

FINAL REPORT

A Data-driven Decision Support System to Identify Optimal Land Use Alternatives for Protecting Species of Concern on DoD and Surrounding Lands

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List of Acronyms

AT – Arroyo Toad

AUC or AUROC – area under the receiver operating characteristic curve

Bd – *Batrachochytrium dendrobatidis* (chytrid fungus pathogen)

CRLF – California Red-legged Frog

CDFW – California Department of Fish and Wildlife

CRMR – Camp Roberts Military Reservation

CSLO – Camp San Luis Obispo

CSUMB – California State University at Monterey Bay

DOD – Department of Defense

DNA – deoxyribonucleic acid

DSS – decision support system

eDNA – environmental DNA

ERC – energy release component

FHL – Fort Hunter-Liggett

FSim – large fire simulation software

FYLG – Foothill Yellow-legged Frog

GBIF – Global Biodiversity Information Facility

GCM – global climate model

GIS – Geographic Information System

HSM – habitat suitability model

LPNF – Los Padres National Forest

Marxan – fusion of Marine and SPEXAN. SPEXAN is an acronym for Spatially Explicit Annealing

MBC – metabarcoding

NDVI – normalized difference vegetation index (NDVI)

PCR – polymerase chain reaction

PRISM - Parameter-elevation Relationships on Independent Slopes Model

PROTAX – probabilistic method for taxonomical classification software

qPCR – quantitative polymerase chain reaction

RF – random forest

rRNA – ribosomal ribonucleic acid

SCP – systematic conservation planning

SDM – species distribution model

SOC – species of concern

SOM – species occupancy model

TSS – true skill statistic

USU – Utah State University

VAFB – Vandenberg Airforce Base

WPT – Western Pond Turtle

WSFT – Western Spade-foot Toad

Keywords

Decision support systems, species of concern, Marxan, freshwater ecosystems, amphibians, turtles, eDNA, habitat suitability models

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Abstract

Introduction and Objectives – Spatial conservation plans are required to optimally manage species of concern while considering other land use needs. Systematic conservation planning identifies areas within species’ ranges that can be conserved at minimal costs or disruption to other land uses. Rare or cryptic species present challenges for conservation planning as the crucial first step is identifying where species exist on a landscape. In addition, understanding landscape-level risks that may undermine conservation actions is critical to designing robust conservation plans. We present the results of a systematic conservation plan developed to conserve threatened and endangered aquatic reptiles and amphibians in south-coastal California. The area is home to a suite of threatened reptiles and amphibians. It is also a multi-use landscape with extensive areas of federal land managed by the Department of Defense and the US Forest Service.

Technical Approach – We combined data from a comprehensive eDNA sampling survey with the locations of previous detections to produce habitat suitability models for four amphibian species and a pond turtle: The California Red-legged Frog (*Rana draytonii*), the Foothill Yellow-legged Frog (*Rana boylei*), the Arroyo Toad (*Anaxyrus californicus*), the Western Spadefoot Toad (*Spea hammondi*), and the Western Pond Turtle (*Actinemys marmorata*). The use of eDNA overcomes many of the challenges associated with detecting cryptic species and also allowed us to quantify the distribution of chytrid fungus, a serious amphibian pathogen, across the study area. We calculated the opportunity costs of restoring current land uses to suitable habitat – i.e., moving or removing existing built infrastructure (roads, buildings, military ranges). Combining information on the location of roads, projected climate change, and the predicted distribution of chytrid fungus produced estimates of the risk that conservation actions would fail. We integrated estimates of spatial variation in predicted habitat suitability, land costs, and risks into a suite of Marxan analyses to identify priority locations for conservation. We then created maps that showed how the costs and land area required to meet different conservation goals influenced which sites had the highest priority for biodiversity conservation.

Results – Our results identified critical landscape areas for protection that balanced the needs of biodiversity and human land uses. Moreover, our results highlight that we may be able to achieve conservation objectives with minimal disruption to important, high-value land uses such as airstrips, testing ranges, and buildings. However, we also demonstrate that conservation in highly-developed landscapes will require encroaching on lower dollar-value infrastructure, as achieving conservation goals was impossible if all areas containing any infrastructure were off-limits for conservation.

Benefits – With increasing development placing pressure on natural landscapes, managers increasingly need tools to aid in balancing biodiversity protection and human land use. Objective, data-driven, spatially explicit decision-support systems can be applied across multi-jurisdictional landscapes to help meet mutually agreed upon conservation goals.

Executive summary

Introduction

Decision support systems are critically needed by natural resource managers wishing to best conserve species of concern while considering other land use needs. However, the effectiveness of decision support systems is currently constrained by both the quality of data describing the known locations of species of concern within a landscape of interest and the need for methods that can objectively evaluate the tradeoffs between conservation and other needs. Effective conservation planning requires knowing where species of concern occur, the costs of different management options applied to individual land parcels, and how habitat conditions influence the viability of different species under changing environmental conditions. The goal is to then identify what land use management scenarios best conserve species while minimizing costs and restrictions on land use activities. Ideally, species occurrences are known with certainty. However, many estimates of species occurrence can be highly uncertain, which compromises their use. Uncertainty in species occupancy occurs because traditional survey techniques (e.g. trapping, netting, electrofishing, visual surveys) capture too small of a portion of the individuals actually present to reliably conclude a species is absent. Such variable, and often unknown, detection probabilities can lead to inaccurate inferences regarding population status, biased habitat suitability or occupancy models, and thus flawed management decisions. This problem is especially pronounced when target species are rare, have clustered distributions, are semi-aquatic, or occur in the substrate.

Surveys based on environmental DNA (eDNA) can overcome many of the constraints associated with traditional survey methods. eDNA refers to DNA fragments found in the environmental media that are derived from organisms in contact with that media. eDNA surveys do not require capture, handling, or even observation of the target organisms, making them especially useful for surveys of species of concern, which can be difficult to capture or even harmed by more invasive traditional survey methods. eDNA-based detection techniques therefore represent a robust, sensitive, and economical alternative to traditional surveys. eDNA monitoring is especially useful in detecting aquatic or semi-aquatic species. In aquatic ecosystems, DNA molecules from species that are patchily distributed, rare, or difficult to physically capture are mixed in the water column. This water can be easily sampled for eDNA, which can subsequently be identified with molecular DNA analysis techniques such as quantitative PCR and sequencing. eDNA approaches can also significantly improve the sampling efficiency and detection probability over what can be achieved with traditional survey methods. The higher sensitivity of DNA-based survey methods can thus reduce the analytical and modeling biases caused by imperfect and variable detection.

Species viability depends on both the availability of suitable habitat and connectivity with other populations, both of which affect the likelihood of local population persistence. Managing species of concern therefore requires knowledge regarding what specific set of local and regional processes influence population occurrence and dispersal and how different threats interact with those processes. Habitat suitability models and occupancy models, which allow prediction of the suitability of unsampled locations, are useful when it is impractical to survey all possible locations that might support a species. Models can be used to identify locations that, if restored, should support viable populations.

Ultimately, successful conservation requires tools that managers can use to objectively evaluate the benefits and costs associated with achieving conservation aims. Natural resource managers are increasingly using systematic conservation planning tools to help identify the specific mixes of land use activities that can achieve desired conservation outcomes at minimal, or acceptable, cost. Decision support tools that are based on principles of optimization and that use sound empirical data and model outputs are especially useful in conservation planning. Rather than rely on expert opinion to identify priority conservation areas, these systematic conservation planning tools can apply optimization techniques to quantitative data on occupancy, local habitat conditions, proximity of populations to one another, and the spatial distribution of threats to identify the specific areas that will best achieve desired levels of conservation for the least cost. Given that a landscape context is required to conduct these analyses, effective use of these tools will often require cooperation and coordination among multiple jurisdictional entities.

Objective

The primary objective of the proposed research was to use eDNA survey data to develop a data-driven, spatially explicit, decision support system that can be used by multiple, collaborating jurisdictions to identify the mixes of land use that optimally conserve species of concern on Department of Defense (DOD) and surrounding lands.

Technical Approach

We developed and tested a systematic conservation planning tool (Marxan) on aquatic species of concern occurring on and around military installations in the south-central coastal region of California. Our focal landscape included Fort Hunter-Liggett (FHL), Camp Roberts Military Reservation (CRMV), Vandenberg Air Force Base (VAFB) – now Vandenberg Space Force Base, Camp San Luis Obispo (CSLO), and federal and state lands near these installations. Prior to initiating data collection and analysis, we scheduled a workshop for stakeholders in the study area to discuss the aims of the project and its design and solicit suggestions for potentially improving both the survey design and how the decision-support tool could be applied across the lands each stakeholder manages. To complete our objective, we needed to (1) identify target species of concern; (2) use eDNA to identify what specific habitats the target species occupied; (3) characterize the geoclimatic environments associated with species' occurrences; (4) compile information on, or model, how climate and fire risk varies across the study region and is likely to change by mid-century (2040-2069); (5) develop empirical, habitat suitability models to predict the likelihood of each species inhabiting non-sampled habitats in the study region; and (6) use habitat suitability predictions, estimates of the opportunity costs of removing infrastructure and restoring ecologically degraded areas to viable habitat, and estimates of the risks of conservation actions failing in specific planning units (1 x 1 km areas) as inputs to Marxan analyses that identify the mix of planning units that achieves conservation targets at the least cost.

Our target species included four amphibians and a freshwater turtle that are either federally protected species or species of concern in California: the Arroyo Toad (*Anaxyrus californicus*), the Western Spade-foot Toad (*Spea hammondi*), the Foothill Yellow-legged Frog (*Rana boylei*), the California Red-legged Frog (*Rana draytonii*), and the Western Pond Turtle (*Emys*

marmorata). We collected eDNA samples at 199 sites across the four military installations and the Las Padres National Forest and used qPCR to assess if each of the target species was present at each of the sites. We also compiled historical occurrence records to augment eDNA detections when constructing habitat suitability models for some species. We also used the eDNA samples to quantify the distribution of the chytrid fungus, *Batrachochytrium dendrobatidis*, a serious amphibian pathogen across the study area.

After completing eDNA sampling, we characterized the geoclimatic setting of each site by compiling data on 173 GIS-scale variables as well as data on current and future (2040-2069) climate conditions and fire regimes. We used these data as predictors in empirical habitat suitability models that we developed for each species. These models allowed us to predict the likely habitat suitability at 30 x 30 m resolution across the entire study landscape. Output from the habitat suitability models was a key input into the Marxan analyses that identified optimum sets of 1 x 1 km planning units for protection or restoration. In addition to these data, we also calculated the opportunity costs of restoring current land uses to suitable habitat – i.e., moving or removing existing built infrastructure (roads, buildings, military ranges).

We integrated estimates of the spatial variation in predicted habitat suitability, land costs, and risks into a suite of Marxan analyses to identify priority locations for conservation. We used information on the location of roads, projected climate change, and the predicted distribution of chytrid fungus as estimates of the risk that conservation actions applied to planning units would fail. We explored two scenarios in these analyses: (1) all planning units were available for selection and (2) only planning units with low to moderate opportunity costs were considered as being available. The second scenario eliminated planning units that contained immovable infrastructure such as buildings and air facilities. We then created maps that showed how spatial variation in both costs and habitat suitability influenced which sites had the highest priority for biodiversity conservation.

Results and Discussion

eDNA sampling was efficient at detecting both target species of concern as well as many other aquatic species in the study area. The eDNA survey also confirmed that most of the target species currently have very limited distributions, which meant too few detections occurred to develop eDNA-based habitat suitability models for all species but the Western Pond Turtle. However, we were able to augment the eDNA detection data with historical data compiled by the Global Biodiversity Inventory Facility to develop habitat suitability models for the California Red-legged Frog and the Arroyo Toad. Too few eDNA detections occurred and too few historical records existed to model habitat suitability for the Foothill Yellow-legged Frog and the Western Spade-foot Toad.

The Marxan analyses identified critical landscape areas for protection that balanced the needs of biodiversity and human land uses. Overall, the results of the Marxan analyses highlight differences across the landscape in the relative conservation priority of different planning units. Crucially, at the qualitative level, we saw no major differences in the relative conservation priority of different planning units as conservation targets increased – scaled from low (e.g., 0.2) to high (e.g., 0.8) amounts of protected planning units. The frequently selected planning units

seemed to be in similar areas for both scenarios regardless of whether conservation targets were set at 0.2 or 0.8. Only the absolute number of selections changed. This consistency in the identification of high-priority areas has important implications for management actions, as we can be more confident in our ability to identify high-priority areas, and important locations for conservation can be selected regardless of what our quantitative conservation targets may be. Given that priority areas can be consistently identified independently of conservation targets, managers could highlight important areas for conservation even if all do not agree on what the actual conservation targets should be. Our results highlight that we may be able to achieve conservation objectives with minimal disruption to important, high-value land uses such as airstrips, testing ranges, and buildings. However, we also demonstrate that conservation in highly-developed landscapes will require encroaching on lower dollar-value infrastructure, as achieving conservation goals was impossible if all areas containing any infrastructure were off-limits for conservation.

The Marxan algorithm operates independently of human biases, but the results can be affected by the initial data that are used, which may have biases or inaccuracies. We estimated how opportunity costs differed across types of land use and infrastructure, and more refined estimates of these costs could lead to somewhat different Marxan solutions. In addition, roads on the landscape formed part of our estimation of risk (planning units failing), with high risks occurring immediately adjacent to roads and decaying as distance from the road increases. However, we did not independently assess the effect of roads on the target species and generalized from studies published on different species and different locations. Based on our results, though, any quantitative discrepancies between responses reported in these studies and what occurs in our study area should lead to quantitative, but not qualitative differences in the relative importance of different planning units – i.e., the important areas would likely remain important, whereas the low-priority areas would remain less important. Only the absolute magnitude of importance would change.

Our Marxan analyses identified locations within the study area that represent high-priority locations for conservation of the target species. As these high-priority locations remained the same regardless of the conservation targets (0.2 - 0.8), we suggest managers in the study region initially use the planning units identified as high-priority when targets are set at 0.2 for conservation, such as the wilderness areas in Los Padres National Forest. The reason is that these planning units represent the locations that were initially selected when the amount of each species' distribution to be conserved was relatively low. Therefore, regardless of what conservation targets managers may eventually agree upon (anywhere between 0.2 and 0.8 of the predicted distribution), these areas should be included in the conservation solution. If agreed-upon targets are low (e.g., 0.2), we can simply choose the areas identified by Marxan. However, if agreed-upon targets are greater than 0.2, then the planning units identified when targets are set at 0.2 will still be identified as high-priority and included, although additional planning units would be needed to meet the higher target, meaning we lose nothing by including the high priority 0.2 planning units from the outset. These results were the same regardless of whether scenario 1 or 2 was adopted. However, given that only scenario 2 was able to meet conservation targets of 0.8 and still avoid areas of built infrastructure at lower levels, we would recommend adopting this strategy. The ability of scenario 2 to meet targets highlights the importance of not excessively constraining possible options and letting the algorithm find the optimal solution.

Implications for Future Research and Benefits

Ideal systematic conservation planning tools are objective, data-driven, quantitative, accurate, and transferrable. This project demonstrated the potential benefits of such an approach in a region with multiple jurisdictions and authorities responsible for making conservation decisions. The landscape-scale analyses we conducted showed that different jurisdictions within that landscape differed in both the amount of natural habitat remaining as well as the amount of potentially high-quality habitat that could be restored to meet mutually agreed upon, region-wide conservation targets.

Another benefit of using systematic conservation planning tools like Marxan is that they can be used widely under many different types of landscapes. This transferability allows direct comparison and evaluation of proposed or already implemented conservation actions and facilitates discussion regarding what types of conservation actions work best and what types of actions, and under what types of environmental settings, do actions not achieve intended conservation targets.

All tools are imperfect, and managers need to determine if the tool we developed is a useful addition to the tools they currently use despite its imperfections. On the ground managers should first examine if the specific outcomes of our analyses make sense. For example, do their individual and collective best professional judgements regarding the conservation value of different planning units agree with the conservation values that Marxan assigned? If so, this Marxan-based analysis is likely useful. However, if best professional judgement disagrees with Marxan assignments, then one or more aspects of the Marxan tool needs to be scrutinized and corrected. Marxan outputs will be especially sensitive to how accurate the habitat suitability models are that we developed. The metrics we used to evaluate model performance indicated our models were generally accurate, but considerable uncertainty exists with respect to individual predictions. If managers find the current Marxan solution too inaccurate for routine use, future research should explore how sensitive overall Marxan solutions are to uncertainty in their habitat suitability inputs. This information would allow managers to directly incorporate estimates of uncertainty into decisions regarding conservation actions.

Objective

The primary objective of the proposed research was to use highly sensitive eDNA survey data to develop a data-driven, spatially explicit, decision support system that can be used by multiple, collaborating jurisdictions to identify the mixes of land use that optimally conserve species of concern on Department of Defense (DOD) and surrounding lands. As part of the project, we tested if species distribution models provide similar predictions as species occupancy models for use in parametrizing the decision support systems.

Background

Decision support systems (DSS) are critically needed by natural resource managers wishing to best conserve species of concern (SOC) while considering other land use needs. However, the effectiveness of DSS is currently constrained by both the quality of data describing the known locations of SOC within a landscape of interest and the need for methods that can objectively evaluate the tradeoffs between conservation and other needs. Effective conservation planning requires knowing where SOC occur, the costs of different management options applied to individual land parcels, and how habitat conditions influence the viability of different species under changing environmental conditions. The goal is to then identify what land use management scenarios best conserve species while minimizing costs and restrictions on land use activities. Ideally, species occurrences are known with certainty. However, many estimates of species occurrence can be highly uncertain, which compromises their use. Uncertainty in species occupancy occurs because traditional survey techniques (e.g. trapping, netting, electrofishing, visual surveys) capture too small of a portion of the individuals actually present to reliably conclude a species is absent. Such variable, and often unknown, detection probabilities can lead to inaccurate inferences regarding population status, biased habitat suitability or occupancy models, and thus flawed management decisions (Kéry and Schmidt 2008, Wilcox et al. 2016). This problem is especially pronounced when target species are rare, have clustered distributions, are semi-aquatic, or occur in the substrate.

Surveys based on environmental DNA (eDNA) can overcome many of the constraints associated with traditional survey methods. eDNA refers to DNA fragments found in the environmental media that are derived from organisms in contact with that media (Taberlet et al. 2012). eDNA surveys do not require capture, handling, or even observation of the target organisms, making them especially useful for surveys of SOC, which can be difficult to capture or even harmed by more invasive traditional survey methods. eDNA-based detection techniques therefore represent a robust, sensitive, and economical alternative to traditional surveys (Mächler et al. 2014, Thomsen and Willerslev 2015, Wilcox et al. 2016). eDNA monitoring is especially useful in detecting aquatic or semi-aquatic species (Pilliod et al. 2013, Rees et al. 2014, Baldigo et al. 2017). In aquatic ecosystems, DNA molecules from species that are patchily distributed, rare, or difficult to physically capture are mixed in the water column. This water can be easily pumped through a filter to concentrate the eDNA, which can subsequently be identified with molecular DNA analysis techniques such as quantitative PCR (qPCR) and sequencing. eDNA approaches can significantly improve the sampling efficiency and detection probability over what can be achieved with traditional survey methods (Pierson et al. 2016, Deiner et al. 2016, Baldigo et al. 2017) and are quickly becoming the method of choice for detecting native aquatic SOC as well

as potentially invasive species (Dougherty et al. 2016). The higher sensitivity of DNA-based survey methods can thus reduce the analytical and modeling biases caused by imperfect and variable detection – e.g., (Schmelzle and Kinziger 2016) showed that eDNA data doubled the probability of detecting the endangered tidewater goby compared with traditional methods, and substantially improved occupancy models.

Species viability depends on both the availability of suitable habitat and connectivity with other populations, both of which affect the likelihood of local population persistence (Akçakaya and Sjögren-Gulve 2000). SOC managers therefore require knowledge regarding what specific set of local and regional processes influence population occurrence and dispersal and how different threats influence those processes. Ecologists are increasingly using species distribution models (aka habitat suitability models) or occupancy models to identify those environmental factors that likely constrain a species' occurrence to certain types of habitats (Elith and Leathwick 2009, MacKenzie et al. 2017). These models are especially useful when it is impractical to survey all possible locations that might support a species (Williams et al. 2009), but managers still need to know at what other locations a species may occur. Models can be used to identify locations that, if restored, might support viable populations (Osborne and Seddon 2012) and thus increase the connectivity between populations and the overall size of regional meta-populations.

Ultimately, successful conservation requires tools that managers can use to objectively evaluate the benefits and costs associated with achieving conservation aims. Natural resource managers are increasingly using systematic conservation planning (SCP) tools to help identify the specific mixes of land use activities that can achieve desired conservation outcomes at minimal, or acceptable, cost (Matthies et al. 2007, Watts et al. 2009, Bagstad et al. 2013). Decision support tools that are based on principles of optimization and that use sound empirical data and model outputs are especially useful in conservation planning (Ball et al. 2009, Hammill et al. 2016). Rather than rely on expert opinion to identify priority conservation areas, these SCP tools can apply optimization techniques to quantitative data on occupancy, local habitat conditions, proximity of populations to one another, and the spatial distribution of threats to identify the specific areas that will best achieve desired levels of conservation for the least cost. Given that a landscape context is required to conduct these analyses, effective use of these tools will often require cooperation and coordination among multiple jurisdictional entities.

Materials and Methods

General approach

We identified 11 tasks that, when completed, would produce the desired decision support system. Completion of those tasks involved developing a general workflow that summarized the linkages between those tasks (Fig. 1). We first conducted a one-day stakeholder workshop (Task 1) in San Luis Obispo to discuss the project with natural resource managers and scientists in the region and obtain both general feedback regarding project goals and objectives and specific recommendations and guidance regarding site selection and access. We then selected survey sites (Task 2), developed species-specific eDNA markers (Task 3), conducted eDNA sampling in the study area (Task 4), processed the eDNA samples (Task 5), conducted eDNA bioinformatics analyses (Task 6), compiled (Task 7) and characterized (Task 8) landscape and climate data

(Task 9) used as predictors in developing habitat suitability models for the target species (Task 10), and conducted Marxan analyses to generate estimates of optimal land use prioritizations (Task 11). We also developed a training video to support stakeholder training in the application and interpretation of Marxan analyses.

Study area, target species, and survey timing

We developed and tested our SCP approach on aquatic SOC occurring on and around military installations in the south-central coastal region of California (Fig. 2). Our focal landscape included Fort Hunter-Liggett (FHL), Camp Roberts Military Reservation (CRMR), Vandenberg Air Force Base (VAFB) – now Vandenberg Space Force Base, and Camp San Luis Obispo (CSLO), along with federal and state lands near these installations. Target species included several aquatic species that are either federally protected species or species of concern in California. The primary target species included the California Tiger Salamander (*Ambystoma californiense*), Arroyo Toad (*Anaxyrus californicus*), Western Pond Turtle (*Emys marmorata*), Western Spade-foot Toad (*Spea hammondi*), Foothill Yellow-legged Frog

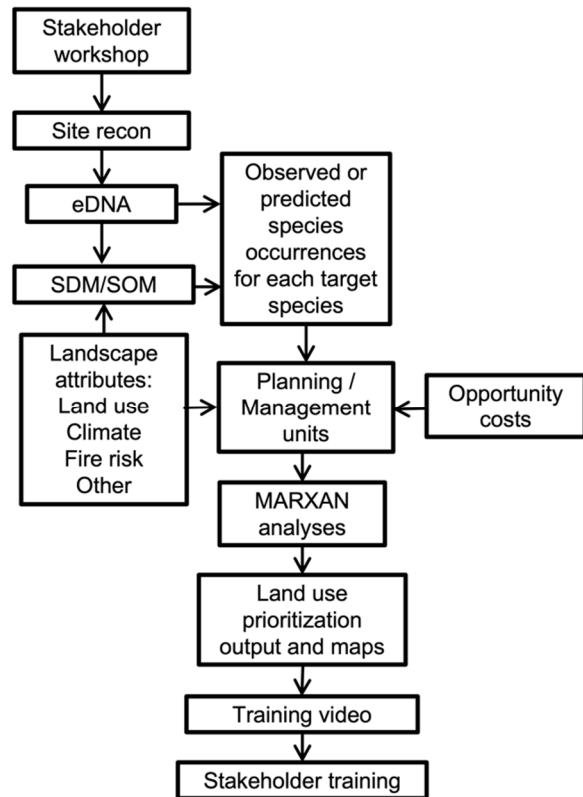


Figure 3. Workflow showing the links between main tasks.

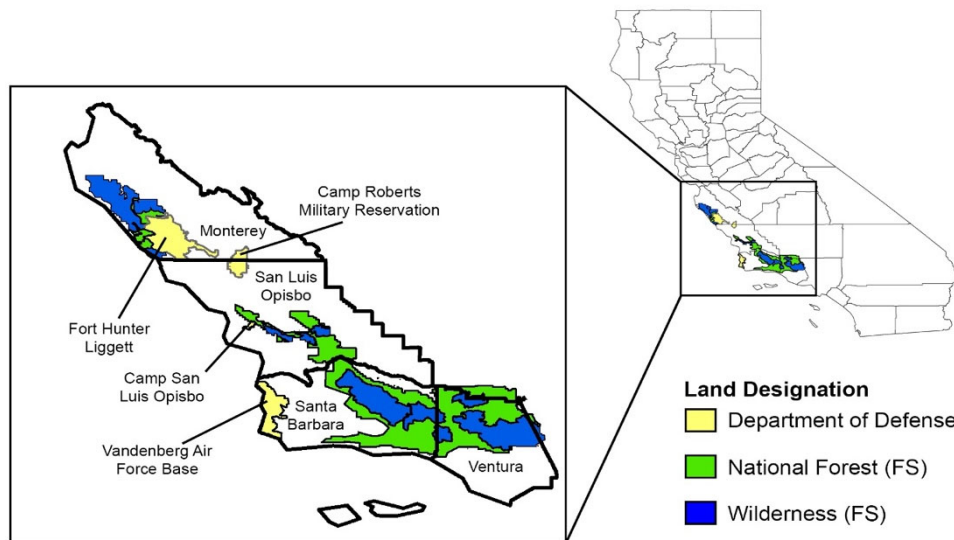


Figure 2. The focal landscape includes military installations and Forest Service lands in central coastal California and consists of all colored regions on the map.

(*Rana boylei*), and the California Red-legged Frog (*Rana draytonii*). We also determined if several two other sensitive species occurred in the study area: Three-spined Stickleback (*Gasterosteus aculeatus*) and Rainbow Trout (*Oncorhynchus mykiss*).

We conducted surveys in March-April of 2019 because all of the target species should be in the water at that time (Fig. 3), and temporary aquatic habitats in the region were still likely to contain water. The specific sites surveyed were selected in consultation with Dr. Laura Patterson, state-wide amphibian specialist for the California Department of Fish and Wildlife (CDFW) and Dr. Kristie Klose (LPNF aquatic ecologist) to maximize the information relevant to all jurisdictions.

Species	Month												Habitat
	1	2	3	4	5	6	7	8	9	10	11	12	
Spadefoot Toad													ponds
Arroyo Toad													streams
California Tiger Salamander													ponds
Foothill Yellow-legged Frog													streams
California Red-legged Frog													ponds
Western Pond Turtle													pond/streams

Figure 3. Timing of life-history events for the six original candidate target species. Colored cells indicate when species are either breeding (orange) or developing in the water (blue). Pond Turtles are in the water the entire year.

Stakeholder workshop

We conducted a stakeholder meeting on 6 November 2018 in San Luis Obispo, California to provide an overview of the project to all interested stakeholders and receive input from which to tailor aspects of the project to the needs of managers of the military installations and adjoining areas. We invited natural resources personnel employed by the participating DOD installations, the US Forest Service, and State of California. Seven DOD stakeholders attended the meeting, but USFS and California Fish and Wildlife personnel were not able to attend. DOD attendees included: Jackie Hancock (Fort Hunter-Leggitt), Kim Guillian (Fort Hunter-Leggitt), Jason Bachiero (Fort Hunter-Leggitt), Rhys Evans (Vandenberg Air Force Base), Michael Moore (Camp Roberts), Michelle Wong (Camp Roberts), and Robert Lovich (US Navy).

Comments from the stakeholders following our overview presentation were extremely valuable in providing guidance that resulted in fine tuning the list of target taxa we will study and where we should focus sampling. The most important recommendations from the stakeholders were to (1) drop the California Tiger Salamander as a target species because it has a very restricted distribution in the study area and, hence, cannot be modeled and (2) consider the addition of either sensitive species of fairy shrimp or the chytrid fungus pathogen, *Batrachochytrium dendrobatidis* (*Bd*), as replacements for the Tiger Salamander. We decided to include the fungus pathogen for eDNA sampling and species distribution modeling because, as a disease agent, it was likely to influence the viability of the amphibian species of interest and thus be an important risk factor in the Marxan analyses. We planned to use eDNA detections of *Bd* to better understand the factors that influence its spatial distribution and hence the waterbody-specific risk it poses to the different amphibian species.

