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Translating eDNA data into conservation action: Partnerships to support imperiled amphibians in coastal California wetlands

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Abstract

Environmental DNA (eDNA) detections of imperiled species have the potential to inform conservation action, but this requires the acceptance of new technologies by decision-makers. Here we describe how engaging stakeholders into a collaborative process led to the successful translation of new eDNA findings into conservation outcomes. We characterized the distribution of three federally listed pond-breeding amphibians across nearly 200 wetlands in coastal California using both traditional field surveys and eDNA sampling; the latter had greater detection rates overall. Regulatory agency staff gained trust in the rigor and effectiveness of eDNA data by joining traditional surveys and through the collaborative development of recommendations for the adoption of eDNA methods. Extensive outreach to the local community within the range of the highly endangered Santa Cruz long-toed salamander resulted in invitations to sample previously unsurveyed wetlands on private property and the detection of new breeding sites. Conservation organizations and resource management agencies were integrated into our core team from the start, and ultimately shaped wetland management actions, siting of new wetlands, and land acquisition priorities informed by the data generated. Thus, this project serves as a model for actionable eDNA science directly affecting conservation.

KEYWORDS

actionable science, amphibians, collaborative approach, conservation, environmental DNA, threatened species

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1 | INTRODUCTION

To be effective and sustainable, conservation of imperiled species must occur at a sufficiently broad geographic scale and in the socio-ecological context of the landscape (Wiens, 2009). This typically requires working across a mosaic of land ownership and jurisdictions, which presents a multitude of challenges. Accuracy and efficiency in data collection, analysis, and the dissemination of results are critical for building trust and informing decision-making across a diversity of stakeholders and rightsholders (Darling et al., 2020). To characterize species' distributions and implement conservation strategies for them at a landscape scale, it is thus essential to use efficient technical methods and collaborative approaches.

The detection and analysis of environmental DNA (eDNA)—genetic material shed by organisms and collected from substrates like soil, water, or air—is a powerful emerging tool in the conservation toolbox that can be used to characterize species distributions rapidly and with less field effort and habitat disturbance than traditional field surveys (Rees et al., 2014). However, eDNA detection success can vary greatly by species, life stage, and environmental conditions (Goldberg et al., 2016). Robust results rely on meticulous species-specific study design and validation (Thalinger et al., 2021) and the interpretation of those results into management actions is best accomplished through well-developed collaborative relationships and communication strategies (Stein et al., 2023). There has been a steep learning curve in the development and application of eDNA methods, and experiences early on that led to distrust by some in the management community about the use of eDNA tools (Darling et al., 2020). Rebuilding that trust relies on transparent demonstrations of validated eDNA detection methods, careful interpretation of results, and direct sharing of information.

Recently, there has been increasing recognition of the need to apply collaborative approaches explicitly designed to increase the use of scientific data for management. Identifying key decision-makers and determining what information will influence their imminent decisions, gathering sufficient evidence, and sharing it openly is critical for actionable science that affects environmental policy and management (Bisbal & Eaton, 2022; Fisher et al., 2020). Success can be defined as respectfully conducted, partner-relevant research that is accessible, understandable, and shared, and thereby can create opportunities for change (Cooke et al., 2020). Such translational ecology, linking ecological knowledge to decision-makers, is increasingly recognized as critical for achieving conservation outcomes (Enquist et al., 2017). In this project, we endeavored to validate and share a standardized, accessible method for eDNA detection of rare amphibians using collaborative approaches to increase the use of this powerful new tool for informing conservation decisions.

We focused on populations of imperiled amphibians breeding in coastal wetlands along the central California coast as a system where new technical and collaborative approaches could support improved landscape-scale conservation. Known and potential wetland breeding sites and adult terrestrial upland habitat for multiple federally listed amphibians occur across a matrix of private and public lands. A variety of governmental and nongovernmental organizations are involved with conservation measures to support them. Better characterization of the distribution of these species, including identification of previously unknown breeding habitats, is considered a fundamental data gap, which eDNA techniques are well-suited to address (Halstead et al., 2018; Kieran et al., 2020; Moss et al., 2022).

