sierra bighorn while hunting their primary mule deer (*Odocoileus hemionus*) prey (Pierce et al. 1999, 2000), an increase in the odds that at least one lion selectively preyed upon Sierra bighorn, or both.

## Recovery Times for the Mt. Langley Herd

The Mt. Langley herd declined substantially during its extreme predation episode and if the pattern of decline observed from 2016–2019 continues, this herd will become extirpated within a few years. Lion-mediated extirpations of bighorn sheep have been documented elsewhere. For example, Rominger et al. (2004) reported that a bighorn sheep translocation effort in the Sierra Ladron mountains of New Mexico failed primarily because of lion predation, noting that as the bighorn population declined, the predation rate actually increased, which they suspected to be the result of lions being subsidized by domestic cattle. Similarly, the functional extirpation of a herd of desert bighorn (i.e., only a single ewe remained at the end of the study) in the San Andreas Mountains of New Mexico occurred when lions killed most of what was left of a herd of nine bighorn in a span of less than a year (Rominger and Weisenberger 2000).

The Mt. Langley herd currently is not a viable source of translocation stock, a change that has reduced the Recovery Program's capacity for translocation by about 25% (i.e., of 73 ewes translocated during 2004–2019, 21 were removed from the Mt. Langley herd). Consecutive years of predation are likely to have a more negative impact on population growth than years interspersed with a lack of predation, even if the overall predation rate remains the same (Pike et al. 2004; Festa-Bianchet 2006). Given its current low abundance, the Mt. Langley herd is unlikely to rebound to its former abundance in a short time frame unless predation is—at least temporarily—sharply reduced. We calculated that it would take ~15 years for the 2019 population of 19 ewes to return to 40 ewes if modest population growth (5% per year) occurs. However, our simple model assumed exponential growth and that  $\lambda$  is always positive, neither of which may be reasonable assumptions. Whether or not the Mt. Langley herd will recover at all is currently unclear and it may take a substantial amount of time because actual population demographics include stochastic effects (e.g., severe winters) not accounted for in our simple model. On the other hand, high population growth had been documented in the past (e.g., the Mt. Langley herd increased from 10 to 45 ewes during 1999–2006) during a period of favorable weather conditions and almost no lion predation, which provides some reason for optimism. To be clear though, because lion abundance is almost certainly not maintained by Sierra bighorn but by their primary prey, mule deer, the decline in abundance of Sierra bighorn at Mt. Langley is unlikely to appreciably impact the lion population (Pierce et al. 2000; Johnson et al. 2013). Thus, continued high lion predation could drive this herd to extirpation, similar to apparent competition situations that occur among caribou (*Rangifer tarandus*), moose (*Alces alces*), and wolf (*Canis lupus*) populations in Canada (e.g., Serrouya et al. 2015).

In contrast to the Mt. Langley herd, the Sawmill-Baxter Complex performed very differently following the extreme predation episode that occurred there during 2007 and 2008, experiencing population growth in the years immediately afterward. We suggest that the reason for these divergent trajectories is primarily related to the different management responses that occurred to mitigate the predation. Removal of lions that preyed upon Sierra bighorn within the Sawmill-Baxter Complex began during the predation episode, which likely limited the number of ewes killed, while removing lions that preyed upon Sierra bighorn at Mt. Langley occurred only after ~40% of the herd had been killed. In addition, during the Sawmill-Baxter Complex extreme predation episode, all of the lions known to prey upon Sierra bighorn within the herd were removed, while during the Mt. Langley extreme predation episode, an adult male and an adult female with at least 3 subadults (all uncollared

animals) were unable to be removed and may have continued preying upon Sierra bighorn. These observations demonstrate that if predation is to be mitigated through lion removal, quick response times (e.g., beginning tracking and capture efforts within 24–48 hours after a Sierra bighorn kill is confirmed) are required.