Site selection and reconnaissance

We had four main goals for site selection:

1. Sample approximately the same number of sites from both DOD and adjacent non-DOD land and ensure adequate sample size for modeling.
2. Select sites that were representative of the natural environmental gradients in the study region to ensure SDMs/SOMs were unbiased.
3. Sample across a range of fire histories so SDMs/SOMs would be able to predict responses of targeted species to fire disturbance.
4. Ensure sites were accessible.

Prior to selecting sites, we built a spatial database of all possible waterbodies (i.e., streams, lakes, ponds, and wetlands) on DOD and non-DOD lands in the study area interest and their environmental characteristics. Stream data were derived from the National Hydrological Dataset Plus Version 2 (Moore et al. 2019). Lake, pond, and wetland data was derived from the National Wetland Inventory (USFWS 2018). We define ponds as open lentic waterbodies with < 2 ha of surface area, whereas lakes have > 2 ha. To minimize the number of waterbodies classified as wetlands but that may be functionally stream channels, we removed any wetland within 100 m of any stream segment with a catchment less than 100 km². We also removed waterbodies that were in DOD restricted access areas (e.g., impact areas). We then summarized the climate, vegetation, topography, and geology associated with each waterbody to characterize its surrounding environment (Table 1). Climate data for lakes and wetlands were derived from the PRISM 30-

Table 1. Environmental gradients used to characterize waterbodies by type. For streams, * = catchment scale and ** = watershed scale.

Lakes and ponds	
Waterbody area	Mean elevation
Precipitation	% barren land cover
Dewpoint	% forest land cover
Minimum mean annual temperature	% shrub land cover
Average mean annual temperature	% woody wetland
Maximum mean annual temperature	% herbaceous land cover
Streams	
Local catchment area	% forest land cover* **
Watershed area	% shrub land cover*
Minimum mean annual temperature*	% woody wetland*
Average mean annual temperature*	% herbaceous land cover*
Maximum mean annual temperature*	Mean % of catchment lithology as calcium oxide*
Mean annual precipitation**	Mean % of watershed lithology as calcium oxide**
% barren land cover*	Minimum elevation

Year Normals (Daly et al. 2008) and the National Land Cover Database 2011 (Wickham et al. 2021). Data for streams were derived from the StreamCat PRISM and NLCD datasets (Hill et al. 2016). We characterized streams and rivers at watershed- or local-catchment scales depending on the environmental variable. We characterized the environments within a 200-m buffer around all ponds, lakes, and wetlands. We also used Fire Perimeters Version 17_1 (CalFire 2018) to characterize fire history as (1) the percent area burned and (2) years since last fire. Soil burn severity (calculated as percentile moderately and severely burned when available) were derived

from Soil Burn Severity data produced by the Burned Area Emergency Response Imagery Support Team.

We then determined the number of sampling sites for each waterbody type to achieve proportional representation of waterbody types on both DOD and USFS lands. Generally, a 10% sample of 5-km stream reaches, of 4-ha portions of wetlands, and individual ponds and lakes across both DOD and LPNF lands provided a reasonably even distribution of sites across installations and waterbody types. Because LPNF had a greater density of streams than the DOD lands, we only selected 2% of available sites in the LPNF instead of 10%. We also selected 20% of lakes to ensure we had a large enough sample to adequately characterize species occurrences in these less common waterbody types. Because we planned to sample lakes in 3 locations and ponds in 1 or 2 locations (depending on size), the allocation of samples was slightly different than that of the number of sites (waterbodies).

We used a random stratified sampling approach to ensure that sampled sites represented the variability across environmental gradients. We accounted for the multiple environmental gradients (Fig. 4) by using separate principal components analysis (PCA) with varimax rotation to reduce the dimensionality of the environmental data associated with streams, wetlands, and lakes to two composite environmental gradients (for this analysis, ponds and lakes were combined). The first two principal components accounted for 44% of the environmental variation among lakes, 41% among streams, and 42% among wetlands. We divided each resulting PCA into nine strata by dividing all sites into three equal groups along each axis (Fig. 4). We randomly selected one site from strata in order of the strata with the greatest number of

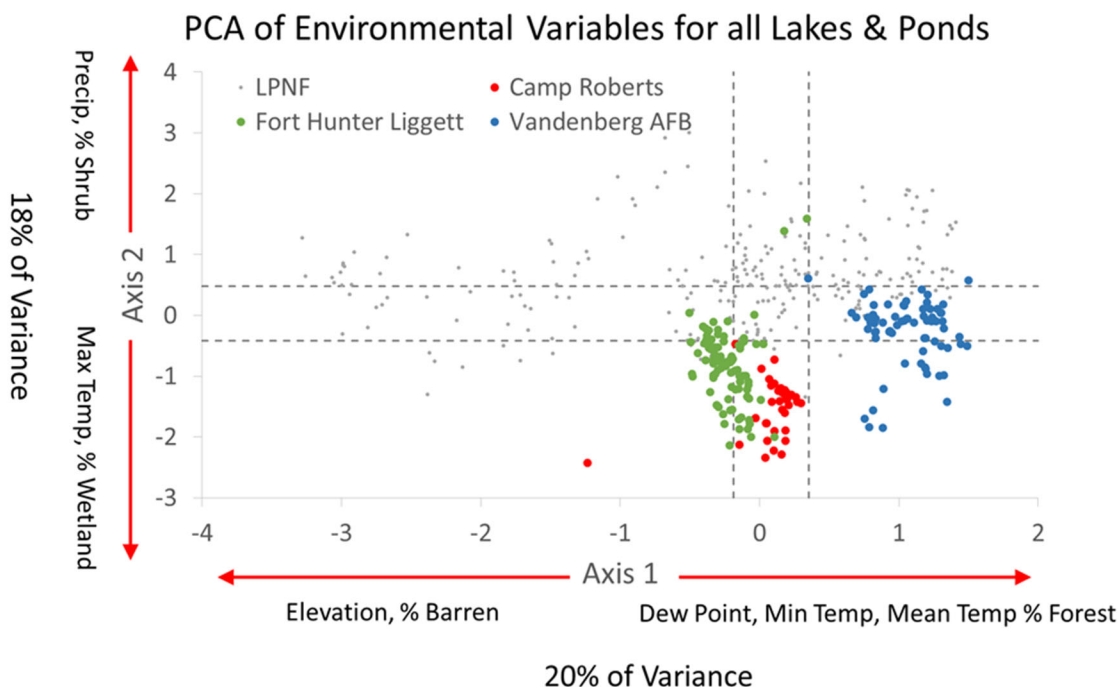


Figure 4. Example of an application of principle components analysis to characterize environmental variation among all lakes and ponds in the study region and the gridding of the PCA ordination space to ensure sample sites were representative of the diversity of environmental conditions in the study area.

potential sites to the strata with the least potential sites. Selection continued in this manner until the targeted number of sites were selected. To account for variability in fire history, selection alternated between burned and unburned sites. However, the random selection method resulted in selecting only sites with 100% area burned for NLNF streams, so we hand selected sites to produce a less skewed distribution.

After initial site selection, we assessed access to sites with GIS and Google Earth. Sites greater than 10 km from the nearest road or occurring in impassable terrain were replaced by a different random site from the same environmental strata. We met with representatives from the environmental office at each installation to arrange for installation access, coordinate with range control, and receive gate keys and radios where needed. We later visited many, but not all, sites to verify physical access and that the sites we selected met the expected environmental conditions (i.e., either lentic or lotic habitat of the expected size and condition). We visited sites along with other team members and representatives from the environmental office of each installation. At each installation, we visited as many sites that could be reached in a day, so sites relatively close to main roads were prioritized. We did not visit sites in SLO because it is relatively small size and Co-PI Olson had previous knowledge of the installation.

eDNA field sampling

We collected eDNA samples following protocols outlined in Carim et al. (2016) with modifications described below. We trained 20 graduate and undergraduate students on use of the protocol in the field at sites representing the range of waterbody types expected. Each week, four 2-person crews sampled sites. Crews were assigned to different installations, with at least one crew working in the north and south sides of the study area each week to avoid confounding of time (day of year) and location. Crews collected samples over 2-4 day periods.

Crews assessed each potential site for safety hazards and suitability for sampling. A sample site was considered suitable if crews could identify 3 separate locations approximately 10 m apart with water at least 5 cm deep. Sites < 5 cm in depth were not sampled and were replaced with an alternate site from the same environmental stratum. Crews classified sites with > 50% emergent or surface vegetation as wetlands, sites with discernable flow and < 50% vegetation as streams, and sites without flow and < 50% vegetation as lakes.

Three samples were collected at each site, 10 m apart. At each sample location, crews pumped 1-5 liters of water through a 1.5- μ m pore size disposable, glass fiber filter with a battery powered peristaltic pump. A separate collection tube was used at each site to prevent contamination. Filter holders were covered with 1-mm mesh to prevent debris or animals from contacting the filter. Water was pulled through the filter and then into a collection bucket marked with 1 and 5 L to assist in monitoring the total sample volume. The actual volume pumped was measured with a 1-L graduated cylinder after the sample had been collected. To minimize potential contamination, one crew member was designated as dirty hands and handled all materials used at previous sites (e.g., pump, water collection bucket) and the other member was clean hands handling site specific materials (filter holder, tube) while wearing gloves. Collection tubes, filter holders, and forceps were reused after being cleaned and soaked in a 50 percent solution of household chlorine bleach for 20 minutes, rinse with deionized water, and allowed to air-dry.

Lake and wetland samples were collected within 3 m of shore by placing the filter just below surface to avoid stirring up bottom sediments. Stream samples were collected by placing the filter on or near the stream bottom. Pumping continued until 5 L of water was filtered. If pumping slowed to > 15 seconds between drops, the filter was considered to be clogged. If < 1 L was sampled with the first filter, then a second filter was collected. Filters were carefully removed in the field with forceps and preserved individually in a 50-mL Falcon tube with silica gel desiccant to preserve the sample. In those situations when filters clogged before 1 L of water was pumped, the second filter was preserved with the first filter. Ancillary data were collected at each sample site, including in situ water chemistry and temperature, local land cover, and natural and human disturbances (see Appendix Supplemental Materials – eDNA Data Collection Sheet). Field data were recorded both electronically and on paper at each site and checked for completeness. All sampling equipment was bleach-sterilized between each waterbody to eliminate cross-site contamination. Field blanks were collected after every 10th site (n = 12) by applying the collection protocol to one L of distilled water brought from the lab. Field blanks served as negative controls for potential field-based between-site contamination. Samples were stored at room temperature until all samples were collected and then shipped to the Molecular Ecology Laboratory at Utah State University.

We collected 3 samples from each river or wetland site, but we sampled 1-3 sites at each lake depending on lake size to improve detections in large, relatively unmixed waterbodies. Goldberg et al. (2018) showed that lake size and number of samples affected detection levels with larger lakes requiring sampling at more sites to provide robust detection probabilities. Based on this work, we sampled lakes <20 m across at 1 site (i.e., 3 samples) lakes 20-30 m across at 2 sites (6 samples) and lakes > 30 m across at 3 sites (9 samples). When 2 or 3 sites were sampled the additional sites were placed equidistant around the lake.

eDNA marker development and analysis

Quantitative PCR marker development. For four species (chytrid fungus [*B. dendrobatidis*], California Red-Legged Frog [*R. draytonii*], Foothill Yellow-legged Frog [*R. boylei*], and Western Pond Turtle [*A. marmorata*]), we used quantitative PCR (qPCR) assays that had been previously developed in other laboratories. For chytrid fungus, we used the assay described in Boyle et al. (2004) and also used successfully by Kamoroff and Goldberg (2017). For the California Red-Legged Frog and the Foothill Yellow-Legged Frog, we used the assays described in Halstead et al. (2018) and Bedwell and Goldberg (2020), respectively. For the Western Pond Turtle, we used an unpublished qPCR assay developed by the lab of Dr. Caren Goldberg with permission (<https://labs.wsu.edu/goldberglab/>, Table 2).

Each of these assays was validated and optimized in our lab prior to running them on eDNA samples. These procedures included testing for species-specific sensitivity and specificity. To test assay sensitivity, we used MiniGene™ synthetic plasmids containing the assay sequence for each species. Plasmids were suspended in 100 µL of IDTE buffer (10 mM Tris, 0.1 mM EDTA), linearized by digestion with the enzyme Pvu1, and then purified with a PureLink™ PCR Micro Kit (Invitrogen, Carlsbad, CA) following standard protocols. The resulting products were quantified on an Invitrogen™ Qubit® fluorometer, and estimated concentrations were converted

to copy number based on molecular weight (Wilcox et al. 2013). These products were then diluted in IDTE buffer to create quantities of 2, 5, 10, 20, 50, and 100 copies/reaction of each plasmid, and each quantity was analyzed in ten qPCR replicates with the multiplex assay to determine assay sensitivity. For California Red-legged Frog, Foothills Yellow-legged Frog, and Western Pond Turtle, we also tested assay specificity by running the assays on tissue samples from all known non-target sympatric amphibian and turtle species from our study area (Appendix Supplemental Materials Table S1). Additionally, for the California Red-legged Frog and Foothills Yellow-legged Frog assays, we optimized the assays to be run in multiplex for economy. All qPCR assays were optimized to run under the same conditions. qPCR reactions included 7.5 μ l TaqManTM Environmental Mastermix 2.0 (Thermo-Fisher Scientific) 900 nM of each primer, 250 nM of probe, and 0.1 ng of tissue template DNA (for specificity testing) or 4 μ l eDNA template (for eDNA samples) in a total reaction volume of 15 μ l. Cycling conditions were 95° C for 10 minutes followed by 45 cycles of 95° C for 15 seconds and 60° C for one minute.

Table 2. Primers and probes used for quantitative PCR. Sources: 1 = Boyle et al. (2004), 2 = Halstead et al. (2018), 3 = Bedwell and Goldberg (2020), 4 = Caren Goldberg lab (unpublished), 5 = Rodgers et al. (2023) /this project.

Target Species	F-primer 5'-3'	R-primer 5'-3'	Probe	Source
<i>B. dendrobatidis</i>	CCTTGATATAATACA GTGTGCCATATGTC	AGCCAAGAGATC CGTTGTCAA	6FAM- CGAGTCGAACAAAAT- MGBNFQ	1
<i>R. draytonii</i>	CATCTGCCGCGATGT TAATAAC	CTCGCCCGATGTG GAAGTAG	6FAM- CATCCTTCTTTTTTATCTG CA-MGBNFQ	2
<i>R. boylii</i>	TCCGCCTCATGATCG AAAAC	GCGAAGCCTGT GAGR	NED- CCCTTTCCACAACCACTA- MGBNFQ	3
<i>A. marmorata</i>	TATCCACAACACAA TGAGGAGAGAC	ATAGGGAGGATG TGAAGTATTATGA GG	6FAM- TCATACATTAAATAATC CCTCC-MGBNFQ	4
<i>A. californicus</i>	TCACCAGCGAATAT CTCCTCTTTAT	GGCTATGGATGT ATCAGCTGTGTAA	6FAM- TCAAATCGCAACTGGAC- MGBNFQ	5
<i>S. hammondi</i>	CGGAAACGTACTAG TCCAATGAATC	GATCCGGTTTCGT GTAGGAATAGA	NED- CGCTTCTTTACTTTTCACT TT-MGBNFQ	5

For the Arroyo Toad (*A. californicus*) and the Western Spadefoot Toad (*S. hammondi*), we developed novel, multiplexed qPCR assays. For assay design, we sequenced mitochondrial DNA from tissue samples from across the ranges of both species (Fig. 5). For the Arroyo Toad, we sequenced 627 base pairs (bp) of the mitochondrial cytochrome b (cytb) gene from 74 previously collected tissue samples, including 60 from California, USA and 14 from Baja California, Mexico (Supplemental Materials Table S2, Fig. 5). For the Western Spadefoot Toad, we sequenced 607 bp of cytb, as well as 530 bp of the mitochondrial 16S rRNA gene (16S) from 42 tissue samples from across California (Supplemental Materials Table S3, Fig. 5). We generated sequence data from two mitochondrial genes for the Western Spadefoot Toad because highly distinct, reciprocally monophyletic northern and southern clades occur within *S. hammondi* (Neal et al. 2018). Given this relatively deep differentiation, we thought it prudent to explore both a more conserved (16S) and a more variable (cytb) gene region for designing an assay capable of detecting both *S. hammondi* clades, in case the cytb gene region proved too variable

to produce reliable amplification across clades. We also sequenced 43 tissue samples from 20 potentially sympatric amphibian and turtle species for *cytb* or 16S (Supplemental Materials Table S4) and obtained additional non-target species sequences from GenBank (Supplemental Materials Table S5) to ensure that we had sufficient data to design species-specific assays that would not amplify DNA from sympatric species.

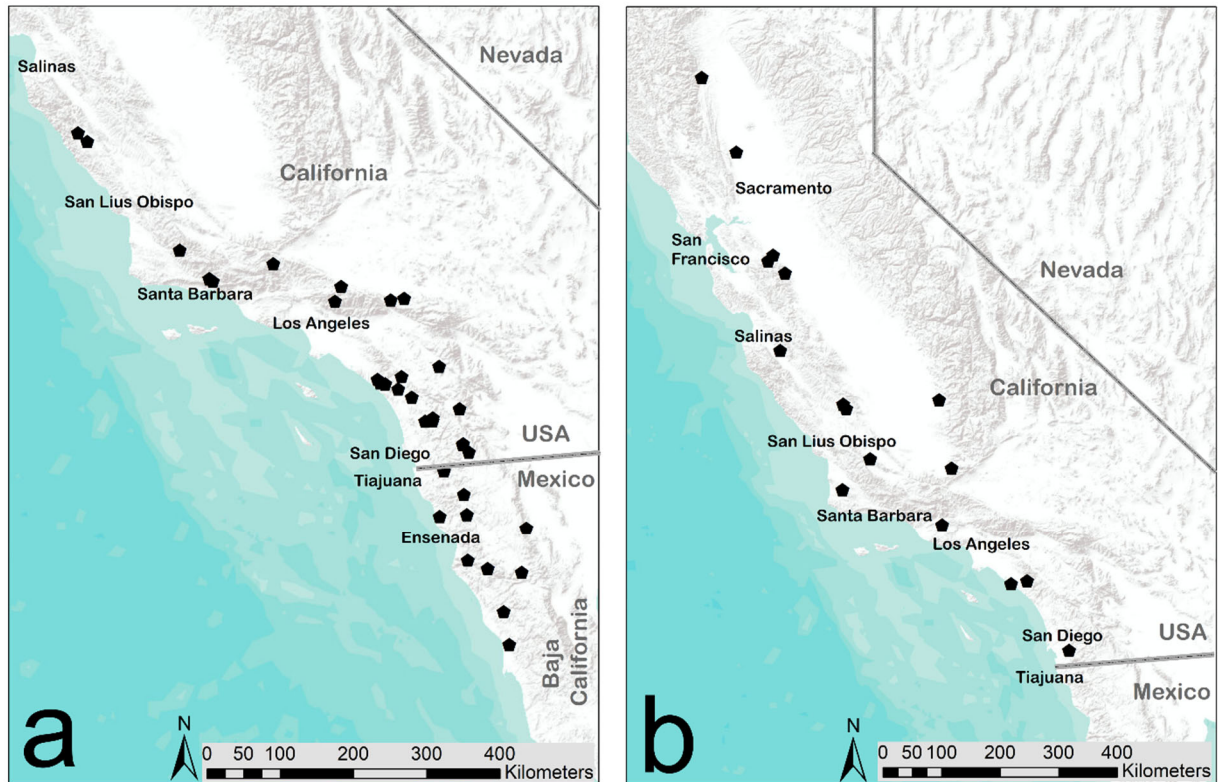


Figure 5. Geographic locations of target-species tissue samples sequenced and used for design of species-specific qPCR assays for (a) *Anaxyrus californicus* and (b) *Spea hammondi*.

Sequence data from all species were aligned with Sequencher software v5.2 (Gene Codes, Ann Arbor, MI), and species-specific PCR primers were generated with the online tool DECIPHER (Wright et al. 2014). For the Arroyo Toad, we initially designed and tested three candidate primer sets targeting *cytb* (Supplemental Materials Table S6). For the Western Spadefoot Toad, we initially designed and tested three candidate primer sets, two targeting *cytb*, and one targeting 16S (Supplemental Materials Table S7). Specificity testing included both *in silico* and *in vitro* components. To evaluate primer specificity *in silico*, we used NCBI Primer-BLAST (Ye et al. 2012) against the NCBI refseq non-redundant (nt) database to assess the likelihood that species occurring within or near the ranges of both species could potentially cross-amplify and produce false positives. Primer-BLAST stringency settings required the primers to have at least two total mismatches to unintended targets, including at least one mismatch within the last 5 bps of the 3' end, and ignored targets that have at least six or more mismatches to the primer.

In the laboratory, we initially evaluated specificity of our six candidate primer sets (Table S6) with SYBR® green qPCR to find a primer set with optimal specificity for each target species. Each primer set was tested with 10 tissue DNA extracts from the target species, as well as with at

least two DNA extracts from each sympatric non-target anuran species (n=9 species; Table S3). qPCR reactions included 7.5 µl Power SYBR®-Green Mastermix (Thermo-Fisher Scientific), 900 nM of each primer, and 0.1 ng of template DNA in a total reaction volume of 15 µl. Cycling conditions were 95° C for 10 minutes followed by 45 cycles of 95° C for 15 seconds and 60° C for one minute, followed by a melt curve to check for primer-dimer formation or off-target amplification. After one optimal primer set was chosen for each species (primer sets ANCA3 and SPHA2; see results), we then tested all four primers in multiplex with SYBR®-Green qPCR on tissue samples of both target species with a melt curve to check for potential primer-dimer formation in the multiplex.

Primer sets and MGB probes were then tested in TaqMan™ qPCR. We tested 10 DNA extracts from each target species, two DNA extracts from each non-target anuran species (n=9 species), and one or two DNA extracts from each non-target urodele (salamander or newt) (n= 8 species) and turtle species (n=4 species) (Table S6). qPCR reactions included 7.5 µl TaqMan™ Environmental Mastermix 2.0 (Thermo-Fisher Scientific) 900 nM of each primer, 250 nM of probe, and 0.1 ng of template DNA in a total reaction volume of 15 µl. Cycling conditions were 95° C for 10 minutes followed by 45 cycles of 95° C for 15 seconds and 60° C for one minute.

Primer concentrations were optimized by running four concentrations of each forward and reverse primer (100 nM, 300 nM, 600 nM, and 900 nM), each in triplicate, with 0.004 ng of target-species tissue DNA per reaction. Primer concentrations with the greatest peak fluorescence and lowest C_t value were selected for subsequent analyses (Table S7). To test assay sensitivity, we used MiniGene™ synthetic plasmids containing the assay sequence as described above.

We field tested our assays with eDNA samples collected from water bodies where our target species had been documented within the prior four years (Hitchcock et al. 2022). We did not conduct exhaustive traditional surveys for our target species at the time of eDNA sampling at all sites. However, brief visual observations were made by eDNA technicians during sampling; and at a subset of sites, traditional visual, dipnet, or seine surveys were conducted by individuals with expertise in morphological identification of our target species either during or shortly before eDNA sampling.

For Arroyo Toad, eDNA samples for assay validation were collected from Marine Corps Base Camp Pendleton (MCBCP) in 2019 (n=8 total samples from seven sites) and 2022 (n=24 samples from eight sites), and from the Los Padres National Forest (LPNF) in Ventura County (n=15 samples from five sites) and Santa Barbara County (n=18 samples from six sites) in 2021. In 2019, we collected a single eDNA sample from each of six sites, and duplicate eDNA samples from one site. In 2021 and 2022 triplicate eDNA samples were collected from each site (Supplemental Materials Table S8). For Western Spadefoot Toad, we collected validation eDNA samples from U.S. Army Base Fort Hunter Liggett (FHL; n=23 samples from eight sites) in 2019, and from MCBCP (n= 12 total samples from four sites), U.S. Marine Corps Air Station Miramar (MCASM; n=3 samples from one site), and two private ranches in Santa Barbara County (n=16 samples from five sites) in 2021. All Western Spadefoot Toad eDNA samples were collected in triplicate from each site, except for one where duplicate samples were collected (Supplemental Materials Table S9).

Metabarcoding marker development. We generated or obtained reference sequence data from the mitochondrial gene 16S for all amphibians and turtle species present in central and southern California for design of a novel metabarcoding primer set. Based on these sequence data, we designed a novel metabarcoding primer set optimized for detecting all amphibian and turtle species present in our study area (Table 3). We tested this marker, along with a published marker (*batra_F* and *batra_R* primers; Table 3) from Valentini et al. (2016), on tissue samples from all amphibian and turtle species expected from our study area, and on 60 pilot eDNA samples from Southern and Central CA.

Table 3. Primers used for metabarcoding of amphibians from eDNA samples. Source: 1= this study, 2 = Valentini et al. (2016)

Primer name	Target gene	Amplicon length (without primers)	primer sequence 5'-3'	Source
16S_TuTS_F	16S	196 bp	GCCTGTTTACCAAAAACATMRC	1
16S_TuTS_R	16S	196 bp	CTCCAYRGGGTCTTCTCGTCTT	1
<i>batra_F</i>	12S	51-53 bp	ACACCGCCCGTCACCCT	2
<i>batra_R</i>	12S	51-53 bp	GTAYACTTACCATGTTACGACTT	2
<i>batra_blk</i>	12S	NA	TCACCCTCCTCAAGTATACTTCAAAGGCA-3SpC3	2

eDNA Sample analysis. Samples were extracted in a dedicated room with the DNeasy Blood & Tissue Kit (Qiagen, Inc. Valencia, CA, USA) following a modified protocol described in (Rodgers et al. 2023). Each round of eDNA extraction (n=39) included one extraction blank negative control consisting of a clean, unused filter.

All eDNA samples were analyzed in triplicate qPCR with 4 µl of eDNA extract, 7.5 µl of TaqMan™ Environmental Mastermix 2.0 (Thermo-Fisher Scientific), optimized primer and probe concentrations, and cycling conditions of 95° C for 10 minutes followed by 45 cycles of 95° C for 15 seconds and 60° C for one minute. All reactions also included a TaqMan™ Exogenous Internal Positive Control (Thermo-Fisher Scientific) to monitor for PCR inhibition. Any samples that showed signs of PCR inhibition according to the IPC were treated with a ZYMO One Step™ PCR Inhibitor Removal Kit and rerun. Each qPCR run (25 samples in triplicate plus controls) included six no-template negative control reactions to monitor for cross-contamination, and a 5-step, 5-fold standard curve (10, 50, 250, 1250 and 6250 copies in triplicate) composed of a synthetic plasmid containing the assay target sequence to quantify DNA copy number. As an extra safeguard against false positives originating from the standard curve, the synthetic plasmid also contained a restriction site so that amplified products could be digested and run on a gel to distinguish true amplification from cross-contamination originating from the high copy-number standard curve (Wilson et al. 2016). To further mitigate cross-contamination, all qPCR reactions were set up under a dedicated PCR hood sterilized with UV radiation prior to each qPCR run (Goldberg et al. 2016).

All eDNA samples were also amplified for next generation Illumina amplicon sequencing (metabarcoding) with two markers, our novel marker targeting 16S, and a published *batra* marker from (Valentini et al. 2016; Table 3). For both markers, all samples were run in triplicate PCR. Reactions contained 2X (12.5 µl) Amplitaq Gold 360 Master Mix (Thermo-Fisher Scientific) 0.2 µM of forward and reverse primer containing illumina sequencing adapters, 0.2

μg/μl of bovine serum albumin, and 4 μM of human blocking primer (*batra* reactions only), and 4 μl of eDNA template in a total volume of 25 μl. Cycling conditions were 95° C for 10 minutes followed by 45 cycles of 95° C for 15 seconds, 55° C (16S marker) or 60° C (*batra* marker) for 30 seconds, and 72° C for 30 seconds, followed by a final extension at 72° for 7 minutes. After the initial marker specific PCR, triplicate PCR products were pooled by sample, diluted 25:1 with sterile H₂O, and this diluted PCR product was used to seed an indexing PCR utilizing Unique Dual Index (UDI) adapters for sample identification (Integrated DNA technologies). Indexing reactions contained 2X (10 μl) Amplitaq Gold 360 Master Mix (Thermo-Fisher Scientific), 1 μl of UDI primer, and 2 μl of pooled and diluted PCR product from the initial PCR in a total reaction volume of 20 μl. Cycling conditions for the indexing PCR were 95° C for 10 minutes followed by 15 cycles of 95° C for 15 seconds, 50° C for 30 seconds, and 72° C for 30 seconds, followed by a final extension at 72° for 10 minutes. Next, PCR products were cleaned-up and normalized with a SequalPrep™ Normalization Plate Kit (Thermo Fisher Scientific) and pooled for sequencing. Three separate libraries were created for each marker, each containing one field-replicate sample from each site. Each library contained a total of 192 reactions, composed of 186 eDNA samples (including field blank and extraction negative controls), two positive control samples composed of a mix of tissue DNA from five exotic amphibian and turtle species not native North America, and four no-template negative control reactions. Each library (n=6) was sequenced on Illumina MiSeq with the v3-600 cycle (25 million read yield) reagent kit.