Here, we illustrate how the development and implementation of an integrated technical and collaborative approach enabled eDNA science to be translated into conservation action. The local goal of the work described here was to support imperiled amphibian conservation along the central California coast. The broader goal was to provide a model useful for a target audience of conservation scientists hoping to affect change with new data, and for regulatory agency staff and on-the-ground practitioners wanting to engage with eDNA experts. The process we employed could be replicated elsewhere: engaging end users from the start, conducting robust comparisons of traditional field methods to eDNA sampling to build trust, and sharing findings in outreach tailored to different end user needs, including recommendations on use of eDNA for regulatory agencies, guidance on wetland management for private landowners, and spatial habitat planning workshops for regional conservation organizations. Thus, we describe here the technical and collaborative approaches undertaken and the results they yielded, both to highlight their contributions to local amphibian conservation efforts, and to serve as a model for actionable eDNA science directly affecting conservation.

2 | METHODS

2.1 | Study species and habitats

Along the central California coast, there are three federally listed wetland-breeding amphibians that are of conservation concern: Santa Cruz long-toed salamanders (SCLTS; *Ambystoma macrodactylum croceum*), California

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tiger salamanders (CTS; *Ambystoma californiense*), and California red-legged frogs (CRLF; *Rana draytonii*). The Central California distinct population segment of CTS is state and federally listed as threatened, CRLF are federally listed as threatened, and SCLTS are state and federally listed as endangered. Of particular concern, SCLTS have been documented to breed in less than 30 wetlands, and very few known reliable breeding sites exist in the southern portion of their restricted range (USFWS, 2019). Many unsurveyed wetlands that are near known breeding sites and which have the potential to support SCLTS breeding are located on private property, where gaining permission to access for surveys presents a significant barrier for the detection of new breeding sites. Work on maintaining known SCLTS breeding sites and associated upland habitat is ongoing, where local agencies and conservation organizations in the area have established a strategic plan to sustain SCLTS populations through a landscape-scale conservation strategy (Camara et al., 2019; RCDSCC, 2019).

Both SCLTS and CTS inhabit terrestrial burrows for much of the year and migrate to ephemeral and permanent wetlands during winter rain events (Anderson & Graham, 1967; Loredo et al., 1996). Although little is known about movement distance for SCLTS, they have been seen to travel up to 1.1 km from breeding sites (D. Laabs and M. Allaback, unpublished data). For CTS, one study estimated that 95% of individuals remained within 1.9 km of their breeding ponds (Searcy & Shaffer, 2011). Similarly, CRLF inhabit wetlands, along with streams, but may remain at wetlands after breeding or disperse to aquatic or upland habitat; they can travel distances of more than 2.8 km (Bulger et al., 2003; Fellers & Kleeman, 2007), making them a highly mobile species relative to SCLTS and CTS. All three target species breed in wetlands with highly variable size, temperature, pH, and percent of emergent, submergent, floating, and perimeter vegetation. Due to this variability, it can be challenging to reliably detect these wetland amphibians.

2.2 | Technical approach

2.2.1 | eDNA sampling and traditional field surveys

We developed an adaptive eDNA sampling strategy that can be used across a variety of central California wetland types. During the spring of 2021 and 2022, we conducted eDNA sampling and traditional field surveys at seasonal, semi-permanent, and perennial wetlands across the Monterey Bay area in Santa Cruz and northern Monterey County, California, to detect the presence of SCLTS, CTS, and CRLF. Paired eDNA and field surveys were conducted at wetlands in 2021 ($n = 67$) and 2022 ($n = 59$) to compare detection rates between the two survey methods and to demonstrate the utility and comparative performance of eDNA analysis. In addition, eDNA-only surveys were conducted at 128 additional wetlands in 2022. We employed an adaptive site-level eDNA sampling strategy where the number of samples collected was dependent upon the estimated surface area of the site at the time of the survey (Table S1, Supporting Information), as species detections in wetlands are limited by the dispersion of eDNA (Goldberg et al., 2018). We recorded environmental and habitat variables that we hypothesized would influence the detection of eDNA from the target species (Table S2).