### Impacts of Lion Removal on the Lion Population

In response to elevated predation within the Sawmill-Baxter Complex during 2007–2009, most independent-aged lions within the count zone were removed. All but one of the lions removed during this period were known to have preyed upon Sierra bighorn. Following lion removals from 2007–2009, lion occupancy in the count zone during 2010–2015 was reduced. During this period, 45 ewes from the Sawmill-Baxter Complex and Mt. Langley herds were translocated to re-establish several previously extirpated herds (Fig. 2; Table 1)—an opportunity that was facilitated by releasing bighorn populations from lion predation. However, the number of lions using Sierra bighorn habitat adjacent to these source herds recovered from a low of two independent lions in 2010–2011 following the period of intense removal to six individuals by 2016, and by 2019 there were as many lions counted ( $n = 9$ ) as at any point since monitoring began. Rapid recolonization by lions may have been possible because the area from which lions were removed was small relative to the entire eastern Sierra lion population, which functions as a source population (Gustafson et al 2019), and/or because of connectivity between the eastern Sierra lion population and western Sierra Nevada and North Coast lion populations of CA as well as the Nevada lion population which exchange migrants in both directions (Gustafson et al. 2019). Such rapid recolonization of vacant lion habitat suggests long-term negative impacts to the number and distribution of lions in the eastern Sierra lion population from removals to protect Sierra bighorn from predation are unlikely. This observation is consistent with our estimate that the majority ( $> 80\%$ ) of the suitable habitat for the eastern Sierra lion population does not overlap Sierra bighorn habitat, indicating that a substantial fraction of the lion population is not subject to removal because those lions do not interact with Sierra bighorn, and (2) the average level of lion removals to date (0.95/yr) equates to 4.14% of the estimated genetically effective population ( $N_e$ ) of 23 eastern Sierra lions (Gustafson et al. 2019), less than 1/3 of the 14% sustainable harvest threshold needed for population stability advocated by Beausoleil et al. (2013), Wielgus et al. (2013), and Beausoleil et al. (2021). Should lion removal rates exceed 3 independent lions/yr, short-term reductions in the eastern Sierra lion population may occur. However, given that numerical abundance is typically considerably higher than  $N_e$  (Frankham 1995), true removal rates are likely considerably lower than worst case estimates we present based on  $N_e$ . In order to cause a reduction in the eastern Sierra lion population, lion removals for Sierra bighorn protection would almost certainly have to increase substantially over any numbers observed to date.

### Conclusion

Lion predation on Sierra bighorn impeded recovery of Sierra bighorn during 1999–2019. Uncontrolled lion predation can contribute to poor population performance, even at typical levels, however, extreme levels of predation appear capable of extirpating Sierra bighorn herds and could seriously inhibit recovery of the subspecies. Because lion predation can be intense enough to cause declines in the largest of Sierra bighorn herds, the entire Sierra bighorn population should be considered susceptible to this phenomenon, even within

herds that have not experienced substantial predation since the Recovery Program began. Our results, combined with the findings of previous work, indicate that years of extreme predation might be expected to occur in approximately 1 of every 10 years in some portion of Sierra bighorn range. However, our observations included years following active predator management (2010–2015) in which the number of lions using Sierra bighorn habitat was substantially reduced. Should predator management not occur in the future, it seems probable that extreme predation events could become more common (and consequently, no longer be considered extreme, in the sense of deviating from the average). Removal of lions that prey upon Sierra bighorn appears to have been effective at improving ewe survival rates and thus using lethal or non-lethal means (e.g., translocation) to remove lions from Sierra bighorn habitat in the future appears necessary if Sierra bighorn are to be recovered. Although we did not have data on cause-specific mortality of neonate Sierra bighorn, lion predation can be a common cause of mortality for neonate desert bighorn (Parsons, Z.D. 2007; Cain et al. 2019); thus, it is possible that lion removal would result in improved recruitment rates as well, which would further accelerate recovery. If such steps are taken, lion abundance within the areas from which lions are removed will almost certainly recover within a short period of time.

While there is a desire within the Department to accommodate natural processes, such as predation, there are currently not enough Sierra bighorn within these herds to simultaneously satisfy the demands of predators and the need to recover this endangered species. High levels of lion predation on Sierra bighorn have already slowed progress toward meeting recovery goals, and if they go unchecked, they could result in the extirpation of entire Sierra bighorn herds. Should Sierra bighorn herds increase in the future and reach recovery targets, an evaluation of what constitutes “acceptable” levels of predation will be warranted; however, predation monitoring and management are likely going to be a part of the management toolbox for Sierra bighorn for the foreseeable future.

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**Front.** The salt marsh harvest mouse is a CESA and ESA listed endangered species restricted to the San Francisco Estuary. In many parts of the range the species overlaps with the morphologically similar western harvest mouse, making it difficult to identify in the field. Photo Credit: William Thein.

**Back.** Belding's savannah sparrow. Photo Credit: USFWS Pacific Southwest Region. CC BY 2.0.



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