Bioinformatic processing of MiSeq data proceeded as follows. After demultiplexing, primer sequences were removed with CUTADAPT v. 1.18 (Martin 2011). Next, data were filtered, denoised, paired-ends were merged, and chimeras were removed with DADA2 (Callahan et al. 2016) within the QIIME2 environment (Bolyen et al. 2019). We then used ncbi BLAST (Altschul et al. 1990) to separate ASVs (Actual Sequence Variants) from aquatic amphibians and fish from those of non-target taxa (e.g., ASVs from bacteria, fungus, mammals, and birds). For the ultimate taxonomic assignment of target ASVs, we used the Naive Bayes classifier Scikit-learn (Pedregosa et al. 2011) within the QIIME2 environment.

Landscape features, climate, and fire modeling

Landscape features and climate. To provide landscape data for habitat suitability modeling and Marxan analyses, we developed a standard analysis framework, assembled and standardized data from multiple sources, and then produced summaries for each analysis unit of each layer. We first created a 50x50m grid encompassing each installation and relevant portions of the LPNF (Figs. 6 and 7). We then projected all landscape and climate data layers into a common coordinate system (projection: NAD_1983_2011_California_Teale_Albers). Some data layers did not cover coastal areas, so we used kriging in ArcGIS to interpolate to any uncovered coastal areas from the existing grid.

We summarized landscape and climate attributes (see details below) for each 50x50 m grid cell, and cells designated as either channel or riparian were also associated with watershed summaries of landscape attributes. We attributed the values of each layer to each analysis cell by using the extract-to-point tool in ArcGIS. This procedure allowed us to assign the values of each landscape layer coincident to the centroid of each 50x50 analysis cell. We then exported csv-delimited files

of each group of layers from a single source (see Appendix Supplemental Materials, Data Dictionary for list of sources) for each installation, with 1 row per analysis unit. Because file sizes were large (often in excess of a million rows), we converted decimal floating point data into integers to minimize storage/manipulation costs. Before conversion we multiplied data by a constant (i.e., 10, 100, or 1000) to retain useful information before converting to integer format (see the Data Dictionary). In addition to characterizing the environmental conditions in each analysis unit, we also needed to characterize upstream environmental influences. To do this, we identified cells influenced by upstream processes (i.e., cells designated as channel or riparian) and then associated each to the catchment produced by the National Hydrologic Dataset Plus for which that analysis cell was in. This process, in turn, allowed us to then link each analysis cell with catchment and watershed environmental summaries in the StreamCat dataset obtained from the USEPA. Nine sample sites were located in isolated upland ponds or wetlands that could not be appropriately characterized with StreamCat watershed data. For these sites we delineated watersheds with ArcGIS and then calculated watershed averages for the same data used in our local analyses. Because of the size of the resulting data sets, we stored each table of environmental variables for each analysis unit in a SQL database. This database could then be queried to extract data for later species distribution modeling or Marxan analysis.

To characterize climate, we used both data based on current observations (Google Earth Engine, PRISM, and BioClim data) and statistically downscaled global climate model (GCM) data from the Multivariate Adaptive Constructed Analogs (MACA) data portal. We selected data from five GCMs: MIROC-ESM-CHEM (Japan), GFDL-ESM2M (USA), IPSL-CM5A-MR (France), inmcm4 (Russia), and GFDL-ESM2G (USA). These models were selected because they span the range of predicted temperature and precipitation conditions. For each GCM we obtained predicted maximum and minimum temperatures and precipitation for both current (1971-2000) and future (2040-2069 under RCP 8.5 conditions) time periods.

Fire modeling. The large-fire simulator FSim is a wildfire model that combines the strengths of statistical models with spatially explicit wildfire simulation models (Finney et al. 2011). FSim is a stochastic, spatially-explicit model that

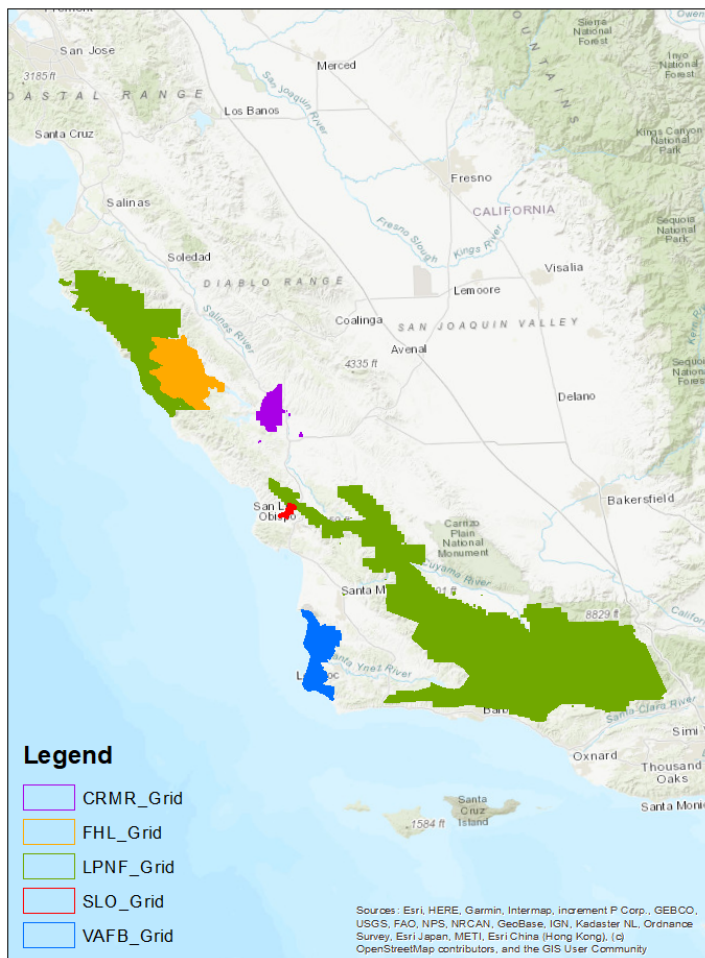


Figure 6. The five 50x50 m grid units used to summarize environmental characteristics for each installation.

incorporates weather, topography, fuels, and spatial patterns of historical ignition probability to simulate the occurrence and spread of large fires by generating tens of thousands of years of statistically plausible weather streams. Fsim produces stable and repeatable estimates of burn probability for any given location on the simulation landscape, and outputs include ignition location, date, final size, and perimeter of each simulated fire. FSim focuses on simulating large fires to maintain computational efficiency and because large fires account for the vast majority of area burned. Following Scott (2014), we used a large-fire threshold of 100 acres (40.5 ha). FSim computes a daily large-fire ignition probability based on the logistic regression between historical large fire ignitions in the study area and the daily Energy Release Component (ERC) for fuel model G of the National Fire Danger Rating System (Andrews et al. 2003, Finney et al. 2011). ERC is a proxy for fuel dryness, and incorporates daily temperature, precipitation duration, humidity, and state of the weather (SOW) (Cohen and Deeming 1985). FSim only simulates ignitions on days where ERC equals or exceeds the 80th percentile ERC for that region (hereafter, these days are referred as burn days), a threshold considered to demarcate the fire season based on the relationship between large fire ignitions and ERC across most of the contiguous United States (Riley et al. 2013).

We applied FSim to the region spanning the Southern California coast from San Francisco to the Mexico border for two adjacent pyromes – i.e., areas of relatively homogenous fire regime defined by fire size, frequency, intensity, and seasonality (Short et al. 2020). Pyrome 33 (hereafter Central Coast) extends south from the southern tip of San Francisco Bay, including the Coast Range and extending east to the edge of the Central Valley. Pyrome 34 (hereafter Southern Coast) extends from the Santa Monica Mountains northwest of Los Angeles to San Diego and the US-Mexico border.

FSim requires specification of three spatial boundaries for each pyrome being simulated. First, the analysis area is the primary region of interest and the area for which final burn probability estimates are valid, equaling the areas of the Central Coast and Southern Coast pyromes. Second, the fire occurrence area (FOA) defines where FSim allows ignitions to start by probabilistically assigning ignition locations using a continuous kernel density raster (40 km nearest neighbor) representing the spatial point

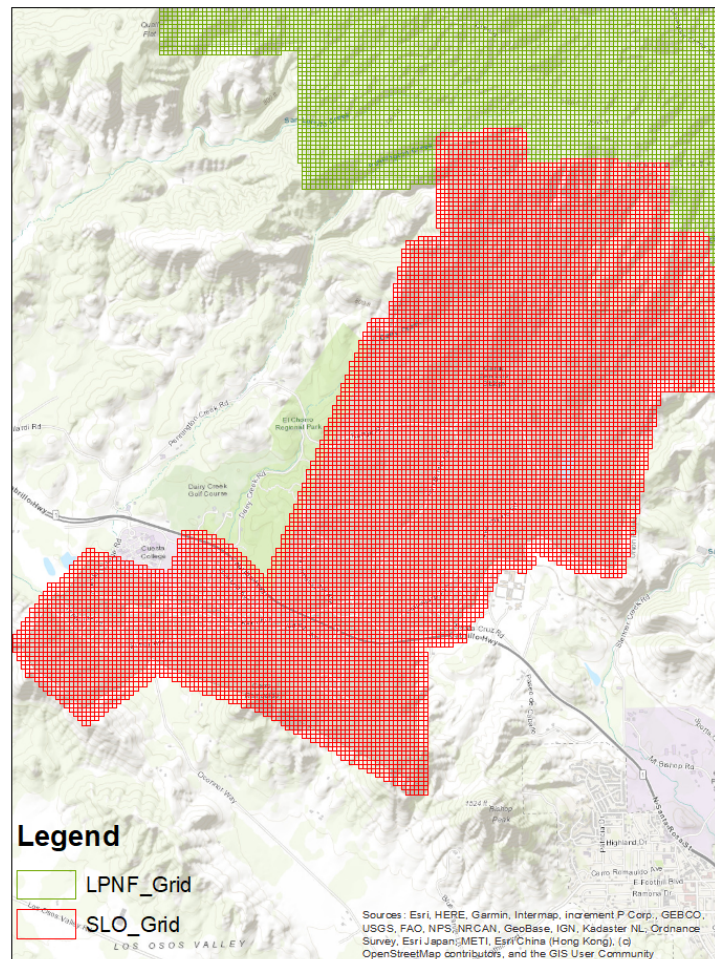


Figure 7. Close up showing individual 50x50 m grided analysis units for Camp San Luis Obispo and a portion of the Los Padres National Forest.

pattern of historical ignition locations (Short et al. 2020). We set the FOA as a 30 km buffer around each pyrome, but did not buffer the border between the two pyromes so that fires could spread between the pyromes without being counted multiple times. Third, FSim requires a fire-modeling landscape (LCP) raster, which we set as a 60 km buffer surrounding the FOA. FSim does not allow ignitions in this buffer, but fires ignited in the FOA can burn outward through the LCP, preventing edge effects that might otherwise occur if fire spread was forced into a hard boundary. We acquired spatial landscape inputs of slope, elevation, aspect, fire behavior fuel model (Scott and Burgan 2005) canopy bulk density, canopy base height, canopy cover, and canopy height from the Landscape Fire and Resource Management Planning Tools, or LANDFIRE, 2016 Remap (LANDFIRE 2018). We resampled the default LANDFIRE 30-m raster grids to 270 m to match our simulation resolution, then combined all eight grids with standard tools in FlamMap software (Finney 2006) to create the LCP raster required for FSim.

We defined four time periods to run simulations: contemporary calibration, baseline, historical, and future. The contemporary calibration period (1992–2015) was selected to calibrate the model because this period best matches the duration of observed fire records. We calibrated the model until both the mean size and annual frequency of simulated large fires were within the 70% confidence intervals of the 1992–2015 observed means (Short 2022). Global climate models (GCMs) simulate climate starting in the past and proceed into the future. As with many other studies that explore climate change impacts into the future, e.g., Barros et al. (2021), Clark et al. (2021), Heidari et al. (2021), we ran FSim with GCM-based climate for both a future period and a recent historical period to fully capture a GCM’s expression of future climate while also capturing its bias by comparison to the historical period. For this study we selected a midcentury period (2040–2069) under RCP8.5 high warming climate change scenario to explore the impacts of significant climate change, while not too far into the future that present-day landscape conditions are excessively implausible. We retained the contemporary landscape for all three time periods to isolate the effect of climate as in Riley and Loehman (2016).

For contemporary calibration period simulations (1992–2015), we used climate from gridMET, an observation-based gridded climate dataset that blends spatial attributes of the gridded climate data Parameter-elevation Relationships on Independent Slopes Model (PRISM) (Daly et al. 2008) with desirable temporal attributes from regional reanalysis using climatically aided interpolation (Abatzoglou 2013). For the historical and future simulation periods (1979–2005 and 2040–2069, respectively), we used the MACA-v2-METDATA downscaled climate dataset (Abatzoglou and Brown 2012), which uses gridMET as the reference dataset for its downscaling algorithm. Conventionally, FSim is driven with weather data from a single representative weather station within the pyrome. For this study, we identified a Remote Access Weather Station (RAWS) with a relatively central location within each pyrome and with long, complete wind records. In the Central Coast, we chose the Fort Hunter Liggett RAWS (36° 00’ 42” N, 121° 14’ 30” W), and in the Southern Coast we chose the Chilao RAWS (34° 19’ 54” N, 118° 01’ 49” W). The wind speed and direction distribution tables required by FSim were constructed from RAWS because spatially-downscaled wind data in gridMET may poorly represent the wind patterns at the selected RAWS locations. Climate for the historical and future simulations were taken from grid cells in gridMET and MACAv2-METDATA that coincided with the RAWS coordinates.

FSim requires ERC timeseries as input, and we used Fire Family Plus (Bradshaw and McCormick 2009) to calculate daily ERC from gridMET and MACAv2-METDATA. State of weather (SOW) is required by Fire Family Plus but is not included in gridMET or MACAv2-METDATA. We calculated SOW from precipitation duration and percent of cloud cover, where cloud cover was inferred from the ratio between solar radiation and potential maximum radiation, and we calculated precipitation duration following methods described by Abatzoglou and Kolden (2013). GCM drizzle is a well-documented GCM phenomenon (Sun et al. 2006, Ahlgrimm and Forbes 2014) and is manifested in MACAv2-METDATA as an unrealistically high frequency of days with extremely light rain in the summer. Because this GCM drizzle unrealistically suppresses ERC values, we adjusted the drizzle days by removing the lowest precipitation days from MACAv2-METDATA for the historical period until the number of drizzle days matched those present in the baseline period gridMET for each month. Similar adjustments were made for the future period, but the target number of drizzle days were altered by the ratio of unadjusted drizzles days in the future to the historical. Total precipitation values were only trivially affected by this adjustment procedure.

FSim is computationally expensive, so out of all downscaled GCMs available in MACAv2-METDATA, we selected a subset of eight GCMs to use for historical and future simulations. Because ERC-based burn days are the key climate threshold FSim uses to ignite and grow a fire, we evaluated all available GCMs in terms of historical bias and projected future change in the number of burn days, where bias is defined as the difference between the historical GCM and baseline gridMET (1979–2005) and future change is defined as the difference between each GCM's future (2040–2069) and historical (1992–2005) periods. We selected eight GCMs that capture the broad range of both historical bias and future change.

Predicting spatial variation in habitat suitability

We originally planned to model the distribution of the target herpetofauna and the chytrid fungus (*Bd*) based on the eDNA occurrence data. However, we had sufficient numbers of eDNA detections to develop models for only the Western Pond Turtle and *Bd* (Table 4). We therefore supplemented occurrences for two species (Arroyo Toad and the California Red-legged Frog) with data from the Global Biodiversity Information Facility database (GBIF, <https://www.gbif.org/>). We could not obtain adequate occurrence data for the other two species to develop models. Prior to modeling, we checked the combined eDNA+GBIF data to ensure that no spatial overlaps existed between our eDNA-detected species locations and GBIF-record locations. In addition, we retained all eDNA species absences that did not overlap with GBIF presences to support presence-absence modeling. We also assembled 44 potential predictors that describe either naturally occurring (e.g., climate, topography/landscape, soil, vegetation) or human-altered environmental conditions (e.g., land use and biological reservoirs of *Bd* – hosts in which disease can persist for a long time without affecting the hosts) (Supplemental Materials Table S10). For modeling and prediction purposes, all predictor variables were rasterized in ArcMap before analysis. We dropped any candidate predictors that had little to no variation across the study area. To avoid collinearity among predictors, we used the *usdm* package (Naimi et al., 2014) to calculate correlations between all predictors and kept only five predictors for *Bd* (precipitation of the wettest quarter, the Normalized Difference Vegetation Index (NDVI),

percent impervious surface, annual mean temperature, and watershed slope) and eight predictors for the herptofauna (minimum temperature, total monthly precipitation, NDVI, watershed slope, distance to flowline, distance to road, distance to a waterbody, and watershed area) that had Spearman correlation coefficients (r) < 0.7 with other predictors.

Table 4: Detections (qPCR and metabarcoding) occurrence records (GBIF) for each target species. MB1 = metabarcoding marker 1, and MB2 = metabarcoding marker 2.

Target species	qPCR	MB1	MB2	GBIF	eDNA absences	Total presences
Chytrid fungus	37				122	37
Arroyo Toad	1	1	0	18	166	20
Foothill Yellow-legged Frog	0	0	0	-	168	0
Western Spadefoot Toad	5	1	0	-	162	6
California Red-legged Frog	12	1	2	27	159	42
Western Pond Turtle	10	13	1	-	138	24

We used Random Forests (RF) models to assess the associations between species detections (presences and absences) and the selected predictor variables. Random forest is a non-parametric modeling technique that uses bootstrapped resampling to produce ensembles of classification or regression trees (Breiman 2001). Random Forest can model non-linear relationships, automatically handles interactions among variables, and is insensitive to overfitting. As a result, RF models have been used in many ecological assessments, including predicting the distributions of invasive species (Cutler et al. 2007, Xie et al. 2016). We set the number of trees to 1000 but otherwise used default settings. The RF algorithm fitted each of the 1000 trees with a randomly selected sample of $\sim 70\%$ of the samples. The other $\sim 30\%$ out-of-bag samples were used to assess model performance.

All species occurrences were unbalanced (Table 4). For example, *Bd* absences were recorded about three times more often than presences (122 vs 37). Unbalanced data are an issue because models will weight predictions toward the majority case to maximize percent correct classification. To improve the balance between model sensitivity and specificity, we used the *samplesize* function in randomForest, which forces RF to use an equal number of presences and absences (Chen et al. 2004, Benkendorf and Hawkins 2020).

After building the RF models, we assessed model performance in terms of four performance metrics derived from out-of-bag samples. These performance metrics included sensitivity, specificity, area under the receiver operator curve statistic (AUC), and true skill statistic (TSS). Sensitivity is the percentage or proportion of species presences predicted correctly. Specificity is the percentage or proportion of species absences predicted correctly. AUC can vary between 0 and 1, where values less than 0.5 indicate a model that performed no better than random, and values closer to 1 indicate a highly performing model. AUC is a widely-used model performance metric, but it is often criticized for being unable to identify if models differ in their ability to predict presences and absences correctly (Hanczar et al. 2010). Like AUC, the TSS also combines both sensitivity and specificity into a single metric of model performance; however, TSS (derived as sensitivity+specificity-1) reflects the balance between accurately predicting presences and absences (Allouche et al. 2006). TSS scores range from -1 to 1 , where 1 indicates perfect model agreement and values of zero or less indicate an uninformative model. We used

the PresenceAbsence R package to calculate all the performance metrics. We also determined which predictors contributed the most to model predictions by calculating the mean decrease in accuracy that occurred when removing each predictor from the model. We then used partial dependence plots to visualize the relationships between predicted probabilities of *Bd* occurrence (our measure of habitat suitability) and each predictor variable. We then used the fitted RF models to map predicted *Bd* and herpetofauna habitat suitabilities across the study area. Finally, to project how the distribution of herpetofauna was expected to shift in response to climate change, we replaced current temperature and precipitation conditions with mid-century temperature and precipitation conditions (2040-2069) for each species model. We used the worst-case climate change scenario for this analysis – i.e., climate projections by the Geophysical Fluid Dynamics Laboratory Earth System Model (GFDL-ESM2) under the Representative Concentration Pathway 8.5 emissions scenario.

Because we had adequate triplicate detections for *Bd* across our study sites, we were also able to develop *Bd* occupancy models. Unlike species distribution models, species occupancy models (SOM) use repeat observations to estimate both the probability that a study site is occupied and the probability of detecting the species of interest. We leveraged the replicate samples to fit *Bd* occupancy models with the R package unmarked (Fiske and Chandler 2011). To facilitate comparisons between RF and SOM approaches, we used the same five predictors used in the RF model to estimate both the probability of a site being occupied by *Bd* and the probability of detection as additive functions. We then produced partial dependence plots of the relationships between *Bd* occurrence probability and each predictor. Finally, we compared the mapped predictions of habitat suitability produced by the two modeling approaches.

Marxan analysis

Marxan analyses inform spatial decisions by identifying areas on a landscape that meet user-specified targets for the least cost (<https://youtu.be/znQEhOcDdNE>). Marxan analyses require spatially explicit information about where on the landscape species exist, as well as the costs associated with using different parts of the landscape for conservation (Margules and Pressey 2000). The risk of conservation actions failing can also be included in the analyses and form an important component of spatial decisions (Hammill et al. 2016). We used the output from the habitat suitability modeling as the basis for running the Marxan analysis. For each species we aimed to include between 10% - 90% of the predicted distribution. The habitat suitability models give a predicted suitability of each planning unit for each species. To use this predicted suitability as a conservation target, the Marxan algorithm calculates the total value of the landscape, then identifies the planning units that meet the user specified target. For example, in a landscape of five planning units with 0.8, 0.7, 0.4, 0.0, and 0.5 predicted suitabilities, the total suitability is 2.4. If we set a target of conserving 50% of the species' predicted habitat suitability, we would need to include planning units that collectively represent 1.2 suitability units, so either units 1 and 2, 1 and 3, 1 and 5, or 2 and 5. Setting targets based on the habitat suitability models ensures that we are including areas where the species is present while also accounting for likely suitable habitat in unsampled locations. The spatial distributions of each species habitat suitability values were combined with information on costs relating to land use and risk of failure to inform spatial decisions. Below we go through these processes in greater detail.

Planning units. We divided the project landscape into 1x1 km grid cells (hereafter termed planning units) (Fig. 8). These planning units represent the operational units of management within the project landscape. Each planning unit was parameterized with each species' predicted habitat suitability values and was assigned an opportunity cost based on the cost of managing land parcels, the value of the infrastructure it contained and the cost of removing that infrastructure, and the risk of the planning unit failing to support its conservation potential due to factors outside of our control such as disease. For two species (Arroyo Toad and Western Spadefoot Toad) we had insufficient detections to complete the species distribution models. We felt, however, that it was important to include these species in the Marxan analysis, and so we constrained the analysis such that any planning unit in which we had a confirmed detection of either of these species (10 planning units total) was forced to be part of the Marxan solution. Forcing planning units to be part of the solution means that all detected locations of the rarest species are conserved.

Opportunity costs. Opportunity costs were calculated by combining the cost of managing a 1*1 km land parcel (estimated as \$1500) with the costs associated with the potential impact on any infrastructure present in the planning unit (Fig. 9). Infrastructure costs were estimated based on the predicted costs of moving or restoring any infrastructure present in the planning unit (Table 5).

Risks of failure. - Risks of failure account for the probability that should a planning unit be selected for conservation, the species it contains will not survive or the habitat itself will become unsuitable. Habitats may become unsuitable for a variety of reasons, such as the presence of diseases that may lead to mortality or the occurrence of a hazardous

disturbance. In our analyses, risks were based on the probability that a planning unit contains *Bd* (as predicted by the *Bd* habitat suitability model) and the proximity of roads. Estimates of the risks of roads to the target species were based on previous empirical assessments of the impact of roads on amphibian populations conducted in the western USA (Beebe 2013). Based on the data presented in Beebe (2013), we generated a logistic decay curve describing the impact of roads, with a 95% mortality rate associated with roads zero m from the planning unit, declining to zero impact for roads >1000 m from the planning unit. The final risk value assigned to each planning unit was based on the probability of the planning unit not being affected by either *Bd* or roads. For example, if a planning unit had a 0.25 chance of being contaminated with *Bd* and had a 0.40 chance of being affected by roads, its overall risk was $(1-0.25)*(1-0.40) = 0.45$.

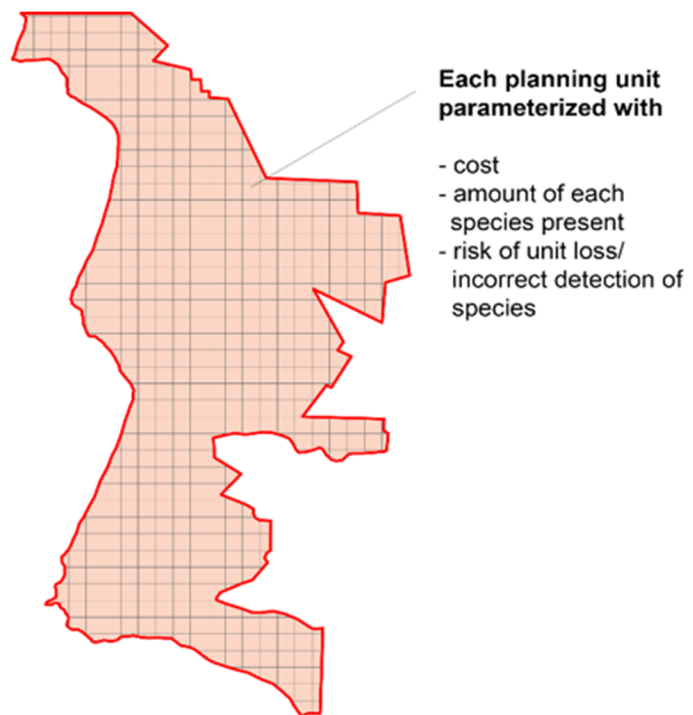


Figure 8. Illustration of planning units to be used at Vandenberg Air Force Base and the data assigned to each unit.

Running and presenting the analyses. Marxan analyses were run with stand-alone Marxan software (<https://marxansolutions.org/>). For each conservation target level, we performed 100 Marxan runs, each of which included 1,000,000 iterations. In addition, we set the probability of meeting our conservation target value (ptarget1D) at 0.95, indicating that when the risk of planning units failing is accounted for, we have a 95% chance of meeting our target. The ptarget1D parameter needs to be accounted for whenever risk of failure is being included in the analysis because given the uncertainty associated with risk of failure, we can never be sure a conservation target has been met. We did not attempt to produce clumped solutions with the boundary length modifier in Marxan because of the potentially small home range sizes of each species. From the 100 individual Marxan runs, we report the total cost and number of planning units selected associated with the best performing run (that which meets the targets for the least cost). We use these data to demonstrate how costs and the number of planning units required increases as conservation targets increase. We also provide illustrative maps of the solutions provided by Marxan across a range of different conservation targets. We present these maps in two different forms indicating the relative importance of each planning unit. These maps illustrate how often each planning unit was selected in the 100 Marxan runs performed for each conservation target. Planning units that are selected in a large number of the Marxan runs are of a higher conservation importance than those that are selected less frequently. Complementary to these relative importance maps, we also present maps showing the planning units selected in the best performing run for each conservation target.