We collected eDNA samples using single-use Whirl-Pak® bags and new gloves for each sample. A single surveyor collected the eDNA sample by standing on the shore without entering the water, when possible, and dragging the bag on the surface to collect approximately 250–500 mL of water. However, if water access was difficult then the surveyor would slowly enter the water and collect the sample without splashing or disturbing sediments. Boots were thoroughly cleaned and decontaminated using 10% bleach for ≥5 min and rinsed with tap water between each site to reduce the probability of introducing exogenous DNA (as is common practice, e.g., Smith & Goldberg, 2020). We stored clean supplies separately from used field gear to prevent DNA contamination. If an abundant amount of floating vegetation or debris was present on the surface, the surveyor gently cleared the organic matter with a gloved hand before sample collection. After sample collection, samples were stored in an individual bleach-cleaned, reusable plastic container within coolers on ice packs. In addition, prior to eDNA sample collection, we created one distilled water control at each site and stored it in the cooler, along with the other eDNA samples, so that the control sample was exposed to every step of the sample collection process.

We concentrated the eDNA from water samples following the filtration protocol established by Goldberg et al. (2011). Water samples were filtered through 47 mm 5 μm polyethersulfone filters in single-use filter funnels (Sterlitech Corp.) using 1 L vacuum flasks and hand pumps. Each water sample was filtered until the 250 mL target volume was reached or until the filter clogged. We then folded each filter in half with bleach-cleaned forceps and stored it at room temperature in 99% molecular-grade ethanol (in 2021) or a coin envelope in an individual plastic bag containing indicator silica beads (in 2022). This change in preservatives was due to supply chain issues.

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For wetland sites with paired eDNA and traditional surveys, at least two surveyors conducted traditional surveys using visual surveys and dip nets and/or seines after the eDNA samples were collected. Dip netting occurred throughout all accessible areas of the site, particularly along perimeter areas with abundant emergent vegetation. Similarly, we hauled seines along with dip netting around the perimeter or shallow areas of the site but were often limited to sites with limited vegetation or debris which, if abundant, would make seining ineffective. To measure traditional survey effort, we recorded the number of person-hours spent dip netting (Figure S1) and the number of seine hauls performed. We conducted traditional surveys until the entire site was surveyed, with all accessible areas sampled at least once, or at least 10 individuals of each target species were caught. We note that only one traditional survey was conducted for comparative purposes, although up to three visits spaced at least 10 days apart in March, April, and May are required before reporting a negative finding according to current USFWS protocol for SCLTS and CTS (USFWS, 2003, 2012). However, our field survey efforts met or exceeded those required during a single visit (i.e., at least 50 dip net sweeps per wetland and seine sweeps covering more than 50% of accessible water) for SCLTS and CTS, as well as for CRLF (USFWS, 2005).

2.2.2 | Laboratory analysis

We extracted eDNA from filter samples using the Qiashredder/DNeasy Blood and Tissue Kit (Qiagen) protocol from Goldberg et al. (2011). One half of each filter was extracted, while the other half remained preserved. Each set of extractions included a negative extraction control to monitor for contamination. Extracted samples were then analyzed in triplicate for all three target species with validated species-specific assays that we developed (Table S4) that included internal positive controls. Furthermore, we developed a competitive qPCR tiger salamander assay that can distinguish between the mitochondrial DNA of native CTS and non-native barred tiger salamanders (BTS, *Ambystoma mavortium*) and excludes SCLTS (Table S4). We designed gBlocks Gene Fragments standards (Integrated DNA Technologies) for each of the four species as positive controls and to monitor the efficiency of the qPCR reactions (Text C in Data S1). We considered a qPCR reaction positive if the curve indicated an exponential signal increase at any point and a sample positive if it tested positive in all three reactions or on at least one of the reactions on each of two triplicate runs (Figure S2). If the Cq of the internal positive control was ≥3.0 above those of the standard curve and negative controls, then we considered the sample inhibited (inhibitors interfere with the amplification of target DNA). Inhibited samples were cleaned using the OneStep™ PCR Inhibitor Removal Kit (Zymo Inc.) and re-tested. If the samples were still inhibited, then they were diluted 10-fold until they tested positive or were no longer inhibited.

2.2.3 | Statistical analyses

In order to assess the effectiveness of eDNA detections and validate the eDNA survey protocol across a variety of wetland habitat characteristics, we analyzed species detections from the Spring 2022 eDNA data using a Bayesian multi-species occupancy modeling framework to identify relationships between environmental and habitat variables (e.g., wetland vegetation and upland land cover classes) with eDNA detection for each target species across the study area (Text D in Data S1). In addition, we investigated the influence of habitat variables on the proportion of inhibited eDNA samples per wetland site using generalized linear models (Text E in Data S1).

2.3 | Collaborative approach

The initial impetus for this project came from staff of the Elkhorn Slough Reserve (co-authors KW, SKF), California Department of Fish and Wildlife (co-author DF) and the recovery lead for SCLTS at US Fish and Wildlife Service (co-author CJM). We desired to improve conservation of SCLTS by learning more about the distribution of the species in the southern end of its range in Monterey County. We assembled the core team (including CSG) and wrote a grant proposal to the U.S. Cooperative Endangered Species Conservation Fund, which supported the work described here from 2021 to 2023. This core collaborative team (Table S7) met regularly throughout the project, ensuring diverse stakeholders could provide formative feedback on all steps, ranging from initial planning to preparation of final projects. Other collaborators joined the project as it progressed. Collaborative efforts had three target goals: (1) building trust in these eDNA methods within regulatory agencies; (2) engaging private landowners in amphibian conservation; and (3) informing actions of conservation organizations. Throughout the project, we consistently centered the needs of agencies and stakeholders for clear and validated eDNA methods that could be used to complement existing survey and conservation strategies.

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2.3.1 | Building trust in eDNA methods within regulatory agencies

The objective of this component of our collaborative approach was "to improve conservation outcomes for state or federally listed species by providing an additional source of information to be used in decision-making through increasing acceptance of validated eDNA methods by regional regulatory agencies." Our core team included representation of the key state (California Department of Fish and Wildlife-CDFW) and federal agency (US Fish and Wildlife Service-USFWS) with authority over the focal species (Table S7). They were engaged in all team meetings and joined some of the traditional surveys and observed the eDNA sampling. These team members further engaged colleagues within their agencies for additional feedback. They helped to identify a key need: better understanding of the reliability of eDNA sampling relative to traditional methods, which resulted in the development of rigorous and extensive comparison described in the technical methods above. They also indicated the importance of written recommendations on the use of eDNA by regulatory agencies. We thus generated a document of such recommendations and solicited and received extensive formative feedback on them from agency team members and additional representatives, which shaped the final document (Goldberg et al., 2023).

2.3.2 | Engaging private landowners to increase understanding and stewardship

One objective of this component of our collaborative approach was "to identify previously unknown breeding sites for the focal species to enhance understanding of true distributions and thereby inform conservation planning on adjacent lands. A second objective was to foster interest in the species and habitats and thereby potentially inspire improved habitat management by the landowners who discover they have imperiled species on their property." We thus conducted outreach aimed at reaching landowners within the range of the focal species, via social media and email listserv postings, partner relationships, and a dedicated webpage explaining our goals and asking landowners willing to allow surveys on their lands to contact us via a web portal. The top priority unanimously identified by the core team was the highly endangered SCLTS, so we posted flyers near known SCLTS breeding sites and in nearby community centers. We held an initial webinar to increase understanding of the focal species and their habitats and to invite participation. We then engaged with landowners who expressed willingness, sampling on their lands and sharing the results for their property as soon as they became available. We generated and disseminated a summary brochure of general findings and organized a final webinar to share results.

2.3.3 | Informing action by regional conservation organizations

The objective of this component of our collaborative approach was "to improve decision-making by regional conservation organizations and change on-the-ground action by providing enhanced information about distribution of breeding sites for these species." In addition to the four conservation organizations on our core team, we contacted dozens of others in the region, and ultimately worked closely with 15 organizations (Table S8). For example, we sampled wetlands owned by the Santa Lucia Conservancy, Monterey Peninsula Regional Park District, the Elkhorn Slough Foundation, the Bureau of Land Management, California State Parks, the Center for Natural Lands Management, and the Land Trust of Santa Cruz County. All these organizations had an interest in better understanding listed species distributions to inform management of their wetlands and surrounding uplands. Our core team also identified a need for enhanced spatial data and tools, in particular for the Elkhorn Slough watershed, in order to inform conservation planning (land acquisition strategies, new wetland creation sites, etc.). Our approach thus included preparation of spatial data management tools and a workshop to disseminate them to conservation organizations.