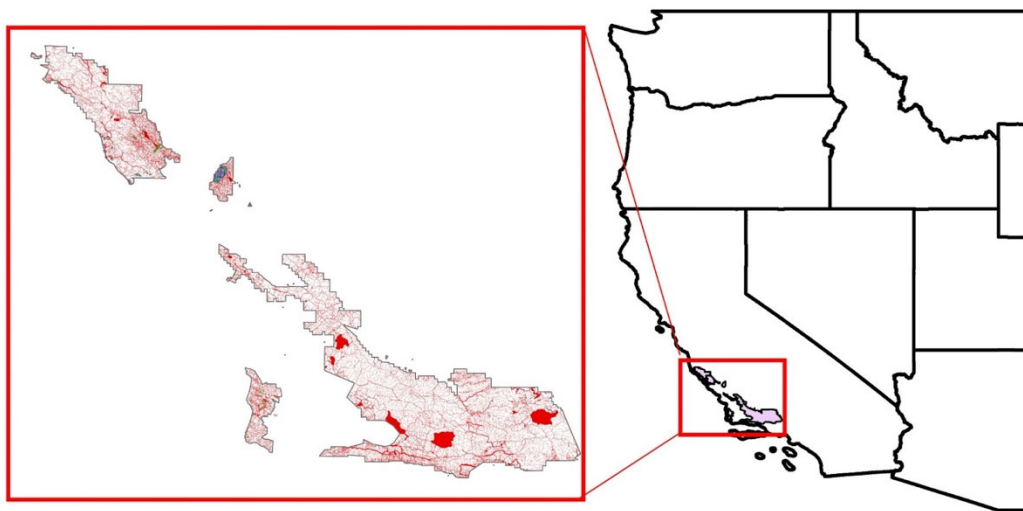


Figure 9. Locations of infrastructure within the study area. Infrastructures include roads, buildings, testing zones, and golf courses amongst others. The costs associated with different infrastructures are given in Table 5. Infrastructures are shown here to give an indication of the level of development present in the study area.

We used multiple scenarios to quantify the effects of adopting two different philosophies to conservation. In the first scenario, we specify that any planning unit that contains built infrastructure not related to transportation (i.e., buildings and silos, but not roads or railways) was off limits to conservation. This scenario represents a situation where managers would not want to remove expensive infrastructure to restore conservation value. Effectively, this scenario

represents the biological cost of protecting existing infrastructure. In the second scenario, we made all planning units available for conservation on the proviso that financial costs associated with removal and restoration of infrastructure would be paid. This second scenario effectively represents the economic cost of prioritizing conservation over disrupting existing activities.

Results and Discussion

Site selection and reconnaissance

We sampled 199 locations distributed across 161 primary sites split approximately evenly between sites on DOD and LPNF lands (Table 6, Fig. 10). Because of the differences in the relative number of stream segments in the LPNF and the greater number of wetlands on DOD lands, site selection of these waterbody types was not uniform between DOD and LPNF (Fig. 11). The final selection produced a reasonable distribution of percent area burned (65%), years since last burn (2.5 years), and moderate soil burn intensity (20%) across all sites (Fig 12).

We visited ~ 40-50% of sites at each of the DOD installation and found that sites were accessible and suitable for sampling about 80% of the time. We also attempted to visit 10% of the selected sites on LPNF land but encountered considerable access problems (roads damaged by recent rains, or areas closed to allow recovery from fire). We received a Forest Service gate key that provided access to many areas, and reassigned sites within the LPNF to other areas with better access.

eDNA sampling

We collected triplicate eDNA samples from 175 sites at 132 separate waterbodies (lakes were sampled at multiple sites) on DOD and LPNF lands across the California central coast (Fig. 11). These sites were generally evenly distributed across ownership, waterbody type, and environmental gradients (Table 6). We also collected 12 field blanks. Approximately 25% of the sites selected for sampling were either inaccessible or were dry. Access problems were primarily caused by difficulties obtaining permission to cross private land surrounding LPNF lands or because sites were on private holdings within the LPNF. Some LPNF sites were also inaccessible because of roads conditions. The sites that were dry were mostly low-precipitation, low-elevation drainages that are naturally ephemeral.

Table 5. Estimated costs of removing different types of infrastructure. Costs were based on whether the planning unit contained any of the features listed.

Feature	Removal cost (\$)
Military target	100K
Agricultural water tank	10K
Military range area	5M
Impact area	35M
Surface danger zone	10K
Cantonment	2M
Railroads	1.5M
Parking	3M
Roads	3M
Heliports	750K
Golf courses	20K
Shed	1M
Hazardous fuel treatment	5M
Building	10M
Airfield	100M
Spring box	1K

Of the 175 sites sampled, 106 (61%) were on DOD land and 69 (39%) were within the LPNF. Of the 106 sites sampled on DOD lands, 31 sites were sampled on Vandenberg Air Force Base, 61 on Fort Hunter-Liggett, 6 on Camp Roberts, and 8 on Camp San Luis Obispo. These sites included 54 streams (31% of sites), 41 wetlands (23% of sites), and 80 sites distributed across 37 ponds/lakes (Fig. 11). Sites were well distributed spatially (Fig. 10) and environmentally (Table 6), but low-precipitation, low-elevation (i.e., coastal) sites were under sampled compared to the other environments because many of these systems were too dry to sample. Fifty-three of the sites selected for sampling were not sampled because they were either dry (12 sites) or inaccessible (41 sites). Accessibility problems mostly occurred on LPNF lands, but a few DOD sites were also inaccessible because of impassable road conditions.

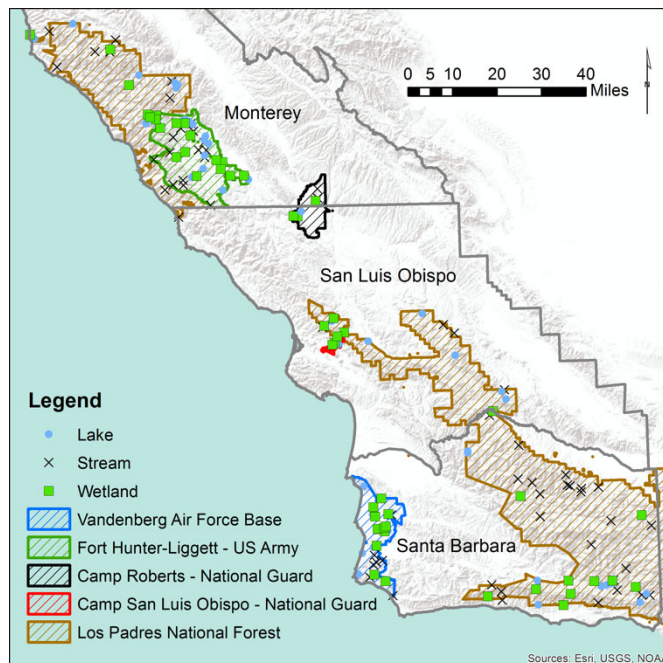


Figure 10. Location of the eDNA sampling locations within the study area.

eDNA marker development and analysis

Quantitative PCR marker development. The existing qPCR assays for chytrid fungus (Boyle et al. 2004), California Red-legged Frog (Halstead et al. 2018), Foothill Yellow-legged Frog (Bedwell and Goldberg 2020) and Western Pond Turtle (Caren Goldberg lab; unpublished) (Table 2), all amplified reliably under our uniform conditions. All four assays were sensitive to ten copies/reaction. Assays for California Red-legged Frog, Foothill Yellow-legged Frog, and Western Pond Turtle were also species-specific when tested against tissue DNA from all sympatric amphibian and

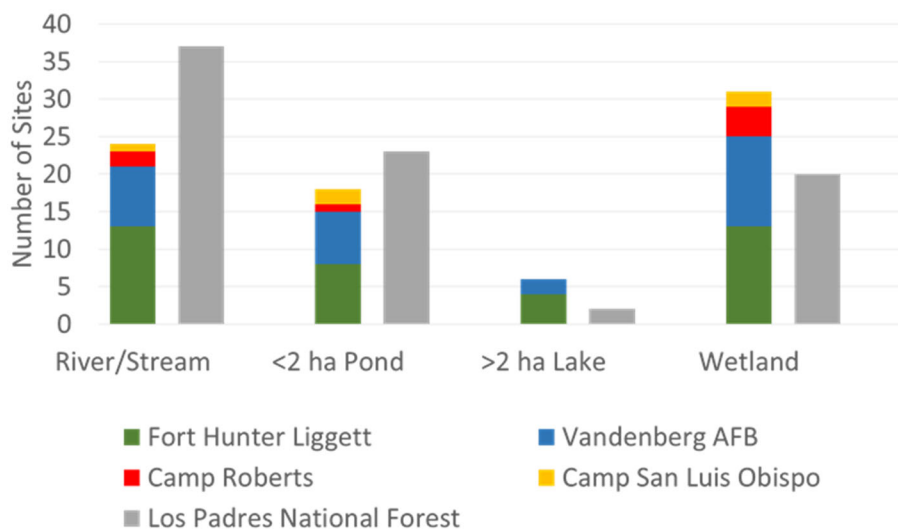


Figure 11. Distribution of sites selected by waterbody type for each for DOD and LPNF installations.

turtle species from our study area (Table S1). We were not able to test specificity for the chytrid fungus assay because we did not possess tissue DNA from sympatric fungal species; however, we trust that the specificity testing conducted by (Boyle et al. 2004) was sufficient.

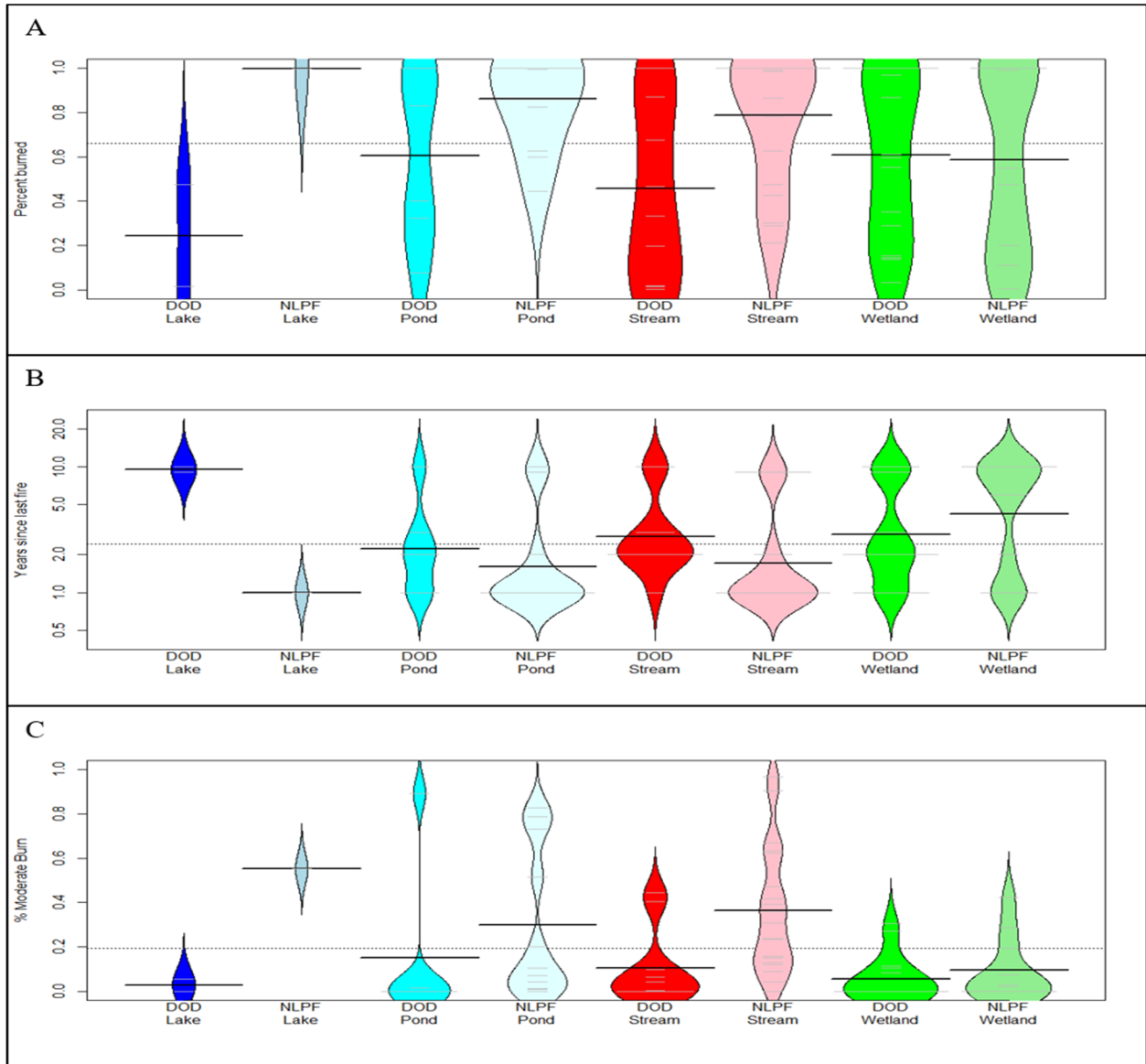


Figure 12. Distributions of (A) percent area burned, (B) years since last burn, and (C) percent moderate burn for all selected burned sites by waterbody type. Dotted lines indicate mean of all data, solid black lines = group means, gray bars = individual data points, and colored areas = indicate distribution of data.

The final species-specific assays we developed for Arroyo Toad and Western Spadefoot Toad target a 120 bp and 147 bp fragment of *cytb*, respectively (Table S7). Ultimately, the more conserved 16S region was not necessary in designing an assay to detect both Western Spadefoot Toad clades. Both assays perfectly matched all available sequences from both target species at both primers and the probe.

In silico evaluation of primer specificity with NCBI Primer-BLAST for Arroyo Toad identified no species with the potential to cross-amplify. For Western Spadefoot Toad, Primer-BLAST identified several species with the potential to cross-amplify (Table Supplemental Materials S11). Of the taxa identified, just one occurs in North America, the congener *Spea bombifrons*, which is not known to occur in California or Baja California. However, all 32 *S. bombifrons* cytb sequences present on Genbank (accessed Nov 7, 2022) possess a terminal mismatch at the 3' end of primer SPHA2-R such that cross-amplification is unlikely.

Table 6. Distribution of sites and samples among waterbody types and installations. FHL = Fort Hunter Liggett, CR = Camp Roberts, CSLO = Camp San Luis Obispo, VAFB = Vandenberg Air Force Base, Total DOD = sum of FHL, CR, CSLO, and VAFB, LPNF = Los Padres National Forest.

Installation	Streams	Ponds	Lakes	Wetlands	Total sites	Total samples
FHL	13	8	4	13	38	50
CR	2	1	0	4	7	8
CSLO	1	2	0	2	5	6
VAFB	8	7	2	12	29	37
Total DOD	24	18	6	31	79	101
LPNF	37	23	2	20	82	98
Total	61	41	8	51	161	199

The *in vitro* evaluation of primer specificity with SYBR® Green qPCR for Arroyo Toad determined one of the three primer sets tested (ANCA3) was superior with regards to specificity. For primer set ANCA1 and ANCA2, tissue DNA from multiple non-target species amplified within 7-10 Cycles of Arroyo Toad tissue samples, a range which may not confer complete specificity once TaqMan™ probe is added. For primer set ANCA3; however, amplification was observed in just one non-target tissue sample from *Rana boylei*, and this sample amplified >19 cycles later than Arroyo Toad tissue samples, a range which is more than suitable for specificity once a TaqMan™ MGB probe is added. No other non-target sample amplified with primer set ANCA3, and all Arroyo Toad samples amplified with a mean C_t of 24.169. Additionally, the melt curve produced a single sharp peak indicating no primer-dimer formation or off-target amplification. Thus, we proceeded with primer set ANCA3 for further TaqMan™ qPCR testing.

For Western Spadefoot Toad, *in vitro* evaluation of primer specificity with SYBR® Green qPCR indicated that all three primer sets were species-specific amongst sympatric amphibian species. For all three primer sets, all non-target tissue samples either did not amplify, or amplified > 14 cycles later than target samples, a range which is more than suitable for specificity once a TaqMan™ MGB probe is added. We chose primer set SPHA2 to proceed with further TaqMan™ qPCR testing because SPHA2 had the lowest mean C_t for Western Spadefoot Toad tissue samples (23.29) and displayed the highest peak fluorescence of the three primer sets tested. For SPHA2, all non-target samples amplified >16 cycles later than target samples or did not amplify at all, and the melt curve produced a single sharp peak indicating no primer-dimer formation or off-target amplification. In the SYBR® Green test with both primer sets ANCA3 and SPHA2 in multiplex, the melt curve produced a single sharp peak indicating no primer-dimer formation between the four primers in the multiplex.

The *in vitro* evaluation of assay specificity with inclusion of TaqMan™ MGB probes indicated that both assays are species-specific amongst the sympatric amphibian samples tested and amplify DNA from only the target species. For Arroyo Toad, all target samples amplified with a mean C_t of 27.42. For Western Spadefoot Toad, all target samples amplified with a mean C_t of 28.07. No amplification of non-target tissue DNA was observed with either TaqMan™ assay.

For both species, 100% of qPCR replicates amplified down to a template concentration of ten copies per reaction. For Arroyo Toad 80% (8 of 10) of reactions containing five copies per reaction amplified, and 50% (5 of 10) of reactions containing two copies per reaction amplified. For Western Spadefoot Toad, 90% (9 of 10) of reactions containing five copies per reaction amplified, and 60% (6 of 10) of reactions containing two copies per reaction amplified.

For Arroyo Toad, we collected 65 total eDNA samples at 26 sites from ten rivers and streams (Table 7, Table S8) with recent documentation of species presence (Hitchcock et al. 2022) to validate our novel assays. At 14 of the 26 sites, either Arroyo Toad larvae or juveniles were observed either at the time of eDNA sampling (n=12) or by United States Geological Survey (USGS) biologists conducting traditional surveys two weeks prior to our eDNA sampling (n=2; Robert Fisher, USGS, personal communication). All visual observations of larvae or juveniles were from MCBCP in 2019 and 2022. No Arroyo Toads of any life stage were visually observed during eDNA sampling in the LPNF during 2021, although the individual who conducted the eDNA sampling in 2021 was not an expert in the identification of Arroyo Toad larvae, which can be cryptic and difficult to differentiate from other sympatric anuran tadpoles.

Table 7. Summary of environmental DNA sampling for Arroyo Toad (*Anaxyrus californicus*) and Western Spadefoot Toad (*Spea hammondi*) for field validation of quantitative PCR assays.

Year	Number of sites/samples	Number of sites with visual observation	Number of sites/samples with eDNA detection	% of sites/samples with eDNA detection at sites with visual observation
<i>A. californicus</i>				
2019	7 / 8	6	7 / 7	100 / 100
2021	11 / 33	0	0 / 0	Na / Na
2022	8 / 24	8	5 / 11	63 / 46
Total	26 / 65	14	11 / 18	7980 / 58
<i>S. hammondi</i>				
2021	18 / 53	9	11 / 30	89 / 85

Our assay detected Arroyo Toad eDNA in 18 samples from 11 sites in four different rivers/streams (Table S8). All eDNA detections were from sites where Arroyo Toad larvae or juveniles were visually observed at the time of eDNA sampling, or by USGS crews previous to eDNA sampling. In sampling at MCBCP in 2019, Arroyo Toad eDNA was detected in all samples where larvae or juveniles were observed. Arroyo Toad eDNA was not detected in any eDNA water samples collected from the LPNF in 2021. For samples collected from MCBCP in 2022, eDNA was detected from 11 of 24 samples at 5 of 8 sites where larvae were observed at

the time of sampling or by USGS biologists two weeks prior. eDNA failed to detect Arroyo Toad presence at 3 of 8 sites where they were visually confirmed as present.

For Western Spadefoot Toad, we collected 53 total eDNA samples at 18 sites from 13 ponds with historical documentation of species presence. At nine of the 18 sites sampled, Western Spadefoot Toads were visually observed at the time of eDNA sampling. Sites with visual observation included three sites at FHL, two sites on private ranches in Santa Barbara County, and all sites at MCBCP (Table S9). Our assay detected Western Spadefoot Toad in 30 eDNA samples at 11 sites from seven lakes/ponds. These sites included eight of the nine sites where Western Spadefoot Toad larvae were observed at the time of eDNA sampling. At one pond in Santa Barbara County, two Western Spadefoot Toad larvae and one nearly metamorphosed individual were netted, but eDNA was not detected. Additionally, eDNA was detected at three sites from a single pond on FHL where Western Spadefoot Toad larvae were observed in 2017 but were not observed at the time of eDNA sampling in 2019 (Table S9).

Metabarcoding marker development. Testing of both our novel 16S metabarcoding marker and the *batra* marker from Valentini et al. (2016) (Table 3) on tissue DNA and pilot eDNA samples demonstrated that both markers worked well in amplifying DNA from all our primary and secondary target species, as well as detecting many fish species. Our new metabarcoding marker should be useful for future eDNA metabarcoding studies of amphibians and turtles in California, and it may also work well in other locations worldwide as evidenced by the fact that this marker amplified DNA from all five exotic species in our mixed, positive control sample, which included tissue DNA from three anuran species, one turtle species, and one newt species, all from Africa or Asia.

eDNA Sample analysis. We observed qPCR amplification for all our target species from our field-collected eDNA samples (Table 8). Chytrid fungus was the most commonly detected of our target species, followed by Western Pond Turtle and California Red-legged Frog. Our least commonly detected species were Western Spadefoot Toad, Arroyo Toad, and Foothill Yellow-legged Frog, with Foothill Yellow-legged Frog only detected in two samples.

Our metabarcoding analysis detected all of our target vertebrate species (the markers we ran were not capable of detecting chytrid fungus) from our field-collected eDNA samples (Table 9).	Table 8. Summary of eDNA amplification from quantitative PCR of target species across the study area.			
	Species	Common name	# samples	# sites
	<i>B. dendrobatidis</i>	Chytrid fungus	67	42
	<i>R. draytonii</i>	California Red-legged Frog	42	21
	<i>R. boylii</i>	Foothill Yellow-legged Frog	2	2
	<i>A. marmorata</i>	Western Pond Turtle	54	41
	<i>A. californicus</i>	Arroyo Toad	9	6
	<i>S. hammondi</i>	Western Spadefoot Toad	21	11

Additionally, we detected seven other amphibian species and three other turtle species. Our novel 16S marker generally detected our target species in more samples than the *batra* marker from (Valentini et al. 2016), except in the case of California Red-legged Frog, which was detected in more samples with the *batra* marker (33 samples with *batra* vs. 26 samples with 16S; Table 9). This difference was most notable for Western Pond Turtle (47 samples for 16S vs. 5 for

batra) and Western Spadefoot Toad (26 samples for 16S vs. 5 for *batra*). This discrepancy is not surprising, as our 16S marker was specifically designed to amplify turtle DNA in addition to amphibians, whereas the *batra* assay (Valentini et al. 2016) was only designed for detection of amphibian species. Thus, the *batra* marker may have lower sensitivity for detecting turtle species, even though it still provided some detections. Both metabarcoding markers also incidentally detected fish species. For the *batra* marker, however, taxonomic power was low for assigning species from sequences, and thus we only attempted taxonomic assignment for fish with our 16S marker. The 16S marker detected a total of 21 total fish species (Table 10).

Table 9. Summary of amphibian and turtle species detected from metabarcoding of eDNA samples. 16S refers to the novel metabarcoding marker we designed for this study, and *batra* refers to a second, previously developed marker.

Species	# Samples	
	16S	<i>batra</i>
<i>Actinemys marmorata</i>	47	5
<i>Rana draytonii</i>	26	33
<i>Spea hammondi</i>	26	5
<i>Rana boylei</i>	5	2
<i>Anaxyrus californicus</i>	3	0
<i>Pseudacris hypochondriaca/sierra</i>	247	193
<i>Anaxyrus boreas</i>	91	116
<i>Lithobates catesbeianus</i>	69	35
<i>Taricha torosa</i>	27	16
<i>Ambystoma californiense/tigrinum</i>	20	18
<i>Trachemys scripta</i>	10	0
<i>Pseudacris cadaverina</i>	8	11
<i>Xenopus laevis</i>	6	0
<i>Sternotherus odoratus</i>	2	0
<i>Chrysemys picta</i>	1	1

Environmental DNA sampling has many benefits, including rates of detection that are often higher than traditional methods (Fediajevaite et al. 2021). However, one downside is the potential for false positive samples, in which amplification occurs, or DNA is detected for a given species in a sample when that species was not truly present at the site where the sample was collected (Pfrender et al. 2017, Darling et al. 2021). Although false positive rates for eDNA are typically very low, they are nearly always non-zero, and thus must be considered when performing downstream analyses. Bias caused by false positives can be especially problematic when eDNA is used for the study of rare taxa. This issue is caused by the base rate fallacy (Bar-Hillel 1980, Darling et al.

Table 10. Summary of fish species detected from eDNA samples with our novel 16S metabarcoding marker.

Species	# samples
<i>Dorosoma petenense</i>	8
<i>Catostomus spp.</i>	57
<i>Cyprinus carpio</i>	32
<i>Lavinia exilicauda</i>	50
<i>Pimephales promelas</i>	4
<i>Gila orcutti</i>	2
<i>Rhinichthys osculus</i>	25
<i>Gambusia affinis</i>	24

2021) in which false positives occur at a greater rate than true detections for rare species. To mitigate these factors, we chose a conservative approach in determining which sites we would consider as true positive detections for our downstream analyses to avoid any potential biases introduced by false positives that could affect the outcome of our models. For our downstream analyses, we only considered a site to be positive if the species was detected in more than one field replicate from a single site with any of our three markers (qPCR, 16S, or *batra*), or with at least two different markers from the same sample. Another way of putting this is that for each site, we had nine genetic replicates, three from qPCR and three from each of the two metabarcoding markers. We classified sites as true presences if eDNA for the species was detected in 2 of 9 or more of these genetic replicates. Because false positives are typically stochastic in nature, and the rate of false positives is low, the probability of two false positives occurring at the same site should be exceptionally low, and thus mitigate the potential risks of false positive bias from eDNA sampling in our end models.

Landscape features, climate, and fire modeling

Landscapes and climate. We obtained 171 layers depicting various environmental variables from a variety of sources (summarized in Table 11 and detailed in Supplemental Materials, Data Dictionary).

Fire modeling. Our calibrated simulations produced averages of 1.75 and 3.56 large fires per year per million acres, with average sizes of 2,059 and 3,505 acres for the Central Coast and Southern Coast, respectively. All numbers were well within the 70% confidence intervals of the observed fire records. Seasonally, simulated median fire size and frequency of the contemporary calibration followed similar patterns as the observational record

Table 11. Number of variables measured for each type of major environmental factor.	
Environmental factor	# Variables
Climate	33
Geology	12
Hydrology	3
Soils	13
Vegetation	8
Human influence/Infrastructure	41
Managed/Protected	11
Recent fire history	4
Topography/Landscape position	16
Current/Future modeled climate	30

with some exceptions, notably an underrepresentation of median fire size during the fall and winter months in the Southern Coast and peak fire frequencies that lagged by about one month in both pyromes. In our baseline gridMET simulations run on the 1979–2005 period, monthly fire size and frequency had similar patterns, but were lower than the 1992–2015 calibration period because of reduced ERC from the slightly cooler and wetter climate during this time period. Average annual burn probability for the baseline gridMET simulations (1979–2005) was about four times higher for the Southern Coast at 0.0080 (0.0078) than the Central Coast at 0.0023 (0.0025). Localized areas of high burn probability corresponded to places with historically high ignition density and fuel types with high rates of spread.

When burn probability was averaged over the entire pyrome (landscape-wide burn probability), five simulations projected a future increase and three simulations projected a decrease in the Central Coast pyrome, whereas six simulations projected a future increase and two projected a decrease in the Southern Coast pyrome. Regardless of whether the simulations projected an overall increase or decrease, each individual simulation exhibited local spatial variability. For

example, even though the simulation based on GFDL-ESM2M projected the highest overall decrease of burn probability in the Southern Coast, some areas also had localized increases.

The spatial variability was more pronounced for the GCM simulations that had an overall decrease of burn probability, resulting in a higher degree of spatial uncertainty. In the Central Coast pyrome, three simulations projected an overall decrease in landscape-wide average burn probability. But, all three of these simulations simultaneously agreed on a projected decrease in only 19.2% of all burnable pixels. In contrast, for the simulations that projected a landscape-wide increase in burn probability, 91.3% of the burnable pixels agreed across GCMs on a projected increase in burn probability. Across the study area, the majority of burnable areas had 5-6 simulations that all agreed on projections of burn probability increase, but few pixels had unanimous increases or decreases. In the Southern Coast, 89.41% of burnable pixels had 5-6 simulations that agreed on a burn probability increase, and in the Central Coast, 74.55% of burnable pixels had 5-6 simulations that agreed on a burn probability increase.