3 | RESULTS

3.1 | Technical approach

3.1.1 | eDNA and traditional field surveys

We surveyed a total of 195 wetlands across 2021 and 2022, where we detected SCLTS, CTS, and CRLF at 32, 22, and 52 wetlands, respectively (Figure 1 and Table S9). All field and lab negative controls tested negative for all species across both survey years. In 2021, 11% ($n = 28$) of eDNA samples collected were originally inhibited across 15 wetlands, and in 2022, 12% of eDNA samples ($n = 80$) were originally inhibited across 38 wetland sites. We detected all three species at more sites with eDNA surveys than with traditional surveys (Table 1).

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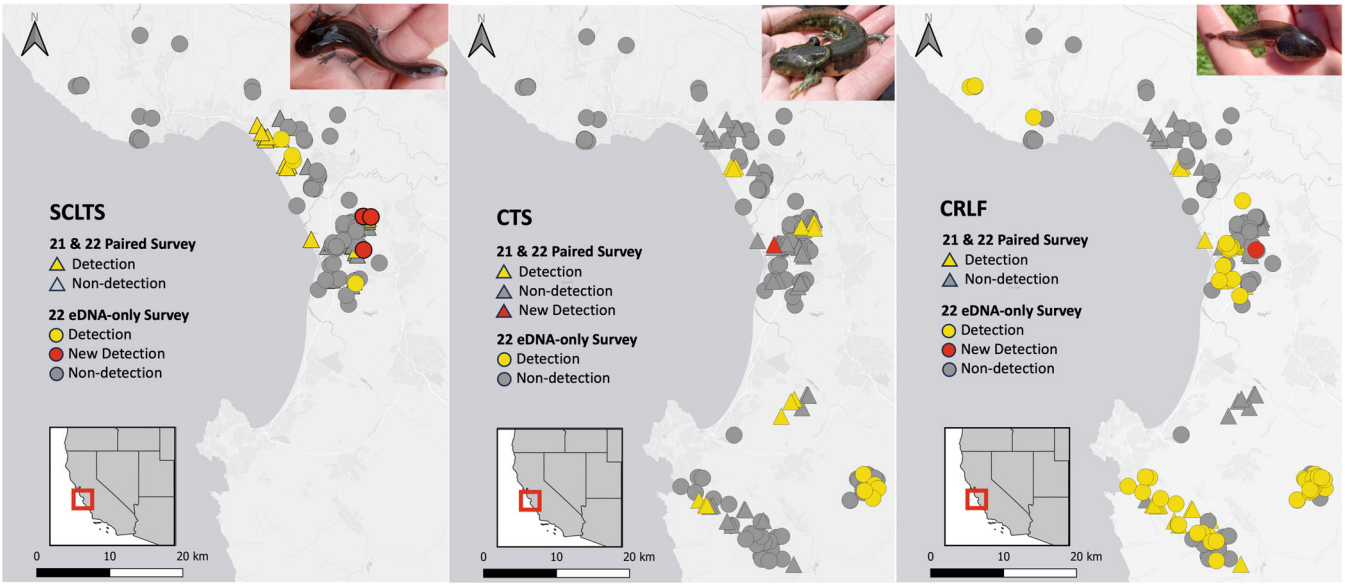

FIGURE 1 Geographic overview of SCLTS (left), CTS (middle), and CRLF (right) detections in California wetlands. Paired eDNA sampling and traditional surveys were conducted at the same sites in 2021 and 2022, and survey detections refer to when either method detected the target species. The eDNA-only surveys were conducted at additional wetlands in 2022 only. New detections refer to detections where the target species had not been previously detected. Photo credit: Mitchell J. Ralson.

TABLE 1 Summary of amphibian detections from traditional surveys and eDNA sampling.

	Species	Traditional survey detections	eDNA detections	Total detections	Traditional survey non-detections	eDNA non-detections
2021 paired	SCLTS	14	18	18	4 (22%)	0 (0%)
	CTS	7	11	11	4 (36%)	0 (0%)
	CRLF	22	27	28	6 (21%)	1 (3%)
2022 paired	SCLTS	13	17	18	5 (27%)	1 (5%)
	CTS	11	10	11	0 (0%)	1 (9%)
	CRLF	19	21	23	4 (17%)	2 (8%)
2022 eDNA-only	SCLTS	-	7	-	-	-
	CTS	-	5	-	-	-
	CRLF	-	46	-	-	-

Note: The 2021 and 2022 paired surveys are where both survey methods were applied ($n = 67$ and $n = 59$, respectively). The 2022 eDNA-only surveys are when only eDNA sampling was applied ($n = 128$ in 2022). The columns with detections provide the number of wetlands where each species was detected. The columns with non-detections provide the number and percentage of wetlands where one method detected the species while the other method did not detect the species.

Santa Cruz long-toed salamanders

We detected SCLTS with eDNA at 18 wetlands in 2021 and 24 wetlands in 2022 (Table 1), including three new wetlands where they had not previously been recorded. Environmental DNA surveys consistently had greater SCLTS detection rates compared to traditional surveys, where traditional surveys missed 22% and 27% of the paired surveys with eDNA detections in 2021 and 2022, respectively. Across both years, eDNA surveys missed one SCLTS detection overall; these samples had an extremely high level of inhibition (still inhibited after 1:1000 dilution).

California tiger salamanders

We had the lowest number of wetland detections for CTS during both survey years; they were detected at 11 wetlands in both 2021 and 2022 (Table 1). We detected CTS at one wetland with both survey methods where they had not been previously recorded. We had higher CTS detection rates using eDNA surveys than with traditional surveys; we did not detect the species with traditional surveys at 36% of the sites where we detected them with eDNA in 2021. In 2022, we detected the species with traditional surveys at every site where we detected them with eDNA, but we did not detect them with eDNA at

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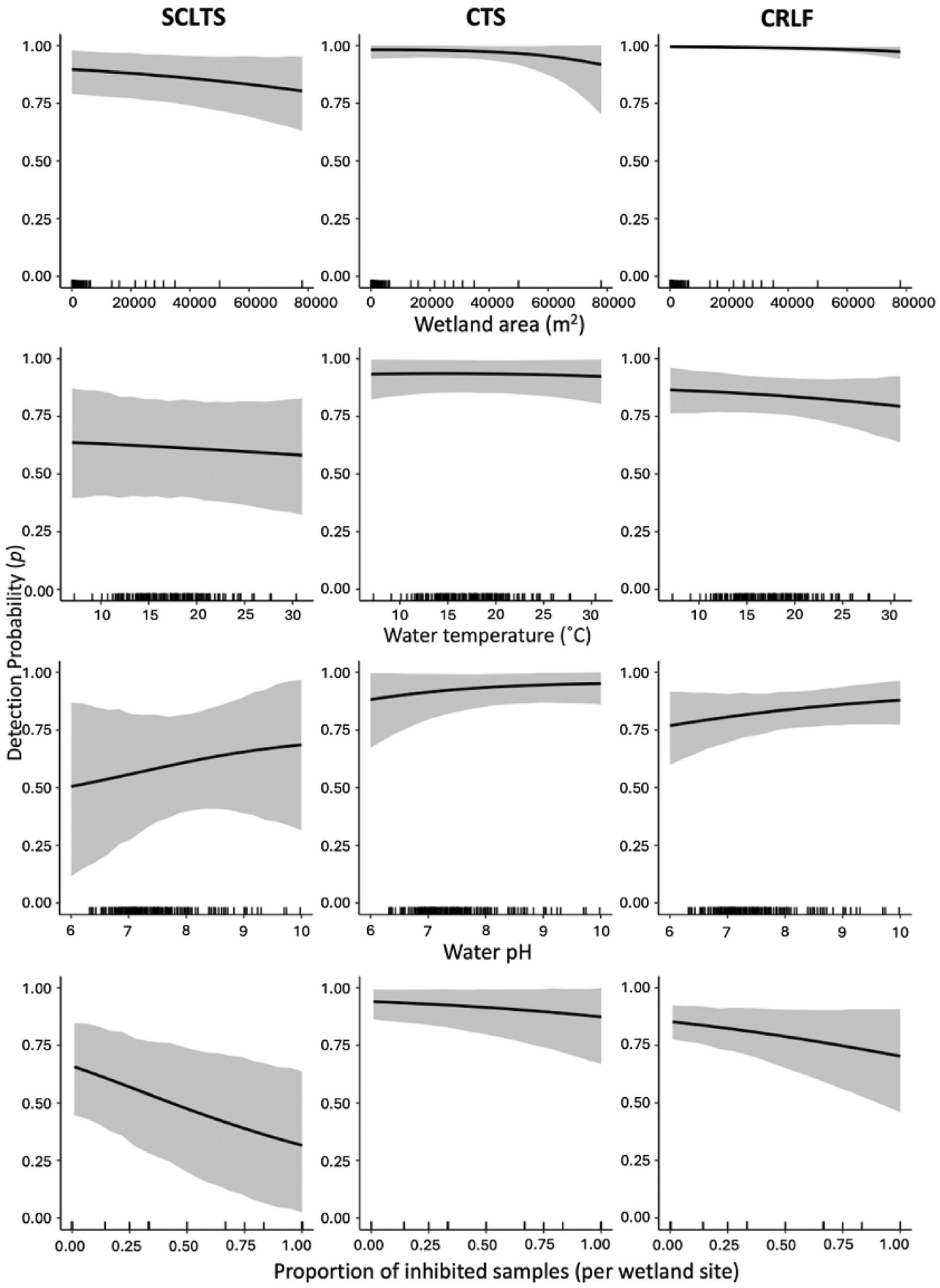