Generally, relationships between large fire frequency and size were strong and related to burn probability, where larger and more frequent fires produced higher burn probabilities. In the Central Coast, projected changes in fire frequency ranged from 0.94 to +1.45 fires per year per million acres, whereas projected changes in fire size ranged from 788.34 to +1,341.79 acres per year. In the Southern Coast, projected changes in ignition frequency ranged from 0.45 to +1.67 fires per year per million acres, whereas projected changes in fire size ranged from 759.52 to +1,056.79 acres.

The complexity of climate change impacts on projected burn probability derives from the temporal variability of ERC and climatic variables (temperature, precipitation, and relative humidity) from which ERC is computed, the spatial variability of factors that contribute to fire growth (e.g., fuel types, topography, and characteristics of canopy), and interactions of both the temporal and spatial variability. Wide variability in projections of climate change are not uncommon for Southern California, particularly for future precipitation projections which are notoriously uncertain in the region (Neelin et al. 2013, Chang et al. 2015, Swain et al. 2018).

FSim simulations in this study reflect regional spatial patterns of the underlying contemporary fire record in Southern California, simulating high burn probability in regions that frequently experienced wildfire historically, whereas spatial patterns in the future projections are modified by the magnitude and seasonality of interacting climate changes. In reality, however, other factors will also influence the spatio-temporal patterns of fire in the future, in addition to climate. The spatial distribution of vegetation types was held constant in our simulations across all three timesteps to isolate the effect of changes in climate. However, vegetation distribution and conditions are unlikely to remain constant throughout the century as fire, drought, and insect and disease outbreaks catalyze long-term type shifts in California (Forrestel et al. 2011, Keeley and Brennan 2012, Syphard et al. 2019).

We evaluated eight plausible climate change scenarios across Southern California, varying in the direction, seasonality, and magnitude of projected change. By incorporating each projection into a high-resolution spatial fire behavior simulator, we were able to consider the effects of each of these projections on changes in annual burn probability, both at a local 270 m resolution pixel

and regionally for Southern California. High resolution future projections, such as presented in this study, enable exploration of wildfire impacts on communities (Ager et al. 2019), buildings (McEvoy et al. 2021), and evacuation dynamics (Dye et al. 2021). Our results describe a range of uncertainties related to the impact of climate alone on fire, but there are many compounding factors yet to be considered that will interact with climate effects, including changes to management practices (Williams and Abatzoglou 2016), vegetation (Hurteau et al. 2019), population distribution (Syphard et al. 2019), and Santa Ana wind strength and seasonality (Guzman-Morales and Gershunov 2019), and modifications to our modeling approach that include these interactions would be highly impactful for continuing to understand the complexity of future fire projections.

Modeling and predicting *Batrachochytrium dendrobatidis* habitat suitability

Of the 159 sites sampled within the study area, we detected *Bd* at 23% ($n = 37$) sites. We also found that *Bd* occurrences were unevenly distributed across the study landscape. *Bd* was most frequently observed at waterbodies within Vandenberg Air Force Base and Fort Hunter Liggett (Fig. 13).

The RF model performed moderately well (sensitivity=0.61, specificity=0.76, AUC=0.70, and TSS=0.37). The importance of the five predictors, in rank order, were NDVI, precipitation of the wettest quarter, watershed slope, annual mean temperature, and percent impervious surface within the watershed contributed to the model fit (Table 12).

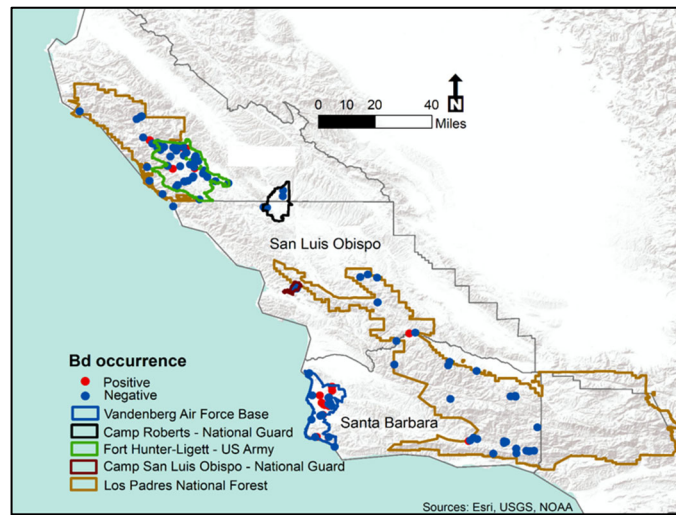


Figure 13: Map of the study area showing locations of *Bd* presences and absences ($n = 37$ and $n = 122$ respectively).

The RF model showed that *Bd* habitat suitability was associated with all five environmental predictors (Fig. 14). *Bd* occurrence increased with both increasing NDVI and percent impervious

Table 12. Predictors used for modeling *Bd* distribution and their importance scores in rank order.

Predictors	Mean Decrease Accuracy
Normalized Difference Vegetation Index	10.9
Precipitation of the wettest quarter	7.7
Watershed slope	7.6
Annual mean temperature	6.8
Percent impervious surface	4.0

surface, but *Bd* occurrence decreased with increasing watershed slope. *Bd* occurrence increased with increasing precipitation of the wettest quarter up to ~400 mm and then plateaued. *Bd* occurrence was low between 11 and 13 °C annual mean temperature but increased from ~13 to ~15 °C (Fig. 14).

The partial dependence plot for the SOM showed similar patterns but also revealed relatively high model uncertainties (Fig. 15). Unlike the RF model, partial dependence plots from the SOM are linear reflecting the linear structure of the SOM, and hence do not capture non-linear responses. In general, the probability of *Bd* occurring at a site increased with increasing NDVI, annual mean temperature, and percent impervious surface. In contrast to the RF model, though, *Bd* occupancy was not associated with precipitation. However, any actual non-linear responses would be manifested as increased uncertainty around the linear trend lines. Additionally, the probability of *Bd* detection decreased with increasing annual mean temperature (Fig. 15). *Bd* suitability maps from both the RF and occupancy models also revealed generally similar spatial patterns – i.e., predicted *Bd* habitat suitability was low across most of the study area but high in a few places (Fig. 16 and 17). The regions with moderate to high *Bd* predicted habitat suitability included Camp San Luis Obispo, most parts of the Vandenberg Airforce Base, and pockets of Fort Hunter-Liggett and the Los Padres National Forest. However, the variation in *Bd* suitability predicted with the occupancy model (Fig. 17) was more extreme than predicted from the RF model, with areas with the highest and lowest probabilities of predicted *Bd* suitability much more distinct compared with the RF suitability predictions (Fig. 16).

Modeling and predicting herpetofauna habitat suitability

Distribution of eDNA-detected locations.

With the exception of the Western Pond Turtle, eDNA samples indicated that our target species occurred at too few sites to support habitat suitability modeling (Table 13). However, we were able to obtain supplemental occurrence data from GBIF for the Arroyo Toad and California Red-legged Frog for random forest modeling. eDNA detections of the Western Pond Turtle were widely distributed across the study area, whereas eDNA detections of the California Red-legged Frogs were clustered on Vandenberg Air Force Base (Fig.18). Unfortunately, too few combined observations (eDNA, GBIF, data from other researchers) were available in and near the study area to support modeling for the Western Spadefoot Toad and Foothill Yellow-legged Frog.

Model performances for Arroyo Toad, California Red-legged Frog, and Western Pond Turtle. Models for the Arroyo Toad and California Red-legged Frog were robust and performed very well with AUCs of 0.89 and

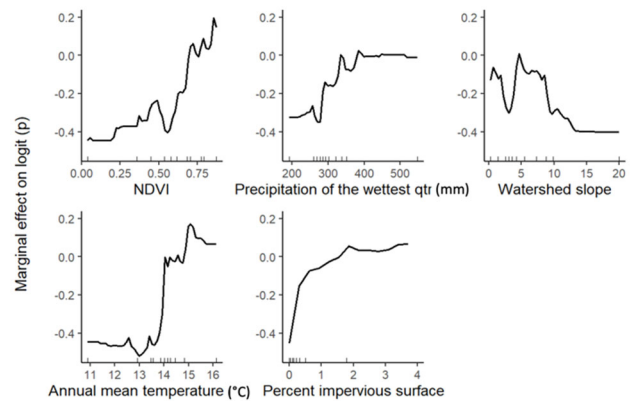


Figure 14. Partial dependence plots of the five predictors in rank order of their influence on the random forest model predicting the probability of *Bd* occurrence:

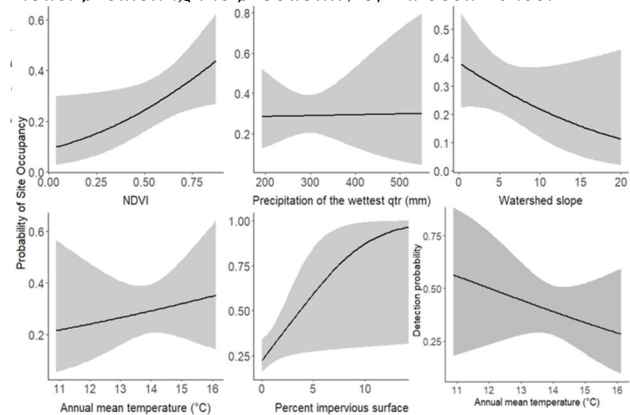


Figure 15. Partial dependence plots for the *Bd* species occupancy model predictors and the plot showing how probability of detection varies with temperature.

0.90 and TSSs of 0.76 and 0.68 respectively (Table 14). Both models performed well because they were good at correctly predicting both presences and absences correctly, hence the high TSS scores. With an AUC of 0.74 and a TSS of 0.40, the model for Western Pond Turtle performed less well. The model was not particularly good at correctly predicting presences, resulting in low sensitivity and TSS (Table 14).

In general, NDVI, distance to flowline, minimum temperature, and total precipitation were the most important predictors of species occurrences (Fig. 19), whereas distance to waterbody and watershed area were the least important predictors. For the California Red-legged Frog and Western Pond Turtle, minimum temperature and NDVI were consistently in the top four predictors of species occurrences (Fig. 19, Fig. 20, Supplemental Materials Table S12). However, for the Arroyo Toad, only NDVI was in the top four predictors (Fig. 21, Table S12). Distance to flowline was consistently in the top four predictors of species occurrence for all three species (in the top two for both the Arroyo Toad and Western Pond Turtle).

Predicted species distributions across the study area are both similar and different for the three species. There was a high likelihood of Arroyo Toad and California Red-legged Frog occurrences in waterbodies of Los Padres National Forest, with the highest habitat suitability around the southernmost extent of the national forest. Waterbodies in the Vandenberg Airforce base

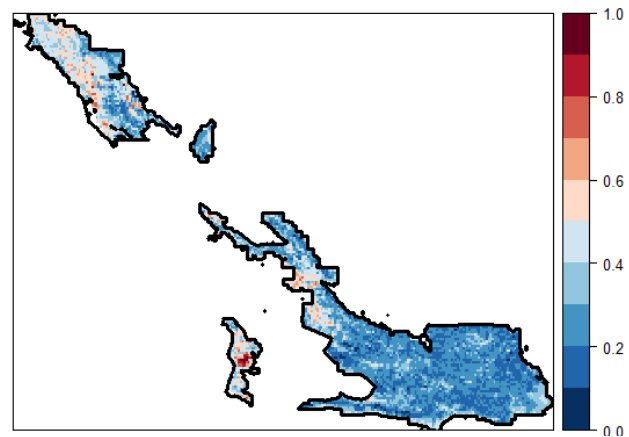


Figure 16. Heat map showing spatial variation in predicted Bd habitat suitability by the random forest model. Red = highest suitability and blue = lowest suitability.

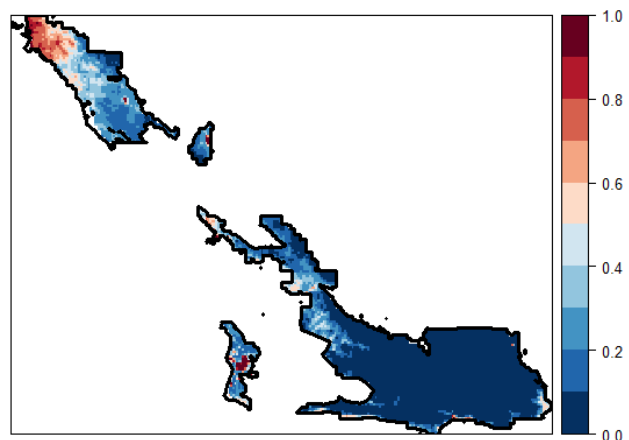


Figure 17. Heat map showing spatial variation in predicted Bd habitat suitability by the species occupancy model. Red = highest suitability and blue = lowest suitability.

Table 13. Detection (eDNA) or GBIF records for each target species. MBC1 = metabarcoding marker 1. MBC2 = metabarcoding marker 2.

Target species	qPCR	MBC1	MBC2	GBIF	Absences	Total presences
Chytrid fungus	37				122	37
Arroyo Toad	1	1	0	18	166	20
Foothill Yellow-legged Frog	0	0	0	-	168	0
Western Spadefoot Toad	5	1	0	-	162	6
California Red-legged Frog	12	1	2	27	159	42
Western Pond Turtle	10	13	1	-	138	24

were predicted to be suitable for California Red-legged Frogs, but not for the Arroyo Toads (Fig. 23). Conversely, the model showed there is a likelihood of finding Western Pond Turtle across the study area, but a low likelihood of occurrence in the Vandenberg Airforce Base and Camp Roberts Military Reservation.

Predictions of future species distributions.

The mean change in habitat suitability was small for all species, but the direction was important. For example, the mean change in habitat suitability (occurrence probabilities) for the Arroyo Toad and the Western Pond Turtle are -0.11 and -0.09 (Table 15) respectively, suggesting that waterbodies across the study area that were hitherto suitable may become slightly unsuitable for the two species by the midcentury (Fig. 23). Conversely, the mean change in habitat suitability for the California Red-legged Frog is +0.13, suggesting that future climatic conditions may favor the occurrence of the species by the midcentury.

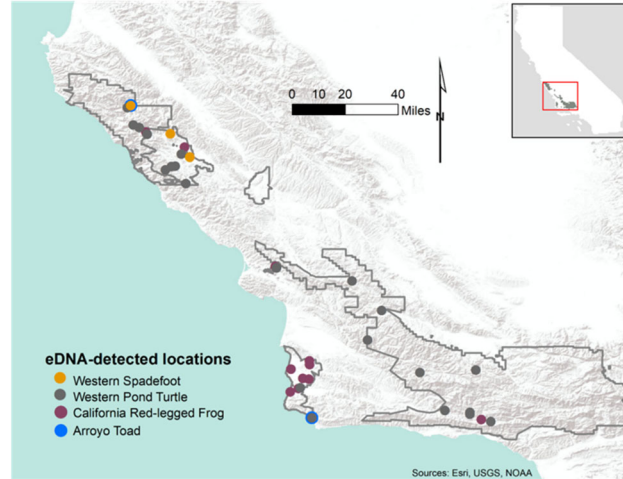


Figure 18. Locations of eDNA detections for four target species.

Table 14: Performance metrics for the random forest models for the California Red-legged Frog (CRLF), Arroyo Toad (AT), and Western Pond Turtle (WPT) and top four predictors for each species in rank order of importance.

Species	Sensitivity	Specificity	AUC	TSS	Top four predictors
CRLF	0.84	0.84	0.90	0.68	minimum temperature, NDVI, total precipitation, distance to flowline
AT	0.86	0.89	0.89	0.76	NDVI, distance to flowline, distance to road, distance to waterbody
WPT	0.68	0.72	0.74	0.40	distance to road, distance to flowline, minimum temperature, total precipitation

Conservation implications. The reliability of model predictions is dependent on the quality and source of input data and the modeling methods used (Abdulwahab et al. 2022). In documenting the occurrences of *Bd* in the study landscape, we used eDNA samples that were carefully collected and processed to minimize false positives that can introduce errors in the models. We therefore have confidence that our protocols correctly identified both *Bd* positive and negative sites. However, it is important to note that because we surveyed the sites only in spring 2019, our *Bd* detections may not be reflective of *Bd* occurrence in the study area across all seasons. Seasonal variation in the occurrence and intensity of *Bd* has been reported in several studies (Kriger and Hero 2007, Sonn et al. 2019, Garnham et al. 2022). We therefore recommend that

monitoring programs that span all seasons be used in the future. An extensive seasonal survey of *Bd* across the study area will better identify those habitats that are disease free and of low risk to amphibians, as well as identify locations that may require more targeted management strategies to curb amphibian declines (e.g., translocation, antifungal treatments, habitat manipulation, and bioaugmentation with commensal bacteria). Additionally, collecting *Bd* occurrence data across seasons will facilitate the building of more robust SDMs. The random forest modeling approach that we used can identify, and model, complex interactions between predictors, but other predictors correlated with the final five predictors that we used in our model might be the actual drivers of *Bd* occurrence. As with all correlative models, we acknowledge that associations imply, but do not establish, causation. In addition, *Bd* occurrence at a site may not necessarily reflect disease dynamics at the microhabitat level. To fully understand *Bd* dynamics at the microhabitat level, research on specific host-*Bd* interactions will be needed. Despite the moderate performance of our models and the inherent uncertainties associated with them, our results were ecologically interpretable, and they should be useful in helping guide *Bd* management actions.

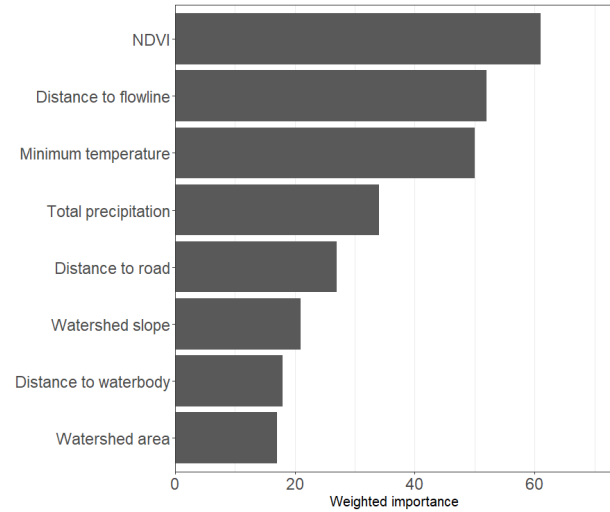


Figure 19. Weighted importance of the eight predictors used in the random forest habitat suitability models for the California Red-legged Frog, Arroyo Toad, and Western Pond Turtle.

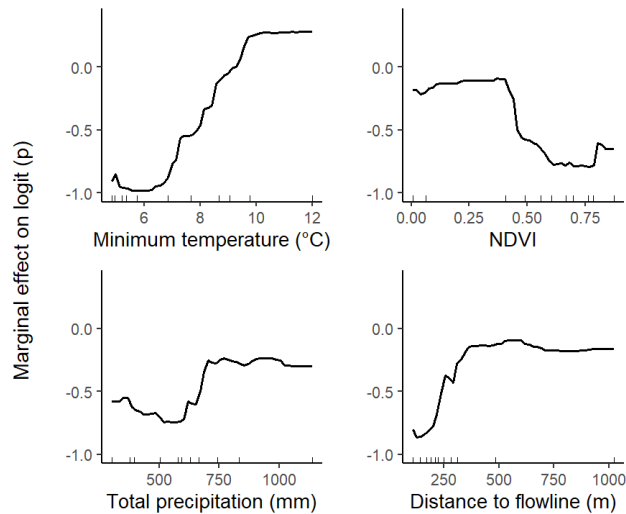


Figure 20. Partial dependence plots for the top four predictors in the California Red-legged Frog random forest model.

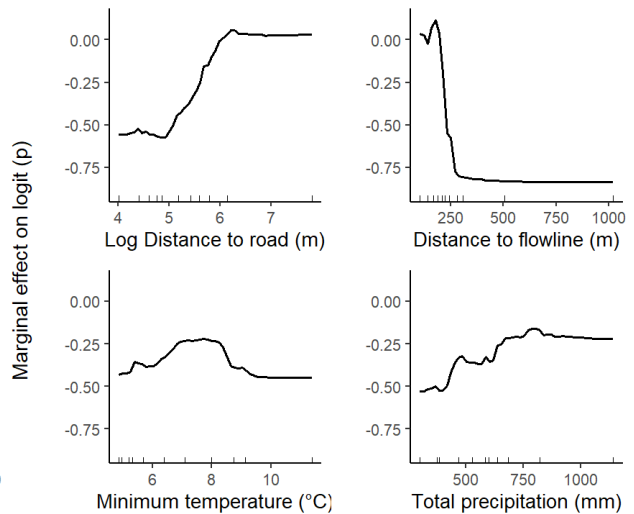


Figure 21. Partial dependence plots for the top four predictors in the Western Pond Turtle random forest model.

Our results have important implications for amphibian research and conservation management. Currently, in-situ management of *Bd* is impractical, and it is almost unavoidable that *Bd* will eventually spread to areas that are currently disease-free (Lips et al. 2008). Consequently, the likely *Bd* drivers that we identified further emphasize the view that once *Bd* establishes, managing it will be difficult. However, identifying *Bd* hotspots and areas of low *Bd* habitat suitability are important first steps toward designing effective management strategies. Designing a robust conservation plan requires information on the target species, the estimated cost of managing the land parcels where the target species occur, and the risks that the target species will face. Certain areas on the landscape may therefore act as refugia, requiring increased levels of isolation to reduce the invasion of *Bd* (Pauza et al. 2010). Given that our modeling approach produced reasonable and interpretable results, our findings should serve as an important measure of risk when identifying the mixes of land for conservation prioritization.

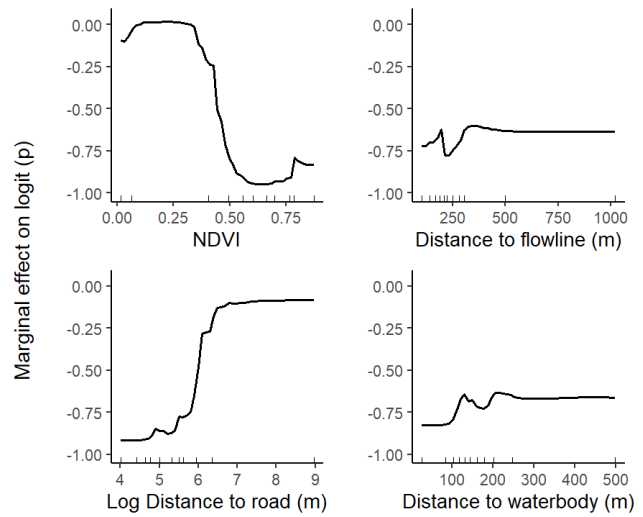


Figure 22. Partial dependence plots for the top four predictors in the Arroyo Toad random forest model.

Table 15. Mean habitat suitability values of the California Red-legged Frog (CRLF), Arroyo Toad (AT), and Western Pond Turtle (WPT) currently and in the future (2040-2069) and the difference between current and future values.

Species	Predicted mean habitat suitability		
	Current	2040-2069	Difference
CRLF	0.56	0.69	+0.13
AT	0.56	0.45	-0.11
WPT	0.49	0.40	-0.09

eDNA sampling is much more efficient at detecting rare species than traditional manual approaches, and the fact that we detected too few occurrences to model habitat suitability for most of the species of concern reinforces the view that these species persist at very few locations. As a consequence, it is imperative that remaining populations be conserved and degraded, but previously suitable, habitats be restored. Our Marxan analyses were a first step toward identifying what habitat units can best achieve that goal.

Marxan analyses

We found that regardless of whether minimizing infrastructure removal (scenario 1) or conservation (scenario 2) were prioritized, the number of planning units required to meet conservation targets increased linearly with the amount of each species range to be protected (Fig. 24). This relatively linear relationship between the area to be protected for amount of each species and the number of planning units required is to be expected, as increasing conservation necessarily requires increased area (Roberts et al. 2020). In addition, given the amount of overlap in species distributions we observed, increasing the proportion protected would require new areas to be identified for each species. However, we found that under scenario 1 (built

infrastructure unavailable for conservation) it was impossible to meet conservation targets for all three species when targets were set at 0.8 or greater (Fig. 24 A). When targets were this high, the algorithm was effectively selecting all available planning units on the landscape, and even this was insufficient to conserve all species. Under scenario 2 (all planning units available, costs must be paid), it was possible to conserve all species regardless of conservation target level (Fig. 24).

With respect to financial cost, under scenario 1 costs to meet all conservation targets below 0.7 were low. However, at conservation targets greater than or equal to 0.8, the costs increased substantially even though not all conservation targets were met. This outcome occurred because when targets are 0.8 or above, it is impossible to meet the target with the available planning units (i.e., ones without built infrastructure) as greater than 0.2 of each species' predicted distribution was in areas with built infrastructure. However, the algorithm was still trying to effectively conserve each species, so it was including all planning units (including very expensive ones containing roads or airfields). This inclusion of expensive, but poor-quality, planning units is what led to the high costs. In the case of scenario 2, where all planning units were available for use in conservation, as conservation targets increased, the estimated costs associated with increasing conservation remained relatively low until we attempted to conserve more than 0.7 of each species' distribution, at which point estimated costs increased exponentially (Fig. 24B). This sudden rapid increase in estimated costs reflects the observed patterns under scenario 1 and indicates that when conservation targets are set at greater than 0.7, we had to include locations that contained high-cost infrastructure in areas for conservation. When conservation targets were below 0.7, we were able to meet conservation targets with areas that did not contain infrastructure, meaning that costs were kept low. This

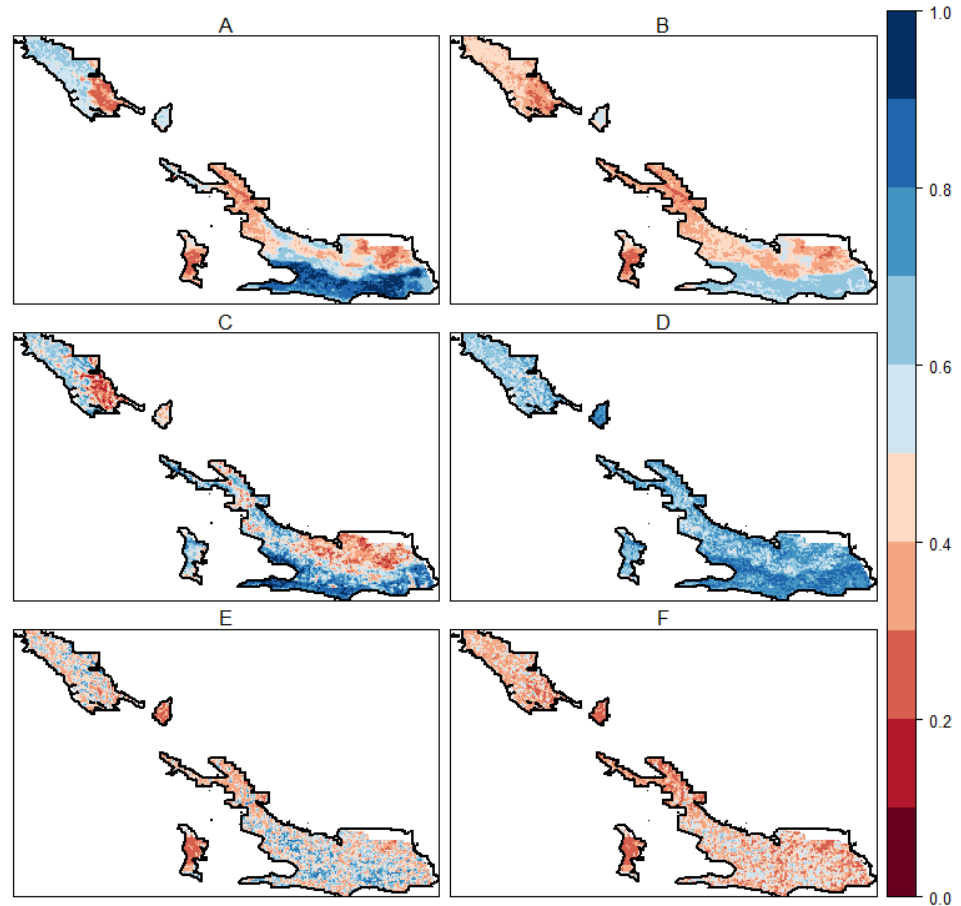


Figure 23. Predicted current (A, C, E) and future (2040-2069)(B, D, F) habitat suitability for the Arroyo Toad (A and B), California Red-legged Frog (C and D), and Western Pond Turtle (E and F) in the study area.

exponential relationship between the proportion of species' distributions to be protected and costs has been previously observed (Withey et al. 2012) and highlights the potential issues with attempting to achieve high levels of conservation in developed landscapes with multiple land use pressures. However, even as costs increased exponentially under scenario 2, they still remained lower at high-conservation targets than for scenario 1, and scenario 2 was able to meet conservation targets. This result highlights that to achieve high levels of conservation, some removal and restoration will need to take place. Therefore, managers face a choice, either set conservation targets at lower levels (0.7 or below) or accept that achieving high levels of conservation will require removal of some existing infrastructure.