FIGURE 2 Legend on next page.

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one site where they were detected with traditional surveys. This site had a low density of CTS larvae as observed by traditional surveys. No evidence of BTS was detected with eDNA surveys (thus there was no evidence of CTS-BTS hybrids in these wetlands).

California red-legged frogs

Among target species, the majority of survey detections were for CRLF for both years (Table 1). We detected CRLF at one wetland during an eDNA-only survey where they had not previously been recorded and detected them with eDNA at one wetland where traditional methods yielded negative results and the species had not been detected for 7 years. We had higher CRLF detection rates with eDNA compared with our traditional surveys; we missed 21% and 17% of detections with traditional surveys in 2021 and 2022, respectively. We did not detect this species with eDNA surveys at one site in 2021 and two sites in 2022 where they were detected with traditional surveys. All three of these wetlands had a low density of CRLF as observed by traditional surveys, and two of them had only one adult and no larvae observed at each wetland.

3.1.2 | Statistical analyses

We found that CTS had the highest estimated detection probability per eDNA sample (0.96) compared to SCLTS (0.75) and CRLF (0.89) (Table S10). For species occupancy, CRLF had the highest estimated occupancy probability across sites (0.37) compared to SCLTS (0.21) and CTS (0.14) (Table S10). Across all three target species, the slope of relationships between detection probability and environmental variables from the occupancy model were in the expected directions, but 95% credible intervals overlapped zero (Figures 2 and S3). We found evidence for a strong negative relationship (95% credible intervals did not overlap zero) between the proportion of inhibited samples per wetland site and detection probability for the SCLTS only (Figure S3). There was some evidence for relationships between the probability of occupancy and land cover classes surrounding wetlands for SCLTS, CTS, and CRLF (Figure S4). There was strong evidence that the proportion of floating vegetation covering the surface of a wetland site was positively related to the proportion of inhibited samples for that site (Figure 3).

3.2 | Collaborative approach

3.2.1 | Building trust in eDNA methods within regulatory agencies

Partners on this project worked extensively together throughout planning, field data collection, and evaluation of results in the context of applied conservation (Figure 4), resulting in a set of recommendations describing best practices for including this validated eDNA sampling protocol as an option for input into regulatory decision-making for wetland amphibians in coastal California (Goldberg et al., 2023). We developed a tabular comparison of the advantages and limitations of traditional surveys versus eDNA sampling, flow charts for combining the two methods (Figure 5) and addressed considerations of factors such as seasonal timing, spatial configuration of samples, negative controls, and qPCR sample inhibition.