Overall, the spatial distribution of high- and low-priority conservation areas highlights how increasing conservation targets leads to increased conflict between conservation and infrastructure. When conservation targets were set relatively low (e.g., 0.2, Fig. 25B), the highest priority locations were in the wilderness areas contained in the western portion of the Los Padres National Forest. This region contains many planning units that were selected in 50 - 75 of the 100 Marxan runs, indicating their high priority. In addition, the wilderness zones to the north of Fort Hunter Liggett were selected in > 50 of the 100 Marxan runs, with some areas selected in > 75 of the runs. As both of these locations are in wilderness areas, they contain very little infrastructure. This lack of infrastructure means that not only was the algorithm able to work around the planning units with built infrastructure (which are unavailable), but the risks of failure posed by proximity to roads was also low (Beebee 2013). Conversely, areas in Fort Hunter Liggett, Vandenberg Air Force Base, and Camp Roberts that contained large areas of infrastructure were unavailable and never selected (Fig. 24B). Each of these areas contained high densities of roads and other forms of infrastructure. Not only were large areas unavailable, but the high density of roads means that even areas in close proximity to infrastructure may likely fail to meet conservation objectives, even if they contain no infrastructure themselves and are relatively cheap. In terms of the planning units identified as the optimal set to meet the conservation target of 0.2, only six were in Vandenberg Air Force Base and only two were in Camp Roberts. Conversely a lot of the optimally selected planning units were found in the wilderness areas of Los Padres National Forest and the wilderness areas to the north of Fort Hunter Liggett (Fig. 26). When conservation targets were set at this low level, there was very little difference in the spatial distribution of priority planning units between scenarios 1 and 2.

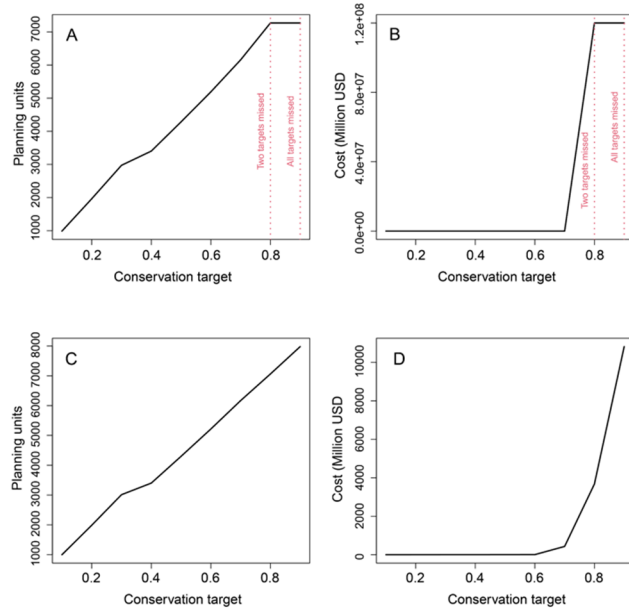


Figure 24. Relationships between the amount of each species' predicted area to be protected and the number of planning units required (A and C) and predicted costs (B and D). Panels in the top row represent scenario 1, where planning units containing non-transport related built infrastructure were unavailable for selection. Panels in the bottom row represent scenario 2, where all planning units could be selected, but removal and restoration costs had to be paid.

This lack of difference stems from the costs associated with using planning units containing infrastructure for conservation. In scenario 2 planning units with infrastructure were available, but their high financial costs meant they were not selected. As a result, regardless of whether infrastructure planning units were unavailable or just costly, the algorithm was still able to meet conservation targets by selecting other areas.

As conservation targets increased, the number of planning units identified as high priority also increased (Fig. 25 C - E, Fig. 27 C - E) as did the number of planning units included in the optimal solution (Fig. 26 C - E, Fig. 28 C - E). When conservation targets were set at 0.4 or 0.6, the relative priorities of different planning units increasingly diverged in both scenarios. With targets set at 0.4, planning units tended to be selected in > 25 of the 100 runs (with many being selected in > 50) or not selected at all (Fig. 25 C, Fig. 26 C). This divergence in planning unit priorities likely stemmed from a combination of more planning units being required to meet conservation targets (leading to many planning units being selected > 25 times) while also achieving conservation goals without using planning units containing large amounts of built infrastructure. As conservation targets increased to 0.6, the selection of planning units differed even more, with a very large number of planning units being selected in > 50 of the Marxan runs (and many being selected > 75 times), whereas others were not selected at all (Fig. 25 D, Fig. 27 D). This increase in the number of selections, and therefore relative priorities, again stemmed from more planning units being required to meet conservation targets, but not so many that planning units containing expensive infrastructure needed to be included. Across the whole spatial extent, when conservation targets were set at 0.6, very few planning units were selected in only 1 - 25 of the Marxan runs (Fig. 25 D, Fig. 27 D). This small number of infrequently selected planning units indicates how constrained the Marxan algorithm had become with respect to which planning units it was selecting, and demonstrates how as conservation targets increase, the number of options available to meet those targets decrease (Margules and Pressey 2000, Wilson et al 2000). However, even when conservation targets were set at 0.6, the algorithm could still meet the targets without requiring areas containing expensive infrastructure, meaning that even when these locations are available, they were selected in 0 out of 100 runs (Fig. 25 D.). When conservation targets were increased to 0.8, the divergence in selection of planning units changed somewhat. In scenario 1 (non-transport, built infrastructure was not

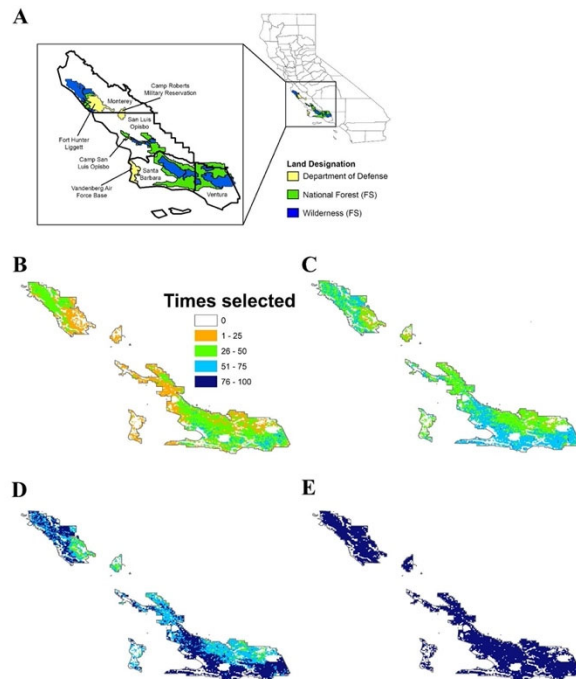
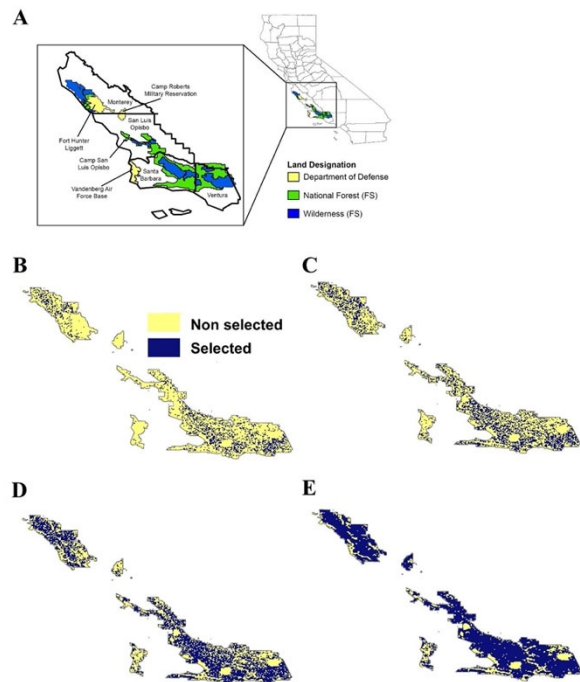


Figure 25. A: The spatial extent of this study, with different land types identified. B – E: The relative importance of each planning unit for conservation under scenario 1 (number of times selected out of 100 possible runs) as conservation targets increase. Conservation targets were set at either 0.2 (B), 0.4 (C), 0.6 (D), or 0.8 (E). Non-transport infrastructure is unavailable for conservation.

available), all planning units that were available for selection were selected, meaning that the distribution was completely different (Fig. 25 E). When targets were set at 0.8 in scenario 1, it was impossible to meet the target for two species (insufficient available area); however, in an attempt to meet the target, the algorithm selected every available planning unit. This selection of every available unit drove up costs, reinforcing the results shown in Fig. 24, where even though targets were not met in scenario 1, the cost of selecting planning units was greater than for scenario 2. When all planning units were available for selection (scenario 2) and targets were set at 0.8, the vast majority of all planning units that did not contain infrastructure were selected in > 75 of the 100 Marxan runs. In addition, many of the planning units that did contain infrastructure were selected in 1 - 25 of the runs (Fig. 26 E). This selection of planning units containing infrastructure was a consequence of both conservation targets being set high and the rapid increase in costs seen in Fig. 24 D. When conservation targets were set so high, w planning units were regularly selected in Vand 27).

Caveats. The Marxan algorithm operates independently of human biases, but the results can be affected by the initial data that are used, which may have biases or inaccuracies. In this study, we estimated how opportunity costs differed across types of land use and infrastructure, and more



refined estimates of these costs may lead to somewhat different Marxan solutions. In addition, roads on the landscape formed part of our estimation of risk (planning units failing), with high risks occurring immediately adjacent to roads and decaying as distance from the road increases. However, we did not independently conduct experiments to directly assess the effect of roads on the target species and instead relied on previously published estimates (Carr and Fahrig 2001, Benítez-López et al. 2010, Beebe 2013). These studies were not conducted in the same study location or focused on our target species. However, based on our results associated with changing conservation targets, any quantitative discrepancies between responses reported in these studies and what occurs in our study area should lead to quantitative, but not qualitative differences in the relative importance of different planning units – i.e., the important areas would likely remain important, whereas the low-priority areas would remain less important. Only the absolute magnitude of importance would change.

Recommendations. The results from our Marxan analysis identified locations within the study area that represent high-priority locations for conservation of the target species. As these high-priority locations remained the same regardless of the conservation targets (0.2 - 0.8), we can make some initial recommendations. We suggest initially using the planning units identified as high-priority when targets are set at 0.2 for conservation, such as the wilderness areas in Los Padres National Forest. The reason is that these planning units represent the locations that were initially selected when the amount of each species' distribution to be conserved was relatively low. Therefore, regardless of what conservation targets managers may eventually agree on (anywhere between 0.2 and 0.8 of the predicted distribution), these areas should be included in the conservation solution. If agreed-upon targets are low (e.g., 0.2), we can simply choose the areas identified by Marxan (Fig. 26 B). However, if agreed-upon targets are greater than 0.2, then the planning units identified when targets are set at 0.2 will still be identified as high-priority and included, although additional planning units would be needed to meet the higher target, meaning we lose nothing by including the high priority 0.2 planning units from the outset. These results were the same regardless of whether scenario 1 or 2 was adopted. However, given that only scenario 2 was able to meet conservation targets of 0.8 and still avoid areas of built infrastructure at lower levels, we would recommend adopting this strategy. The ability of

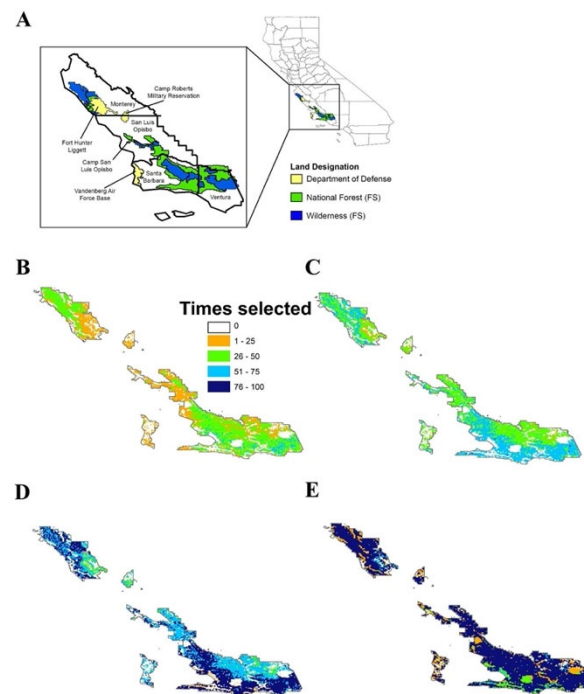


Figure 27. A: The spatial extent of this study with different land types identified. B – E: The relative importance of each planning unit for conservation under scenario 2 (number of times selected out of 100 possible runs) as conservation targets increase. Conservation targets set at either 0.2 (B), 0.4 (C), 0.6 (D), or 0.8 (E). All planning units are available for selection.

scenario 2 to meet targets highlights the importance of not excessively constraining possible options and letting the algorithm find the optimal solution.

Stakeholder training

As part of stakeholder training, we produced a video to help both practitioners and other interested individuals understand how Marxan works and how its outputs should be interpreted (<https://youtu.be/znQEhOcDdNE>).

Conclusions and Implications for Future Research/Implementation

Ideal systematic conservation planning tools are objective, data-driven, quantitative, accurate, and transferrable. This project demonstrated the potential benefits of such an approach in a region with multiple jurisdictions and authorities responsible for making conservation decisions. The landscape-scale analyses we conducted showed that different jurisdictions within that landscape differed in both the amount of natural habitat remaining as well as the amount of potentially high-quality habitat that could be restored to meet mutually agreed upon, region-wide conservation targets.

Another benefit of using systematic conservation planning tools like Marxan is that they can be used widely under many different types of landscapes. This transferability allows direct comparison and evaluation of proposed or already implemented conservation actions and facilitates discussion regarding what types of conservation actions work best and what types of actions, and under what types of environmental settings, do actions not achieve intended conservation targets.

All tools are imperfect, and managers need to determine if the tool we developed is a useful addition to the tools they currently use despite its imperfections. On the ground managers should first examine if the specific outcomes of our analyses make sense. For example, do their individual and collective best professional judgements regarding the conservation value of different planning units agree with the conservation values that Marxan assigned? If so, this Marxan-based analysis is likely useful. However, if best professional judgement disagrees with Marxan assignments, then one or more aspects of the Marxan tool needs to be scrutinized and corrected. Marxan outputs will be especially sensitive to

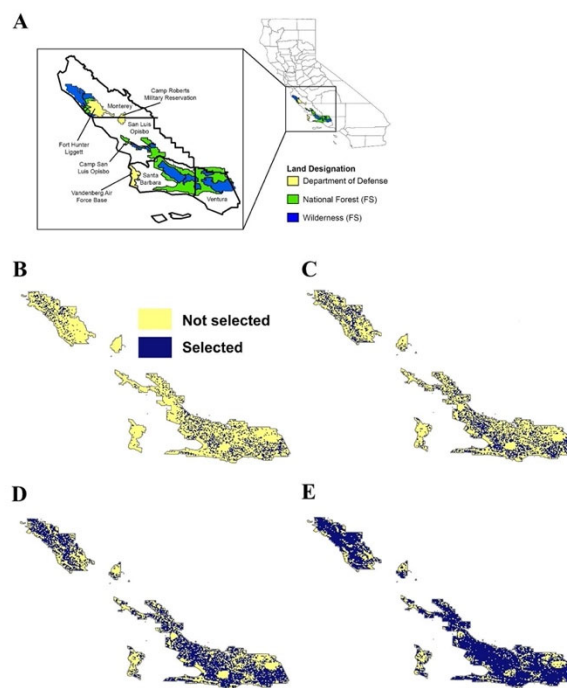


Figure 28. A. The spatial extent of the study. B – E: The optimal planning units identified as being required to meet the conservation targets of 0.2 (B), 0.4 (C), 0.6 (D), and 0.8 (E) under scenario 2. All planning units were available for selection.

how accurate the habitat suitability models are that we developed. The metrics we used to evaluate model performance indicated our models were generally accurate, but considerable uncertainty exists with respect to individual predictions. If managers find the current Marxan solution too inaccurate for routine use, future research should explore how sensitive overall Marxan solutions are to uncertainty in their habitat suitability inputs. This information would allow managers to directly incorporate estimates of uncertainty into decisions regarding conservation actions.

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Appendices

Supporting Data

eDNA sample collection data sheet

eDNA Sample Collection Data Sheet									
Site Code				Latitude (Dec Degree)					
Water Body Type	Stream	Wetland	Lake	Longitude (Dec Degree)					
Crew Initials				Date					
Hose #				Rain in previous 48 hrs? Y N	Flow? Y N				
Water Temp (°C)				Vegetation	Trees (circle all): Conifer Hardwood NA				
pH				% Cover (1 "x"/row)	0	<10	10-40	40-75	>75
DO Conc (mg/L)				Trees > 5m					
DO Saturation (%)				Veg 0.5 – 5 m					
Sp Conductivity (µS/cm)				Shrubs < 0.5 m					
Sampling	Sample 1	Sample 2	Sample 3	Herb/grass					
Time Start				Barren/Rock					
# Filters				Human or Natural Disturbances (Note distance and intensity):					
Time Stop									
Volume (L)									
Depth (cm)									
Substrate									
Substrate Codes: RG: Rock/gravel, SD: sand, SL: silt									
Notes (clogging, amphib sightings, etc.):									

eDNA Sample Collection Data Sheet									
Site Code				Latitude (Dec Degree)					
Water Body Type	Stream	Wetland	Lake	Longitude (Dec Degree)					
Crew Initials				Date					
Hose #				Rain in previous 48 hrs? Y N	Flow? Y N				
Water Temp (°C)				Vegetation	Trees (circle all): Conifer Hardwood NA				
pH				% Cover (1 "x"/row)	0	<10	10-40	40-75	>75
DO Conc (mg/L)				Trees > 5m					
DO Saturation (%)				Veg 0.5 – 5 m					
Sp Conductivity (µS/cm)				Shrubs < 0.5 m					
Sampling	Sample 1	Sample 2	Sample 3	Herb/grass					
Time Start				Barren/Rock					
# Filters				Human or Natural Disturbances (Note distance and intensity):					
Time Stop									
Volume (L)									
Depth (cm)									
Substrate									
Substrate Codes: RG: Rock/gravel, SD: sand, SL: silt									
Notes (clogging, amphib sightings, etc.):									

Data Dictionary and list of 171 candidate predictor variables (see separately uploaded Excel file RC18-1034-DataDictionary-20230314.xlsx)

Table S1. Tissue samples used for specificity testing of eDNA assays. MVZ refers to the Museum of Vertebrate Zoology collection number, CAS refers to the California Academy of Sciences herpetology collection number.

Sample Name	species name	common name	source
RT173	<i>Actinemys marmorata</i>	Western Pond Turtle	MVZ:Herp:16499 4
RT174	<i>Actinemys marmorata</i>	Western Pond Turtle	MVZ:Herp:16499 5
RT85	<i>Ambystoma californiense</i>	California Tiger Salamander	CAS-211677
RT86	<i>Ambystoma californiense</i>	California Tiger Salamander	CAS-211684
RT123	<i>Ambystoma californiense</i>	California Tiger Salamander	CAS-214885
RT108	<i>Anaxyrus boreas halophilus</i>	Western Toad	CAS-206465
RT150	<i>Anaxyrus boreas halophilus</i>	Western Toad	CAS-253026
RT97	<i>Anaxyrus californicus</i>	Arroyo toad	CAS-175636
RT162	<i>Anaxyrus californicus</i>	Arroyo toad	MVZ:Herp:14523 0
RT163	<i>Anaxyrus californicus</i>	Arroyo toad	MVZ:Herp:15001 3
RT112	<i>Aneides lugubris</i>	Arboreal Salamander	CAS-208660
RT113	<i>Aneides lugubris</i>	Arboreal Salamander	CAS-208671
RT121	<i>Aneides lugubris</i>	Arboreal Salamander	CAS-214883
RT169	<i>Batrachoseps gavilanensis</i>	Gablilan Mountains Slender Salamander	MVZ:Herp:26661 4
RT170	<i>Batrachoseps gavilanensis</i>	Gablilan Mountains Slender Salamander	MVZ:Herp:26661 5
RT168	<i>Batrachoseps incognitus</i>	San Simeon Slender Salamander	MVZ:Herp:25193 1
RT145	<i>Batrachoseps luciae</i>	Santa Lucia Mountains Slender Salamander	CAS-252904
RT146	<i>Batrachoseps luciae</i>	Santa Lucia Mountains Slender Salamander	CAS-252906
RT120	<i>Batrachoseps nigriventris</i>	Black-bellied Slender Salamander	CAS-214856
RT110	<i>Bufo boreas halophilus</i>	Western Toad	CAS-208507
RT175	<i>Chrysemys picta</i>	Painted Turtle	MVZ:Herp:25071 2
RT176	<i>Chrysemys picta</i>	Painted Turtle	MVZ:Herp:23858 1
RT148	<i>Ensatina eschscholtzii</i>	Ensatina Salamander	CAS-252913
RT149	<i>Ensatina eschscholtzii</i>	Ensatina Salamander	CAS-252943
RT117	<i>Ensatina eschscholtzii croceator</i>	Ensatina Salamander	CAS-213213

RT140	<i>Ensatina eschscholtzii croceator</i>	Ensatina Salamander	CAS-235932
RT106	<i>Ensatina eschscholtzii eschscholtzii</i>	Ensatina Salamander	CAS-205796
RT119	<i>Ensatina eschscholtzii eschscholtzii</i>	Ensatina Salamander	CAS-214854
RT103	<i>Ensatina eschscholtzii xanthoptica</i>	Yellow-eyed Ensatina Salamander	CAS-203543
RT109	<i>Ensatina eschscholtzii xanthoptica</i>	Yellow-eyed Ensatina Salamander	CAS-207429
RT122	<i>Hyla regilla</i>	Pacific Tree Frog	CAS-214884
RT127	<i>Lithobates catesbeiana</i>	America Bullfrog	CAS-224776
RT151	<i>Lithobates catesbeiana</i>	America Bullfrog	CAS-253042
RT155	<i>Pseudacris cadaverina</i>	California Chorus Frog	CAS-255413
RT164	<i>Pseudacris cadaverina</i>	California Chorus Frog	MVZ:Herp:145381
RT165	<i>Pseudacris cadaverina</i>	California Chorus Frog	MVZ:Herp:145382
RT111	<i>Pseudacris sierra</i>	Sierran Chorus Frog	CAS-208510
RT147	<i>Pseudacris sierra</i>	Sierran Chorus Frog	CAS-252908
RT105	<i>Rana boylei</i>	Foothill Yellow-legged Frog	CAS-205752
RT107	<i>Rana boylei</i>	Foothill Yellow-legged Frog	CAS-205859
RT114	<i>Rana boylei</i>	Foothill Yellow-legged Frog	CAS-208976
RT115	<i>Rana boylei</i>	Foothill Yellow-legged Frog	CAS-209515
RT126	<i>Rana boylei</i>	Foothill Yellow-legged Frog	CAS-223945
RT128	<i>Rana boylei</i>	Foothill Yellow-legged Frog	CAS-225295
RT130	<i>Rana boylei</i>	Foothill Yellow-legged Frog	CAS-226107
RT131	<i>Rana boylei</i>	Foothill Yellow-legged Frog	CAS-226108
RT132	<i>Rana boylei</i>	Foothill Yellow-legged Frog	CAS-226109
RT133	<i>Rana boylei</i>	Foothill Yellow-legged Frog	CAS-226110
RT134	<i>Rana boylei</i>	Foothill Yellow-legged Frog	CAS-226111
RT137	<i>Rana boylei</i>	Foothill Yellow-legged Frog	CAS-233406
RT138	<i>Rana boylei</i>	Foothill Yellow-legged Frog	CAS-234641
RT139	<i>Rana boylei</i>	Foothill Yellow-legged Frog	CAS-234642
RT160	<i>Rana boylei</i>	Foothill Yellow-legged Frog	CAS-259672
RT100	<i>Rana draytonii</i>	California Red-legged Frog	CAS-200288
RT101	<i>Rana draytonii</i>	California Red-legged Frog	CAS-200289
RT102	<i>Rana draytonii</i>	California Red-legged Frog	CAS-200290
RT104	<i>Rana draytonii</i>	California Red-legged Frog	CAS-205717
RT116	<i>Rana draytonii</i>	California Red-legged Frog	CAS-211720
RT144	<i>Rana draytonii</i>	California Red-legged Frog	CAS-245052
RT156	<i>Rana draytonii</i>	California Red-legged Frog	CAS-256602
RT157	<i>Rana draytonii</i>	California Red-legged Frog	CAS-257329
RT158	<i>Rana draytonii</i>	California Red-legged Frog	CAS-257330

RT166	<i>Spea hammondi</i>	Western Spadefoot Toad	MVZ:Herp:23417 4
RT167	<i>Spea hammondi</i>	Western Spadefoot Toad	MVZ:Herp:23417 5
RT118	<i>Sternotherus odoratus</i>	Common Musk Turtle	CAS-214343
RT154	<i>Sternotherus odoratus</i>	Common Musk Turtle	CAS-255136
RT161	<i>Taricha torosa</i>	California Newt	CAS-259679
RT171	<i>Taricha torosa</i>	California Newt	MVZ:Herp:23624 2
RT172	<i>Taricha torosa</i>	California Newt	MVZ:Herp:21791 4
RT95	<i>Taricha torosa sierrae</i>	Sierra Newt	CAS-208714
RT96	<i>Taricha torosa sierrae</i>	Sierra Newt	CAS-212845
RT159	<i>Taricha torosa sierrae</i>	Sierra Newt	CAS-259608
RT135	<i>Trachemys scripta</i>	Pond Slider	CAS-227634
RT141	<i>Trachemys scripta</i>	Pond Slider	CAS-243752
RT177	<i>Trachemys scripta elegans</i>	Red-eared Slider	MVZ:Herp:26566 7
RT178	<i>Trachemys scripta elegans</i>	Red-eared Slider	MVZ:Herp:26566 9
RT142	<i>Xenopus laevis</i>	African Clawed Frog	CAS-244034
RT143	<i>Xenopus laevis</i>	African Clawed Frog	CAS-244035
RT153	<i>Xenopus laevis</i>	African Clawed Frog	CAS-253186

Table S2. *Anaxyrus californicus* tissue samples sequenced for design of a species-specific qPCR assay for detection from environmental DNA. CAS is California Academy of Sciences, MVZ is Museum of Vertebrate Zoology.

Sample name	Country	County	Drainage	Date collected	Latitude	Longitude	Source	GenBank Accession #
RT97	USA	San Diego	Kitchen Creek	8/11/1990	32.7682	-116.45098	CAS 175636	OP701659
RT98	USA	San Diego	Pine Valley Creek	5/8/1991	32.84328	-116.52676	CAS 178994	OP701679
RT99	USA	San Diego	San Luis Rey River	5/7/1991	33.21301	-116.56608	CAS 179041	OP701680
RT162	Mexico	NA	Santo Domingo River	5/20/1977	30.75531	-115.95714	MVZ:Herp:145230	OP718187
RT163	Mexico	NA	Santo Domingo River	5/20/1977	30.75531	-115.95714	MVZ:Herp:150013	OP718192
RL1	USA	Los Angeles	Castaic Creek	Unknown	Unknown	Unknown	Private collection	OP701646
RL10	USA	San Diego	Sweetwater River	3/5/2001	Unknown	Unknown	Private collection	OP701647
RL11	USA	San Diego	Sweetwater River	3/5/2001	Unknown	Unknown	Private collection	OP701660
RL12	USA	San Diego	Sweetwater River	3/5/2001	Unknown	Unknown	Private collection	OP701648
RL13	USA	San Diego	Sweetwater River	3/5/2001	Unknown	Unknown	Private collection	OP701649
RL14	USA	San Diego	Sweetwater River	3/5/2001	Unknown	Unknown	Private collection	OP701650
RL15	USA	San Diego	Sweetwater River	3/5/2001	Unknown	Unknown	Private collection	OP701651
RL19	USA	San Diego	San Luis Rey River	4/14/2001	33.33204	-117.15096	Private collection	OP701661
RL20	USA	San Diego	San Luis Rey River	4/14/2001	33.33202	-117.15083	Private collection	OP701652
RL21	USA	San Diego	Santa Ysabel Creek	4/17/2001	33.11761	-116.89404	Private collection	OP701671
RL22	USA	San Diego	Santa Ysabel Creek	4/17/2001	33.11945	-116.89501	Private collection	OP701672

RL23	USA	San Diego	Santa Ysabel Creek	4/17/2001	33.11905	- 116.89494	Private collection	OP701673
RL24	USA	San Diego	Santa Ysabel Creek	4/17/2001	33.09236	- 116.89591	Private collection	OP701674
RL25	USA	San Diego	Santa Ysabel Creek	4/17/2001	33.11774	- 116.89404	Private collection	OP718188
RL26	USA	San Diego	Santa Ysabel Creek	4/17/2001	33.09216	- 116.89604	Private collection	OP701662
RL27	USA	Orange	Gabino Creek	4/19/2001	33.47949	- 117.53974	Private collection	OP718177
RL28	USA	Orange	Gabino Creek	4/19/2001	33.47965	-117.5397	Private collection	OP718178
RL29	USA	San Diego	DeLuz Creek	4/21/2001	33.4153	- 117.31903	Private collection	OP701653
RL3	USA	San Diego	San Mateo Creek	12/7/2000	33.4683	- 117.47966	Private collection	OP718179
RL31	USA	San Bernardino	Mojave River	5/9/2001	34.34094	-117.2453	Private collection	OP718180
RL32	USA	Riverside	Santa Rosa Plateau	5/9/2001	33.5443	-117.2782	Private collection	OP718189
RL35	USA	Monterrey	San Antonio Creek	5/21/2001	35.99911	- 121.24664	Private collection	OP701629
RL36	USA	Monterrey	San Antonio Creek	5/21/2001	36.00035	- 121.24805	Private collection	OP701630
RL37	USA	Monterrey	San Antonio Creek	5/21/2001	35.99803	- 121.24688	Private collection	OP701631
RL38	USA	Monterrey	San Antonio Creek	5/21/2001	35.91269	- 121.13388	Private collection	OP701632
RL39	USA	Monterrey	San Antonio Creek	5/21/2001	35.91269	- 121.13388	Private collection	OP701633
RL4	USA	San Diego	San Mateo Creek	12/11/2000	33.46888	- 117.47929	Private collection	OP701654
RL41	USA	Santa Barbara	Sata Ynez River	5/22/2001	34.51316	- 119.59407	Private collection	OP701634

RL42	USA	Santa Barbara	Mono Creek	5/22/2001	34.53566	- 119.63026	Private collection	OP701635
RL46	USA	Santa Barbara	Indian Creek	5/22/2001	34.53655	- 119.63342	Private collection	OP701636
RL49	USA	Ventura	Piru Creek	5/23/2001	34.69102	- 118.85123	Private collection	OP718182
RL5	USA	San Diego	San Mateo Creek	4/20/2001	33.46888	- 117.47929	Private collection	OP701655
RL54	Mexico	NA	La Mision	6/8/2001	32.10069	- 116.80988	Private collection	OP718184
RL56	Mexico	NA	La Mision	6/8/2001	32.10066	-116.8099	Private collection	OP718185
RL57	Mexico	NA	La Mision	6/8/2001	32.10066	-116.8099	Private collection	OP701663
RL6	USA	San Diego	Santa Ysabel Creek	1/10/2000	33.08513	- 116.98833	Private collection	OP701664
RL62	USA	Riverside	Bautista Creek	6/22/2001	33.64867	- 116.81616	Private collection	OP701656
RL64	USA	Los Angeles	Littlerock Creek	6/30/2001	34.46017	-118.0194	Private collection	OP701637
RL65	USA	Los Angeles	Littlerock Creek	6/30/2001	34.45898	- 118.01861	Private collection	OP701638
RL66	USA	Los Angeles	Tujunga Creek	6/30/2001	34.31051	- 118.09439	Private collection	OP701639
RL67	Mexico	NA	Guadalupe	3/22/2002	32.11827	- 116.47655	Private collection	OP701665
RL68	Mexico	NA	Guadalupe	3/22/2002	32.11803	- 116.47668	Private collection	OP701666
RL69	Mexico	NA	Las Palmas	3/23/2002	32.3303	- 116.51396	Private collection	OP718190
RL7	USA	San Diego	Santa Ysabel Creek	1/10/2000	33.08525	- 116.98805	Private collection	OP701667
RL70	Mexico	NA	Maecadero	4/5/2002	31.63665	-116.4657	Private collection	OP718171
RL71	Mexico	NA	San Vicete	5/17/2002	31.33415	-116.4657	Private collection	OP718172
RL72	Mexico	NA	San Vicete	5/17/2002	31.33415	-116.4657	Private collection	OP701675

RL73	Mexico	NA	San Vicete	5/17/2002	31.33415	-116.4657	Private collection	OP701676
RL75	Mexico	NA	San Rafael	5/25/2002	31.09876	-116.02539	Private collection	OP718173
RL76	Mexico	NA	San Rafael	5/25/2002	31.09876	-116.02539	Private collection	OP701677
RL8	USA	San Diego	Cottonwood Creek	3/2/2001	32.57298	-116.75718	Private collection	OP701668
RL87	USA	San Diego	San Diego River	Unknown	Unknown	Unknown	Private collection	OP701669
RL88	USA	San Diego	San Diego River	Unknown	Unknown	Unknown	Private collection	OP701670
RL89	Mexico	NA	Santo Tomas	5/26/2004	31.54913	-116.22174	Private collection	OP718174
RL9	USA	San Diego	Sweetwater River	3/5/2001	Unknown	Unknown	Private collection	OP701657
RL90	Mexico	NA	Santo Tomas	5/26/2004	31.54913	-116.22174	Private collection	OP718175
RL91	Mexico	NA	Santo Tomas	5/26/2004	31.54913	-116.22174	Private collection	OP718176
RL93	Mexico	NA	Rio San Telmo	4/14/2005	31.9775	-115.74525	Private collection	OP718186
RL94	Mexico	NA	Rio Santa Maria	4/15/2005	31.51107	-115.80393	Private collection	OP718191
RT185 (RL2)	USA	San Diego	Tecate Creek	5/18/1999	33.51444	-117.57166	Private collection	OP701658
RT186 (RL18)	USA	San Diego	Cottonwood Creek	3/29/2001	32.57368	-116.7565	Private collection	OP701640
RT187 (RL34)	USA	San Bernardino	Mojave River	5/17/2001	34.32394	-117.40901	Private collection	OP718181
RT190 (RL40)	USA	Monterrey	San Antonio Creek	5/21/2001	35.91269	-121.13388	Private collection	OP701641
RT191 (RL43)	USA	Santa Barbara	Mono Creek	5/22/2001	34.53665	-119.63215	Private collection	OP701642
RT192 (RL45)	USA	Santa Barbara	Mono Creek	5/22/2001	34.53665	-119.63215	Private collection	OP701643

RT193 (RL47)	USA	Santa Barbara	Indian Creek	5/22/2001	34.54031	- 119.63641	Private collection	OP701644
RT194 (RL50)	USA	Ventura	Piru Creek	5/23/2001	34.69199	- 188.85308	Private collection	OP718183
RT196 (RL63)	USA	Santa Barbara	Sisquoc River	6/29/2001	34.827	- 119.99904	Private collection	OP701645
RT198 (RL106)	USA	San Diego	Pine Valley Creek	5/5/2008	32.85335	- 116.52256	Private collection	OP701678

Table S3. *Spea hammondi* tissue samples sequenced for design of a species-specific qPCR assay for detection from environmental DNA. CAS is California Academy of Sciences, MVZ is Museum of Vertebrate Zoology, and HBS is private collection of H. Bradley Shaffer.

Sample name	Country	County	Date collected	Latitude	Longitude	Source	GenBank Accession #s	
							cytb	16S
RT125	USA	Tulare	5/19/2001	35.89375	-118.9088	CAS-223542	OP265969	MT137273
RT129	USA	Stanislaus	5/20/2001	37.45318	-121.27483	CAS-225300	OP265970	OP131912
RT166	USA	San Joaquin	11/17/1996	37.60078	-121.53529	MVZ:Herp:234174	OP265971	OP131913
RT167	USA	San Joaquin	1/15/1997	37.67221	-121.45633	MVZ:Herp:234175	OP265972	MT135561
RT199	USA	Glenn	4/7/1990	39.79739	-122.55138	HBS-9764	OP227147	not Sequenced
RT200	USA	Glenn	4/7/1990	39.79739	-122.55138	HBS-9765	OP227158	OP115847
RT201	USA	Yolo	5/16/1998	38.91153	-122.02286	HBS-22298	OP227169	not Sequenced
RT202	USA	Yolo	5/16/1998	38.91153	-122.02286	HBS-22299	OP227179	not Sequenced
RT203	USA	San Luis Obispo	4/28/2017	35.1569	-119.96882	HBS-132798	OP227180	OP115848
RT204	USA	San Luis Obispo	4/28/2017	35.1569	-119.96882	HBS-132799	OP227181	OP115849
RT205	USA	San Luis Obispo	4/28/2017	35.1569	-119.96882	HBS-132800	OP227182	OP115850
RT206	USA	San Luis Obispo	4/28/2017	35.1569	-119.96882	HBS-132801	OP227183	OP115851
RT207	USA	San Luis Obispo	4/28/2017	35.1569	-119.96882	HBS-132802	OP227184	OP115852
RT208	USA	San Luis Obispo	4/14/2017	35.7821	-120.33481	HBS-133582	OP227148	OP115853
RT209	USA	San Luis Obispo	4/14/2017	35.7821	-120.33481	HBS-133583	OP227149	OP115854
RT210	USA	San Luis Obispo	4/14/2017	35.7821	-120.33481	HBS-133584	OP227150	OP115855

RT211	USA	San Luis Obispo	4/14/2017	35.7821	-120.33481	HBS-133585	OP227151	OP115856
RT213	USA	Santa Barbara	5/3/2018	34.768143	-120.393662	HBS-134538	OP227152	OP115857
RT214	USA	Santa Barbara	5/3/2018	34.768143	-120.393662	HBS-134539	OP227153	OP115858
RT215	USA	Santa Barbara	5/3/2018	34.768143	-120.393662	HBS-134540	OP227154	OP115859
RT216	USA	Santa Barbara	5/3/2018	34.768143	-120.393662	HBS-134541	OP227155	OP115860
RT217	USA	Santa Barbara	5/3/2018	34.768143	-120.393662	HBS-134542	OP227156	OP115861
RT218	USA	Kern	3/18/2017	35.03829	-118.71936	HBS-131797	OP227157	OP115862
RT219	USA	Kern	3/18/2017	35.03829	-118.71936	HBS-131799	OP227159	OP115863
RT220	USA	Orange	2/2/2017	33.5635	-117.80741	HBS-132561	OP227160	OP115732
RT221	USA	Orange	2/2/2017	33.5635	-117.80741	HBS-132563	OP227161	OP115733
RT222	USA	Orange	3/21/2017	33.60307	-117.55997	HBS-132145	OP227162	OP115734
RT223	USA	Orange	3/21/2017	33.60307	-117.55997	HBS-132146	OP227163	OP115735
RT224	USA	Ventura	3/5/2017	34.32077	-118.86572	HBS-131710	OP227164	OP115736
RT225	USA	Ventura	3/5/2017	34.32077	-118.86572	HBS-131712	OP227165	OP115737
RT226	USA	San Diego	4/3/2017	32.7125	-116.916	HBS-131669	OP227166	OP115738
RT227	USA	San Diego	4/3/2017	32.7125	-116.916	HBS-131670	OP227167	OP115739
RT228	USA	Monterey	4/10/2017	36.50333	-121.34906	HBS-133222	OP227168	OP115864
RT229	USA	Monterey	4/10/2017	36.50333	-121.34906	HBS-133223	OP227170	OP115865
RT230	USA	Monterey	4/14/2017	35.83887	-120.38189	HBS-133556	OP227171	OP115866
RT231	USA	Monterey	4/14/2017	35.83887	-120.38189	HBS-133557	OP227172	OP115867
RT232	USA	Monterey	4/14/2017	35.83887	-120.38189	HBS-133558	OP227173	OP115868
RT233	USA	Monterey	4/14/2017	35.83887	-120.38189	HBS-133568	OP227174	OP115869
RT234	USA	Monterey	4/14/2017	35.83887	-120.38189	HBS-133569	OP227175	OP115870
RT235	USA	Monterey	4/14/2017	35.83887	-120.38189	HBS-133570	OP227176	OP115871
RT236	USA	Monterey	4/14/2017	35.83887	-120.38189	HBS-133571	OP227177	OP115872
RT237	USA	Monterey	4/14/2017	35.83887	-120.38189	HBS-133572	OP227178	OP115873

Table S4. Non-target species tissue samples sequenced for design of species-specific qPCR assays for detection of *Anaxyrus californicus* and *Spea hammondi* from environmental DNA.

Sample name	Species	Common name	State	County	Source	GenBank Accession #s	
						Cytb	16s
RT100	<i>Rana draytonii</i>	California Red-legged Frog	CA	Contra Costa	CAS-200288	OP391557	MT137263
RT102	<i>Rana draytonii</i>	California Red-legged Frog	CA	Contra Costa	CAS-200290	OP391558	MT137264
RT103	<i>Ensatina eschscholtzii xanthoptica</i>	Yellow-eyed Ensatina Salamander	CA	Santa Cruz	CAS-203543	OP391538	not sequenced
RT105	<i>Rana boylei</i>	Foothill Yellow-legged Frog	CA	Santa Clara	CAS-205752	OP391559	MT137265
RT106	<i>Ensatina eschscholtzii eschscholtzii</i>	Ensatina Salamander	CA	Monterey	CAS-205796	OP391540	MT137266
RT108	<i>Anaxyrus boreas halophilus</i>	Western Toad	CA	Monterey	CAS-206465	OP391560	MT137267
RT109	<i>Ensatina eschscholtzii xanthoptica</i>	Yellow-eyed Ensatina Salamander	CA	San Mateo	CAS-207429	OP391541	MT137268
RT111	<i>Pseudacris sierra</i>	Sierran Chorus Frog	CA	San Luis Obispo	CAS-208510	not sequenced	MT137269
RT112	<i>Aneides lugubris</i>	Arboreal Salamander	CA	Santa Cruz	CAS-208660	OP391542	MT137270
RT118	<i>Sternotherus odoratus</i>	Common Musk Turtle	FL	Taylor	CAS-214343	not sequenced	MT137271

RT120	<i>Batrachoseps nigriventris</i>	Black-bellied Slender Salamander	CA	San Luis Obispo	CAS-214856	OP391543	MT137272
RT121	<i>Aneides lugubris</i>	Arboreal Salamander	CA	Monterey	CAS-214883	OP391544	not sequenced
RT123	<i>Ambystoma californiense</i>	California Tiger Salamander	CA	Monterey	CAS-214885	OP391551	not sequenced
RT127	<i>Lithobates catesbeiana</i>	America Bullfrog	CA	Fresno	CAS-224776	OP391561	MT137274
RT131	<i>Rana boylei</i>	Foothill Yellow-legged Frog	CA	Santa Clara	CAS-226108	OP391562	MT137275
RT135	<i>Trachemys scripta</i>	Pond Slider	GA	Shasta	CAS-227634	not sequenced	MT137276
RT142	<i>Xenopus laevis</i>	African Clawed Frog	CA	San Francisco	CAS-244034	OP391555	MT137278
RT145	<i>Batrachoseps luciae</i>	Santa Lucia Mountains Slender Salamander	CA	Monterey	CAS-252904	OP391545	MT137279
RT146	<i>Batrachoseps luciae</i>	Santa Lucia Mountains Slender Salamander	CA	Monterey	CAS-252906	OP391546	not sequenced
RT147	<i>Pseudacris sierra</i>	Sierran Chorus Frog	CA	Monterey	CAS-252908	OP391563	not sequenced
RT148	<i>Ensatina eschscholtzii</i>	Ensatina Salamander	CA	Monterey	CAS-252913	OP391547	not sequenced
RT150	<i>Anaxyrus boreas halophilus</i>	Western Toad	CA	Kern	CAS-253026	OP391564	MT137280

RT151	<i>Lithobates catesbeiana</i>	America Bullfrog	CA	Kern	CAS-253042	OP391565	MT137281
RT152	<i>Taricha torosa</i>	California Newt	GA	Kern	CAS-253044	OP391552	OP378122
RT153	<i>Xenopus laevis</i>	African Clawed Frog	CA	San Francisco	CAS-253186	OP391556	MT137282
RT154	<i>Sternotherus odoratus</i>	Common Musk Turtle	GA	Macon	CAS-255136	not sequenced	MT137283
RT155	<i>Pseudacris cadaverina</i>	California Chorus Frog	CA	Los Angeles	CAS-255413	OP391566	MT137284
RT159	<i>Taricha torosa sierrae</i>	Sierra Newt	GA	Madera	CAS-259608	OP391553	OP378123
RT160	<i>Rana boylei</i>	Foothill Yellow-legged Frog	CA	Amador	CAS-259672	OP391567	MT137285
RT165	<i>Pseudacris cadaverina</i>	California Chorus Frog	Mexico	Baja Norte	MVZ:Herp:145382	OP391568	MT135560
RT168	<i>Batrachoseps incognitus</i>	San Simeon Slender Salamander	CA	Monterey	MVZ:Herp:251931	OP391539	MT135562
RT171	<i>Taricha torosa</i>	California Newt	GA	San Luis Obispo	MVZ:Herp:236242	OP391554	OP378124
RT173	<i>Actinemys marmorata</i>	Western Pond Turtle	CA	Napa	MVZ:Herp:164994	not sequenced	MT135564
RT174	<i>Actinemys marmorata</i>	Western Pond Turtle	CA	Lake	MVZ:Herp:164995	not sequenced	MT135565
RT175	<i>Chrysemys picta</i>	Painted Turtle	NM	Sierra	MVZ:Herp:250712	not sequenced	MT135566
RT176	<i>Chrysemys picta</i>	Painted Turtle	WA	Spokane	MVZ:Herp:238581	not sequenced	MT135567
RT177	<i>Trachemys scripta elegans</i>	Red-eared Slider	NM	Sierra	MVZ:Herp:265667	not sequenced	MT135568

RT238	<i>Pseudacris hypochondriaca</i>	Baja California Chorus Frog	CA	Riverside	CAS-200610	not sequenced	OP377739
RT239	<i>Pseudacris hypochondriaca</i>	Baja California Chorus Frog	CA	Riverside	CAS-200616	not sequenced	OP377740
RT87	<i>Ambystoma californiense</i>	California Tiger Salamander	CA	Santa Clara	CAS-211685	OP391548	not sequenced
RT93	<i>Ambystoma californiense</i>	California Tiger Salamander	CA	Contra Costa	CAS-238956	OP391549	not sequenced
RT95	<i>Taricha torosa sierrae</i>	Sierra Newt	CA	Fresno	CAS-208714	OP391550	OP378125

Table S5. Sequences obtained from GenBank used in design of species-specific qPCR assays for detection of *Anaxyrus californicus* and *Spea hammondi* from environmental DNA.

Species Name	Common name	Gene	GenBank Accession #
<i>Actinemys marmorata</i>	Western Pond Turtle	cytb	EU787059
<i>Actinemys marmorata</i>	Western Pond Turtle	cytb	EU787064
<i>Batrachoseps gavilanensis</i>	Gabilan Mountains Slender Salamander	cytb	JQ250323
<i>Batrachoseps gavilanensis</i>	Gabilan Mountains Slender Salamander	cytb	JQ250324
<i>Batrachoseps incognitus</i>	San Simeon Slender Salamander	cytb	JQ250328
<i>Chrysemys picta</i>	Painted Turtle	cytb	AF069423
<i>Chrysemys picta</i>	Painted Turtle	cytb	KJ536200
<i>Pseudacris hypochondriaca</i>	Baja California Chorus Frog	cytb	KJ536199
<i>Pseudacris hypochondriaca</i>	Baja California Chorus Frog	cytb	KJ536200
<i>Pseudacris sierra</i>	Sierran Chorus Frog	cytb	KJ536202
<i>Spea hammondi</i>	Western Spadefoot	cytb	AY236788
<i>Sternotherus odoratus</i>	Common Musk Turtle	cytb	MG460002
<i>Sternotherus odoratus</i>	Common Musk Turtle	cytb	MG460122
<i>Trachemys scripta elegans</i>	Red-eared Slider	cytb	EU787024.1
<i>Trachemys scripta elegans</i>	Red-eared Slider	cytb	FJ770617.1
<i>Ambystoma californiense</i>	California Tiger Salamander	16S	AY659995.1
<i>Ambystoma tigrinum</i>	Tiger Salamander	16S	AY659992.1
<i>Ambystoma tigrinum</i>	Tiger Salamander	16S	DQ283407.1
<i>Anaxyrus boreas</i>	Western Toad	16S	AY680242.1
<i>Anaxyrus boreas</i>	Western Toad	16S	AY680244.1
<i>Anaxyrus boreas</i>	Western Toad	16S	DQ158436.1
<i>Anaxyrus boreas</i>	Western Toad	16S	DQ283180.1
<i>Anaxyrus boreas</i>	Western Toad	16S	KF665480.1
<i>Anaxyrus boreas</i>	Western Toad	16S	U52752.1
<i>Aneides lugubris</i>	Arboreal Salamander	16S	DQ105346.1
<i>Aneides lugubris</i>	Arboreal Salamander	16S	DQ105347.1
<i>Aneides lugubris</i>	Arboreal Salamander	16S	DQ105348.1
<i>Aneides lugubris</i>	Arboreal Salamander	16S	DQ105349.1

<i>Aneides lugubris</i>	Arboreal Salamander	16S	DQ105350.1
<i>Batrachoseps attenuatus</i>	California Slender Salamander	16S	EU011483.1
<i>Batrachoseps bramei</i>	Fairview Slender Salamander	16S	JQ035722.1
<i>Batrachoseps diabolicus</i>	Hell Hollow Slender Salamander	16S	EU011256.1
<i>Batrachoseps gabrieli</i>	San Gabriel Slender Salamander	16S	AF199234.1
<i>Batrachoseps gavilanensis</i>	Gabilan Mountains Slender Salamander	16S	EU011260.1
<i>Batrachoseps gregarius</i>	Gregarious Slender Salamander	16S	JQ035727.1
<i>Batrachoseps major</i>	Garden Slender Salamander	16S	DQ642047.1
<i>Batrachoseps nigriventris</i>	Black-bellied Slender Salamander	16S	JQ035712.1
<i>Batrachoseps nigriventris</i>	Black-bellied Slender Salamander	16S	KT368149.1
<i>Batrachoseps simatus</i>	Kern Canyon Slender Salamander	16S	JQ035713.1
<i>Batrachoseps stebbinsi</i>	Tehachapi Slender Salamander	16S	JQ035728.1
<i>Chrysemys picta</i>	Painted Turtle	16S	AF069423.1
<i>Chrysemys picta</i>	Painted Turtle	16S	KF874616.1
<i>Chrysemys picta</i>	Painted Turtle	16S	MN135379.1
<i>Lithobates catesbeiana</i>	America Bullfrog	16S	DQ283257.1
<i>Lithobates catesbeiana</i>	America Bullfrog	16S	KX269208.1
<i>Lithobates catesbeiana</i>	America Bullfrog	16S	KY677817.1
<i>Lithobates catesbeiana</i>	America Bullfrog	16S	M57527.1
<i>Lithobates catesbeiana</i>	America Bullfrog	16S	X12841.1
<i>Pseudacris cadaverina</i>	America Bullfrog	16S	AY291114.1
<i>Pseudacris cadaverina</i>	America Bullfrog	16S	AY843734.1
<i>Pseudacris cadaverina</i>	America Bullfrog	16S	EF472006.1
<i>Pseudacris hypochondriaca</i>	Baja California Chorus Frog	16S	AY291111.1
<i>Pseudacris hypochondriaca</i>	Baja California Chorus Frog	16S	AY843737.1
<i>Pseudacris hypochondriaca</i>	Baja California Chorus Frog	16S	DQ679375.1
<i>Pseudacris hypochondriaca</i>	Baja California Chorus Frog	16S	EF472005.1
<i>Pseudacris hypochondriaca</i>	Baja California Chorus Frog	16S	KY677793.1
<i>Pseudacris hypochondriaca</i>	Baja California Chorus Frog	16S	KY677803.1
<i>Pseudacris regilla</i>	Pacific Chorus Frog	16S	KY677809.1
<i>Pseudacris sierra</i>	Sierran Chorus Frog	16S	AY291112.1
<i>Rana boylei</i>	Foothill Yellow-legged Frog	16S	AY779192.1

<i>Rana boylei</i>	Foothill Yellow-legged Frog	16S	DQ347335.1
<i>Rana draytonii</i>	California Red-legged Frog	16S	DQ283189.1
<i>Rana draytonii</i>	California Red-legged Frog	16S	KP013110.1
<i>Sternotherus odoratus</i>	Common Musk Turtle	16S	KF301330.1
<i>Sternotherus odoratus</i>	Common Musk Turtle	16S	MH308386.1
<i>Sternotherus odoratus</i>	Common Musk Turtle	16S	MH308387.1
<i>Sternotherus odoratus</i>	Common Musk Turtle	16S	MH308388.1
<i>Sternotherus odoratus</i>	Common Musk Turtle	16S	MN135409.1
<i>Trachemys scripta</i>	Pond Slider	16S	FJ392294.1
<i>Trachemys scripta</i>	Pond Slider	16S	HQ123497.1
<i>Trachemys scripta</i>	Pond Slider	16S	L28077.1
<i>Trachemys scripta</i>	Pond Slider	16S	MN135556.1
<i>Xenopus laevis</i>	African Clawed Frog	16S	MH115794.1

Table S6. Candidate primers tested with SYBR Green qPCR for detection of *Anaxyrus californicus* and *Spea hammondi* from environmental DNA. Primers indicated in bold were ultimately chosen for the final analysis.

Primer/probe name	Sequence 5'-3'	Target gene	Tm (°C)
ANCA1-F	GCTCAAATCGCAACTGGACTT	cytb	58.1
ANCA1-R	AATGAGGCTCCATTTGCATGT	cytb	58.1
ANCA2-F	AGCTGATACATCCATAGCCTTTTCAT	cytb	59.4
ANCA2-R	GAATGAGGCTCCATTTGCATGT	cytb	59.9
ANCA3-F	TCACCAGCGAATATCTCCTCTTTAT	cytb	58.4
ANCA3-R	GGCTATGGATGTATCAGCTGTGTAA	cytb	58.3
SPHA1-F	CGGAAACGTACTAGTCCAATGAATC	cytb	59
SPHA1-R	TCCGGTTTCGTGTAGGAATAGAAG	cytb	59.5
SPHA2-F	CGGAAACGTACTAGTCCAATGAATC	cytb	59
SPHA2-R	GATCCGGTTTCGTGTAGGAATAGA	cytb	59.4
SPHA3-F	TCTTAACCAAGAGCCACAGCTCTA	16S	58.4
SPHA3-R	TGTCGATATGGACTCTTGAAATGG	16S	59
SPHA4-F	CCCATGGAGCTTTAAACTTAAACC	16S	58.3
SPHA4-R	CCCCAACCGAAAACATAAGC	16S	58

Table S7 Primers and probes used in the multiplex assay for detection of *Anaxyrus californicus* and *Spea hammondi* from environmental DNA. F and R refer to forward and reverse primers; P refers to the MGB probe.

Primer/probe name	Sequence 5'-3'	T _m (°C)	Optimal concentration (nM)
ANCA3-F	TCACCAGCGAATATCTCCTCTTTAT	58.4	600
ANCA3-R	GGCTATGGATGTATCAGCTGTGTAA	58.3	900
ANCA-P	6FAM-TCAAATCGCAACTGGAC-MGB-NFQ	69	250
SPHA2-F	CGGAAACGTACTAGTCCAATGAATC	59	300
SPHA2-R	GATCCGGTTTCGTGTAGGAATAGA	59.4	900
SPHA-P	NED-CGCTTCTTTACTTTTCACTTT-MGB-NFQ	68	250

Table S8. eDNA samples collected for field validation of our species-specific qPCR assay for *Anaxyrus californicus*.

Sample name	Site Name	Water Body	Sample collection date	Latitude	Longitude	<i>A. californicus</i> visually observed	<i>A. californicus</i> detected with qPCR
eDNA598	Upper San Mateo sample 1	San Mateo River	5/8/2019	33.46897	-117.47608	Y	Y
eDNA599	Upper San Mateo sample 2	San Mateo River	5/8/2019	33.41944	-117.54919	Y	Y
eDNA600	Upper San Mateo sample 3	San Mateo River	5/8/2019	33.41944	-117.54919	Y	Y
eDNA601	Christianitos sample 4	Christianitos Creek	5/8/2019	33.45113	-117.56509	Y	Y
eDNA602	Lower San Onofre sample 5	San Onofre River	5/8/2019	33.39270	-117.53036	Y	Y
eDNA604	Lower Margarita sample 7	Santa Margarita River	5/9/2019	33.28435	-117.37415	N	N
eDNA605	Middle Margarita sample 8	Santa Margarita River	5/9/2019	33.32903	-117.33542	Y	Y
eDNA606	Upper Margarita sample 9	Santa Margarita River	5/9/2019	33.36237	-117.32151	Y	Y
eDNA1694	Agua Blanca-A	Agua Blanca Creek	5/15/2021	34.54213	-118.76582	N	N
eDNA1695	Agua Blanca-B	Agua Blanca Creek	5/15/2021	34.54213	-118.76582	N	N
eDNA1696	Agua Blanca-C	Agua Blanca Creek	5/15/2021	34.54213	-118.76582	N	N
eDNA1697	Mono-A	Mono Creek	6/2/2021	34.54029	-119.62549	N	N
eDNA1698	Mono-B	Mono Creek	6/2/2021	34.54029	-119.62549	N	N
eDNA1699	Mono-C	Mono Creek	6/2/2021	34.54029	-119.62549	N	N

eDNA1700	Indian-A	Indian Creek	6/2/2021	34.54322	-119.64208	N	N
eDNA1701	Indian-B	Indian Creek	6/2/2021	34.54322	-119.64208	N	N
eDNA1702	Indian-C	Indian Creek	6/2/2021	34.54322	-119.64208	N	N
eDNA1703	Santa Ynez-A	Santa Ynez River	6/2/2021	34.51265	-119.59246	N	N
eDNA1704	Santa Ynez-B	Santa Ynez River	6/2/2021	34.51265	-119.59246	N	N
eDNA1705	Santa Ynez-C	Santa Ynez River	6/2/2021	34.51265	-119.59246	N	N
eDNA1706	Hardluck-A	Piru River	6/14/2021	34.68269	-118.84377	N	N
eDNA1707	Hardluck-B	Piru River	6/14/2021	34.68269	-118.84377	N	N
eDNA1708	Hardluck-C	Piru River	6/14/2021	34.68269	-118.84377	N	N
eDNA1709	Sespe-Beaver-A	Sespe Creek	6/14/2021	34.55422	-119.24001	N	N
eDNA1710	Sespe-Beaver-B	Sespe Creek	6/14/2021	34.55422	-119.24001	N	N
eDNA1711	Sespe-Beaver-C	Sespe Creek	6/14/2021	34.55422	-119.24001	N	N
eDNA1712	Sespe-lion 1-A	Sespe Creek	6/15/2021	34.56174	-119.16302	N	N
eDNA1713	Sespe-lion 1-B	Sespe Creek	6/15/2021	34.56174	-119.16302	N	N
eDNA1714	Sespe-lion 1-C	Sespe Creek	6/15/2021	34.56174	-119.16302	N	N
eDNA1633	Sespe-lion 2-A	Sespe Creek	6/15/2021	34.55958	-119.16114	N	N
eDNA1634	Sespe-lion 2-B	Sespe Creek	6/15/2021	34.55958	-119.16114	N	N
eDNA1635	Sespe-lion 2-C	Sespe Creek	6/15/2021	34.55958	-119.16114	N	N
eDNA1639	Lower Piru 1-A	Piru River	6/15/2021	34.52619	-118.75694	N	N
eDNA1640	Lower Piru 1-B	Piru River	6/15/2021	34.52619	-118.75694	N	N
eDNA1641	Lower Piru 1-C	Piru River	6/15/2021	34.52619	-118.75694	N	N
eDNA1642	Lower Piru 2-A	Piru River	6/16/2021	34.53548	-118.75904	N	N
eDNA1643	Lower Piru 2-B	Piru River	6/16/2021	34.53548	-118.75904	N	N
eDNA1645	Lower Piru 2-C	Piru River	6/16/2021	34.53548	-118.75904	N	N
eDNA1680	Hardluck02-A	Piru River	7/14/2021	34.68355	-118.84421	N	N
eDNA1681	Hardluck02-B	Piru River	7/14/2021	34.68355	-118.84421	N	N
eDNA1693	Hardluck02-C	Piru River	7/14/2021	34.68355	-118.84421	N	N

eDNA1716	8C-a	Santa Margarita River	5/10/2022	33.31084	-117.34658	Y	N
eDNA1717	8C-b	Santa Margarita River	5/10/2022	33.31084	-117.34658	Y	N
eDNA1718	8C-c	Santa Margarita River	5/10/2022	33.31084	-117.34658	Y	N
eDNA1719	9D-a	Santa Margarita River	5/10/2022	33.32325	-117.33677	Y	N
eDNA1720	9D-b	Santa Margarita River	5/10/2022	33.32325	-117.33677	Y	N
eDNA1721	9D-c	Santa Margarita River	5/10/2022	33.32325	-117.33677	Y	Y
eDNA1722	9E-a	Santa Margarita River	5/10/2022	33.32495	-117.33669	Y	N
eDNA1723	9E-b	Santa Margarita River	5/10/2022	33.32495	-117.33669	Y	Y
eDNA1724	9E-c	Santa Margarita River	5/10/2022	33.32495	-117.33669	Y	Y
eDNA1725	10B-a	Santa Margarita River	5/10/2022	33.33096	-117.33334	Y	Y

eDNA1726	10B-b	Santa Margarita River	5/10/2022	33.33096	-117.33334	Y	Y
eDNA1727	10B-c	Santa Margarita River	5/11/2022	33.33096	-117.33334	Y	Y
eDNA1728	10D-a	Santa Margarita River	5/10/2022	33.33809	-117.33151	Y	N
eDNA1729	10D-b	Santa Margarita River	5/10/2022	33.33809	-117.33151	Y	N
eDNA1730	10D-c	Santa Margarita River	5/10/2022	33.33809	-117.33151	Y	N
eDNA1732	11F-a	Santa Margarita River	5/11/2022	33.35205	-117.32756	Y	Y
eDNA1733	11F-b	Santa Margarita River	5/11/2022	33.35205	-117.32756	Y	Y
eDNA1734	11F-c	Santa Margarita River	5/11/2022	33.35205	-117.32756	Y	Y
eDNA1735	12A-a	Santa Margarita River	5/11/2022	33.35277	-117.32672	Y	N
eDNA1736	12A-b	Santa Margarita River	5/11/2022	33.35277	-117.32672	Y	Y

eDNA1737	12A-c	Santa Margarita River	5/11/2022	33.35277	-117.32672	Y	Y
eDNA1738	12C-a	Santa Margarita River	5/11/2022	33.35555	-117.32583	Y	N
eDNA1739	12C-b	Santa Margarita River	5/11/2022	33.35555	-117.32583	Y	N
eDNA1740	12C-c	Santa Margarita River	5/11/2022	33.35555	-117.32583	Y	N

Table S9. eDNA samples collected for field validation of our species-specific qPCR assay for *Spea hammondi*.

Sample name	Site name	Location	Sample collection date	Latitude	Longitude	<i>S. hammondi</i> visually observed	<i>S. hammondi</i> detected with qPCR
eDNA1091	FHL_LK_49_SITE1	Fort Hunter Ligget	3/29/2019	35.94575	-121.16048	Y	Y
eDNA1170	FHL_LK_49_SITE1	Fort Hunter Ligget	3/29/2019	35.94575	-121.16048	Y	Y
eDNA1175	FHL_LK_49_SITE1	Fort Hunter Ligget	3/29/2019	35.94575	-121.16048	Y	Y
eDNA1083	FHL_LK_49_SITE2	Fort Hunter Ligget	3/29/2019	35.94712	-121.15973	Y	Y
eDNA1171	FHL_LK_49_SITE2	Fort Hunter Ligget	3/29/2019	35.94712	-121.15973	Y	Y
eDNA1180	FHL_LK_49_SITE2	Fort Hunter Ligget	3/29/2019	35.94712	-121.15973	Y	Y
eDNA1136	FHL_LK_49_SITE3	Fort Hunter Ligget	3/29/2019	35.9481	-121.16034	Y	Y
eDNA1141	FHL_LK_49_SITE3	Fort Hunter Ligget	3/29/2019	35.9481	-121.16034	Y	Y
eDNA1146	FHL_LK_49_SITE3	Fort Hunter Ligget	3/29/2019	35.9481	-121.16034	Y	Y
eDNA936	FHL_LK_51_SITE1	Fort Hunter Ligget	3/29/2019	35.95024	-121.16005	N	N
eDNA947	FHL_LK_51_SITE1	Fort Hunter Ligget	3/29/2019	35.95024	-121.16005	N	N
eDNA958	FHL_LK_51_SITE1	Fort Hunter Ligget	3/29/2019	35.95024	-121.16005	N	N
eDNA937	FHL_LK_51_SITE2	Fort Hunter Ligget	3/29/2019	35.95135	-121.15971	N	N
eDNA948	FHL_LK_51_SITE2	Fort Hunter Ligget	3/29/2019	35.95135	-121.15971	N	N
eDNA959	FHL_LK_51_SITE2	Fort Hunter Ligget	3/29/2019	35.95135	-121.15971	N	N
eDNA998	FHL_LK_78_SITE1	Fort Hunter Ligget	4/7/2019	36.07509	-121.26655	N	Y
eDNA1094	FHL_LK_78_SITE1	Fort Hunter Ligget	4/7/2019	36.07509	-121.26655	N	Y
eDNA1103	FHL_LK_78_SITE1	Fort Hunter Ligget	4/7/2019	36.07509	-121.26655	N	Y
eDNA972	FHL_LK_78_SITE2	Fort Hunter Ligget	4/7/2019	36.07488	-121.265605	N	Y
eDNA999	FHL_LK_78_SITE2	Fort Hunter Ligget	4/7/2019	36.07488	-121.265605	N	Y
eDNA1095	FHL_LK_78_SITE2	Fort Hunter Ligget	4/7/2019	36.07488	-121.265605	N	Y
eDNA1116	FHL_LK_78_SITE3	Fort Hunter Ligget	4/7/2019	36.07482	-121.26525	N	Y
eDNA1104	FHL_LK_78_SITE3	Fort Hunter Ligget	4/7/2019	36.07482	-121.26525	N	N
eDNA1657	Homestead-A	MCAS Miramar	3/22/2021	32.88544	-117.0775	N	N
eDNA1658	Homestead-B	MCAS Miramar	3/22/2021	32.88544	-117.0775	N	N
eDNA1664	Homestead-C	MCAS Miramar	3/22/2021	32.88544	-117.0775	N	N
eDNA1671	RC01-A	Rancho Cebada	4/21/2021	34.69811	-120.36649	N	N
eDNA1672	RC01-B	Rancho Cebada	4/21/2021	34.69811	-120.36649	N	N

eDNA1673	RC01-C	Rancho Cebada	4/21/2021	34.69811	-120.36649	N	N
eDNA1659	RC02-A	Rancho Cebada	4/21/2021	34.70534	-120.37629	N	N
eDNA1667	RC02-B	Rancho Cebada	4/21/2021	34.70534	-120.37629	N	N
eDNA1683	RC02-C	Rancho Cebada	4/21/2021	34.70534	-120.37629	N	N
eDNA1674	RC03-A	Rancho Cebada	4/21/2021	34.70616	-120.367	N	N
eDNA1675	RC03-B	Rancho Cebada	4/21/2021	34.70616	-120.367	N	N
eDNA1676	RC03-C	Rancho Cebada	4/21/2021	34.70616	-120.367	N	N
eDNA1677	RC04-A	Rancho Cebada	4/21/2021	34.70665	-120.34728	Y	N
eDNA1678	RC04-B	Rancho Cebada	4/21/2021	34.70665	-120.34728	Y	N
eDNA1679	RC04-C	Rancho Cebada	4/21/2021	34.70665	-120.34728	Y	N
eDNA1660	TG_5014-A	MCBCP	3/23/2021	33.20552	-117.46005	Y	Y
eDNA1665	TG_5014-B	MCBCP	3/23/2021	33.20552	-117.46005	Y	N
eDNA1666	TG_5014-C	MCBCP	3/23/2021	33.20552	-117.46005	Y	Y
eDNA1689	TG_5040-A	MCBCP	3/23/2021	33.30495	-117.45932	Y	Y
eDNA1690	TG_5040-B	MCBCP	3/23/2021	33.30495	-117.45932	Y	Y
eDNA1691	TG_5040-C	MCBCP	3/23/2021	33.30495	-117.45932	Y	Y
eDNA1662	TG_75009-A	MCBCP	3/23/2021	33.3061	-117.46053	Y	Y
eDNA1669	TG_75009-B	MCBCP	3/23/2021	33.3061	-117.46053	Y	Y
eDNA1685	TG_75009-C	MCBCP	3/23/2021	33.3061	-117.46053	Y	Y
eDNA1686	TG_75016-A	MCBCP	3/23/2021	33.30502	-117.45992	Y	Y
eDNA1687	TG_75016-B	MCBCP	3/23/2021	33.30502	-117.45992	Y	Y
eDNA1688	TG_75016-C	MCBCP	3/23/2021	33.30502	-117.45992	Y	Y
eDNA1661	Willow01-A	Willow Ranch	4/21/2021	34.69582	-120.35518	Y	Y
eDNA1668	Willow01-B	Willow Ranch	4/21/2021	34.69582	-120.35518	Y	Y
eDNA1684	Willow01-C	Willow Ranch	4/21/2021	34.69582	-120.35518	Y	Y

Table S10. Candidate predictors considered for herpetofauna and <i>Bd</i> modeling.		
Environmental factor	Variable	Source
Climate	Annual mean temperature	WorldClim
	Mean diurnal range	WorldClim
	Isothermality	WorldClim
	Temperature seasonality	WorldClim
	Max temperature of warmest month	WorldClim
	Min temperature of coldest month	WorldClim
	Temperature annual range	WorldClim
	Mean temperature of wettest quarter	WorldClim
	Mean temperature of driest quarter	WorldClim
	Mean temperature of warmest quarter	WorldClim
	Mean temperature of coldest quarter	WorldClim
	Annual precipitation	WorldClim
	Precipitation of wettest month	WorldClim
	Precipitation of driest month	WorldClim
	Precipitation seasonality	WorldClim
	Precipitation of wettest quarter	WorldClim
	Precipitation of driest quarter	WorldClim
	Precipitation of warmest quarter	WorldClim
	Precipitation of coldest quarter	WorldClim
	Solar irradiance	WorldClim
	Minimum temperature	PRISM
	Total monthly precipitation	PRISM
	Maximum temperature	PRISM
Topography/ Landscape	Elevation	Calculated in ArcMap
	Watershed slope	Calculated in ArcMap
	Aspect	Calculated in ArcMap
	Latitude	Calculated in ArcMap
	Distance to flowline	Calculated in ArcMap
	Distance to road	Calculated in ArcMap
	Distance to waterbody	Calculated in ArcMap
Soil chemical properties	Watershed area	Calculated in ArcMap
	Soil nitrogen	POLARIS
	Soil organic carbon	POLARIS
Soil physical properties	Soil pH	POLARIS
	Clay content	POLARIS
	Sand content	POLARIS
	Silt content	POLARIS
Vegetation	Soil bulk density	POLARIS
	Normalized difference vegetation index	USGS National Map Service
Humans	Human footprint index	(Venter et al., 2016)
	Percent impervious surfaces	NLCD
Amphibian/Non- amphibian hosts	Amphibian richness	IUCN & CIESIN
	Bullfrog habitat suitability	Modeled
	Red-swamp Crayfish habitat suitability	Modeled

Table S11. GenBank sequences identified by Primer-BLAST with the potential to cross-amplify with the qPCR assay that we designed for *Spea hammondi*. None of the sequences belong to species that are sympatric with *Spea hammondi*.

Genbank accession #	Species	Description
EF508476.1	<i>Acanthopsoidea hapalias</i>	Asian freshwater fish species
HM536795.1	<i>Acrossocheilus monticola</i>	Asian freshwater fish species
KC696534.1	<i>Acrossocheilus yunnanensis</i>	Asian freshwater fish species
KF113879.1	<i>Acrossocheilus barbodon</i>	Asian freshwater fish species
KJ994678.1	<i>Acrossocheilus iridescens</i>	Asian freshwater fish species
FJ752340.1	<i>Ameerega smaragdina</i>	South American anuran species
JQ012377.1	<i>Anchoa parva</i>	Marine fish species
JQ012387.1	<i>Anchoa filifera</i>	Marine fish species
JQ012327.1	<i>Anchoviella cf.</i>	Marine fish species
AB279333.1	<i>Anguilla australis</i>	Australian freshwater eel species
MH800477.1	<i>Anguilla mossambica</i>	African freshwater eel species
GU932709.1	<i>Artibeus obscurus</i>	South American bat species
AF334087.1	<i>Barbus graellsii</i>	European freshwater fish species
AY887784.1	<i>Botia striata</i>	Asian freshwater fish species
KY065276.1	<i>Capoeta capoeta</i>	Asian freshwater fish species
AF187018.1	<i>Carollia brevicauda</i>	South American bat species
AF187020.1	<i>Carollia castanea</i>	South American bat species
KC631284.1	<i>Cirrhinus molitorella</i>	Asian freshwater fish species
AF475153.1	<i>Clarias gariepinus</i>	African freshwater fish species
EF422294.1	<i>Coilia mystus</i>	Marine fish species
AP009581.1	<i>Conocara murrayi</i>	Marine fish species
AY953001.1	<i>Coreius guichenoti</i>	Asian freshwater fish species
AP012148.1	<i>Crossocheilus latius</i>	Asian freshwater fish species
AP013321.1	<i>Crossocheilus langei</i>	Asian freshwater fish species
JX074225.1	<i>Crossocheilus oblongus</i>	Asian freshwater fish species
JX083158.1	<i>Crossocheilus diplochilus</i>	Asian freshwater fish species
DQ422087.1	<i>Galeorhinus galeus</i>	Marine fish species
MH500684.1	<i>Garra cambodgiensis</i>	Asian freshwater fish species
EU552578.1	<i>Gilchristella aestuaria</i>	African freshwater fish species
AF416891.1	<i>Glyptosternon maculatum</i>	Asian freshwater fish species
MT606734.1	<i>Gymnocranius frenatus</i>	Marine fish species
AP012012.1	<i>Hara jerdoni</i>	Asian freshwater fish species
HQ257283.1	<i>Hemibagrus filamentus</i>	Asian freshwater fish species
KJ573466.1	<i>Hemibagrus nemurus</i>	Asian freshwater fish species
KJ624624.1	<i>Hemibagrus wyckioides</i>	Asian freshwater fish species
AP011386.1	<i>Henicorhynchus lineatus</i>	Asian freshwater fish species
AP009582.1	<i>Herwigia krefftii</i>	Marine fish species
KJ158097.1	<i>Ilisha amazonica</i>	South American freshwater fish species

MG958199.1	<i>Ilisha melastoma</i>	Marine fish species
KY132250.1	<i>Kaloula conjuncta</i>	Asian anuran species
EF508526.1	<i>Kottelatlimia pristes</i>	Asian freshwater fish species
GQ853086.1	<i>Labeo fimbriatus</i>	Asian freshwater fish species
MH026027.1	<i>Labeo lankae</i>	Asian freshwater fish species
MH026035.1	<i>Labeo porcellus</i>	Asian freshwater fish species
KT698047.1	<i>Lamiopsis tephrodes</i>	Marine fish species
KT698048.1	<i>Lamiopsis temminckii</i>	Marine fish species
AB080154.1	<i>Lefua echigonia</i>	Asian freshwater fish species
EU036445.1	<i>Lepidorhombus whiffiagonis</i>	Marine fish species
FJ155494.1	<i>Lonchorhina aurita</i>	South American bat species
EU932868.1	<i>Macropodus hongkongensis</i>	Asian freshwater fish species
AB239569.1	<i>Mantella bernhardi</i>	African anuran species
AY157035.1	<i>Mesophylla macconnelli</i>	South American Bat species
HM142578.1	<i>Onychostoma lepturum</i>	Asian freshwater fish species
HQ235764.1	<i>Onychostoma rara</i>	Asian freshwater fish species
JX074246.1	<i>Onychostoma ovale</i>	Asian freshwater fish species
AP004431.1	<i>Ostichthys japonicus</i>	Marine fish species
MT850132.1	<i>Parabotia kiangsiensis</i>	Asian freshwater fish species
AP009619.1	<i>Pellona flavipinnis</i>	South American freshwater fish species
EU552554.1	<i>Pellona castelnaeana</i>	South American freshwater fish species
AF201604.1	<i>Petrocephalus simus</i>	African freshwater fish species
EU770156.1	<i>Petrocephalus odzalaensis</i>	African freshwater fish species
EU770183.1	<i>Petrocephalus christyi</i>	African freshwater fish species
EU770191.1	<i>Petrocephalus balayi</i>	African freshwater fish species
GU982923.1	<i>Petrocephalus stuhlmanni</i>	African freshwater fish species
KJ409658.1	<i>Petrocephalus wesselsi</i>	African freshwater fish species
KJ409663.1	<i>Petrocephalus catostoma</i>	African freshwater fish species
GQ244477.1	<i>Pipa pipa</i>	South American anuran species
MT157615.1	<i>Prolixicheilus longisulcus</i>	Asian freshwater fish species
AY912410.1	<i>Pseudobagrus pratti</i>	Asian freshwater fish species
EU241453.1	<i>Puntius denisonii</i>	Asian freshwater fish species
KF574537.1	<i>Rasbora daniconius</i>	Asian freshwater fish species
GQ406329.1	<i>Rectoris posehensis</i>	Asian freshwater fish species
KF410777.1	<i>Rhodeus aff.</i>	Asian freshwater fish species
AF472583.1	<i>Sardinella maderensis</i>	Marine fish species
FJ463052.1	<i>Scutiger boulengeri</i>	Asian anuran species
FJ945491.1	<i>Scutiger tuberculatus</i>	Asian anuran species
FJ945495.1	<i>Scutiger mammatus</i>	Asian anuran species
KC140561.1	<i>Scutiger ningshanensis</i>	Asian anuran species

EF191043.1	<i>Spea bombifrons</i>	North American anuran species, not sympatric with <i>A. californicus</i> or <i>S. hammondi</i>
AF435136.1	<i>Sturnira lilium</i>	South American bat species
AF435170.1	<i>Sturnira luisi</i>	South American bat species
AF435175.1	<i>Sturnira ludovici</i>	South American bat species
AF435177.1	<i>Sturnira magna</i>	South American bat species
AF435184.1	<i>Sturnira tildae</i>	South American bat species
AF435206.1	<i>Sturnira oporophilum</i>	South American bat species
AF435212.1	<i>Sturnira mordax</i>	South American bat species
AF435240.1	<i>Sturnira erythromos</i>	South American bat species
AF435246.1	<i>Sturnira bogotensis</i>	South American bat species
AF435252.1	<i>Sturnira aratathomasi</i>	South American bat species
KC753793.1	<i>Sturnira hondurensis</i>	South American bat species
KY366229.1	<i>Sturnira adrianae</i>	South American bat species
KP670368.1	<i>Tachysurus adiposalis</i>	Asian freshwater fish species
MN585245.1	<i>Tonatia saurophila</i>	South American bat species
AF435173.1	<i>Vampyressa brocki</i>	South American bat species
NC_044879.1	<i>Xenopus itombwensis</i>	African anuran species

Table S12: Mean decrease in accuracies of all eight predictors used for modeling the target species		
Species name	Predictors	Importance scores (Mean decrease accuracy)
California Red-legged Frog	Minimum temperature	28.9
	Normalized Difference Vegetation Index	19.1
	Total precipitation	14.6
	Distance to flowline	12.2
	Watershed slope	11.7
	Distance to road	11.0
	Watershed area	9.1
	Distance to waterbody	6.7
Arroyo Toad	Normalized Difference Vegetation Index	17.3
	Distance to flowline	10.1
	Distance to road	8.0
	Distance to waterbody	6.1
	Watershed area	5.1
	Total precipitation	4.6
	Minimum temperature	2.5
	Watershed slope	1.2
Western Pond Turtle	Distance to road	7.6
	Distance to flowline	7.3
	Minimum temperature	6.9
	Total precipitation	4.3
	Normalized Difference Vegetation Index	4.1
	Watershed slope	3.1
	Distance to waterbody	0.4
	Watershed area	0.1

List of Scientific/Technical Publications

Articles in peer-reviewed journals

Zhang, X.-U., J. Jin, X. Zeng, W. Yang, C. P. Hawkins, A. A. M. Neto, and G.-Y. Niu. 2022. The compensatory CO₂ fertilization and stomatal closure effects on runoff projection in the western United States. *Water Resources Research* 10.1029/2021WR030046.

Abdulwahab, U. A., E. Hammill, C. P. Hawkins. 2022. Choice of climate data affects the performance and interpretation of species distribution models. *Ecological Modelling* 471:p.110042.

Dye, A. W., P. Gao, J. B. Kim, T. Lei, K. L. Riley, and L. Yocom. 2023. High-resolution wildfire simulations reveal complexity of climate change impacts on projected burn probability for Southern California. *Fire Ecology* 19:1-19.

Abdulwahab, U. A., E. Hammill, T. W. Rodgers, J. R. Olson, and C. P. Hawkins. 2023. Modeling the distribution of the amphibian pathogen *Batrachochytrium dendrobatidis* to inform conservation management. *Landscape Ecology* (submitted, in revision).

Rodgers, T. W., R. E. Lovich, J. A. Walton, D. J. Prince, B. R. Gonzales, H. B. Shaffer, and K. E. Mock. 2022. Multiplex qPCR assays for detection of two imperiled anuran species, *Anaxyrus californicus* and *Spea hammondi*, from environmental DNA. *Freshwater Science* (submitted, in revision)

Additional planned submissions

Comparison of multiplex qPCR and multi-locus metabarcoding in detecting threatened and endangered amphibian and turtle species.

Assessing the habitat suitability of three freshwater species of concern under current and future climatic conditions in south-coastal California.

Incorporating disease risk and built infrastructure into conservation planning for threatened amphibians

Technical reports

None.

Conference or symposium proceedings

None.

Conference or symposium abstracts

Four presentations at the *Department of Defense's Strategic Environmental Research and Development Program (SERDP) and Environmental Security Technology Certification Program (ESTCP) Symposia* (2019, 2020, 2021, and 2022).

Abdulwahab, U.A., Hammill, E., Rogers, T., Olson, J.R., Hawkins, C.P., 2022. Modeling the distribution of the amphibian pathogen *Batrachochytrium dendrobatidis* to inform conservation management. Oral presentation, California Aquatic Bioassessment Workshop and California Society for Freshwater Science Meeting, Sacramento, CA.

Abdulwahab, U. A., E. Hammill, and C.P. Hawkins. 2021. Choice of climate data affects the performance and interpretation of species distribution models. *2021 Society for Freshwater Science Conference*. Virtual Meeting, USA, May 2021. Available at: <https://bit.ly/3J68joO>

Dye, A., P. Gao, K. Riley, J. Kim, and L. Yocom. Shifts in Southern California simulated burn probability by mid-century under 8 climate change scenarios. AFE 9th International Fire Ecology and Management Congress. Nov 30-Dec 3, 2021.

Abdulwahab, U.A. E. Hammill, and C.P. Hawkins. 2020. Sensitivity of two species distribution model approaches. *2020 Ecological Society of America Conference*. Virtual Meeting, USA, August 2020. Available at: <https://bit.ly/3Ee80Ez>

Kim, J.B., A.W. Dye, P. Gao, K.L. Riley, and L.L. Yocom. Using GCM-based future climate data to drive a fire model: how much bias is too much? AFE 9th International Fire Ecology and Management Congress. Nov 30-Dec 3, 2021.

Riley, K., C. Hay, J. Kim, P. Gao, A. Dye, L. Yocom, J. Rowe, and E. Kodra. 2022. Methods for handling uncertainty in climate change projections in fire simulation modeling. Fire & Climate conference, International Association of Wildland Fire.

Dye, A., P. Gao, K. Riley, J. Kim, L. Yocom. 2021. Shifts in Southern California simulated burn probability by mid-century under 8 climate change scenarios. 8th International Fire Ecology and Management Congress, Association for Fire Ecology.

Kim, J., A. Dye, P. Gao, K. Riley, L. Yocom. 2021. Using GCM-based future climate data to drive a fire model: how much bias is too much? 8th International Fire Ecology and Management Congress, Association for Fire Ecology.

Dye, A., P. Gao, K. Riley, J. Kim, L. Yocom. 2019. Examining Southern California wildfire using a stochastic large-fire simulation model. Association for Fire Ecology 8th International Fire Ecology and Management Congress, Tucson, AZ.

Textbooks or book chapters.

None

Other Supporting Materials

None