The recommendations were disseminated within USFWS and CDFW by our core team members from these agencies. The local USFWS lead for recovery of these species is optimistic that the recommendations document will help facilitate incorporation of eDNA sampling techniques into standard agency survey protocols for SCLTS, CTS, and CRLF, and the agency is already using the eDNA data from this project for recovery planning (observation by co-author CJM). The recommendations have also increased CDFW confidence in use of eDNA, and CDFW is moving forward with further incorporation of eDNA as a tool for supporting management of state species of concern (observation by co-author DF).

3.2.2 | Engaging private landowners to increase understanding and stewardship

Our outreach to landowners was conducted in winter 2022, prior to the spring 2022 field sampling season. We posted an invitation to participate on the Elkhorn Slough Reserve volunteer listserv, which reaches about 150 people, virtually all of which live in the study area, and on the Elkhorn Slough Reserve Facebook page (3K subscribers). We also posted it to the Elkhorn Slough Foundation Facebook page (8.3K subscribers; this specific post was viewed by 4399 people) and on Instagram (3.2K subscribers). The social media posts provided a link

FIGURE 2 Estimated SCLTS, CTS, and CRLF detection probabilities as a function of three environmental parameters and of inhibition. Solid lines represent the mean predicted estimate and shading represents the 95% credible intervals. Model predictions were estimated by randomly sampling 10,000 draws from the posterior probability distributions, while holding all other parameters at their mean posterior probability distribution value. Observed data ranges for each variable represent the rug plot along the *x*-axes.

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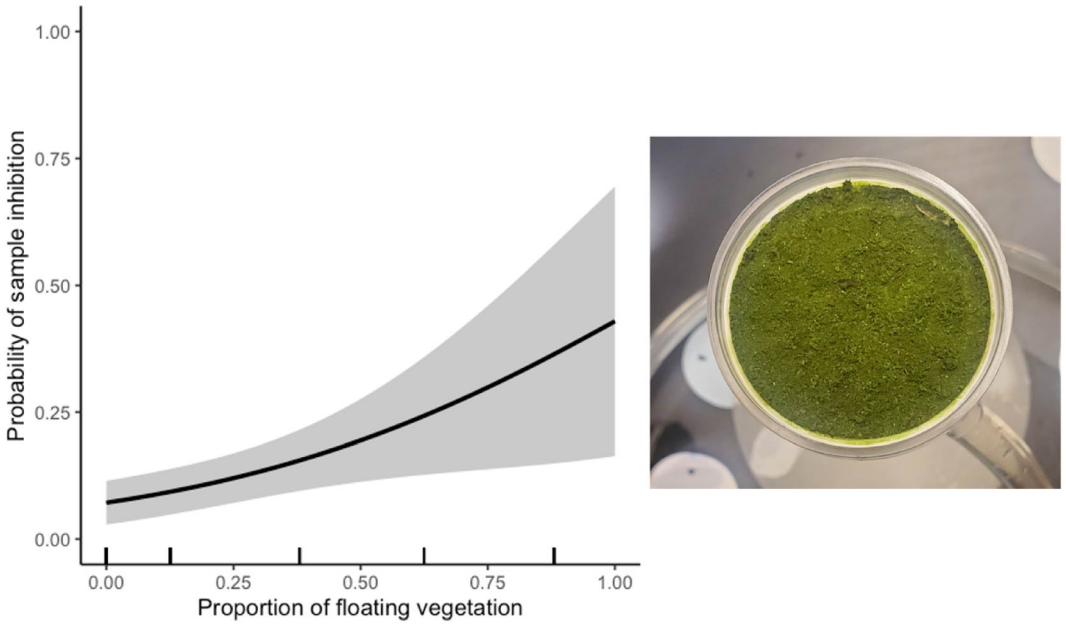

Models	QAICc	Weights	Δ QAICc
Inhibition ~ flv	137.59	0.47	0
Inhibition ~ flv + willow	138.78	0.26	1.19
Inhibition ~ flv + emv	139.23	0.21	1.64
Inhibition ~ flv + emv + oak + grass + scrub + willow	142.40	0.04	4.81
null	144.66	0.01	7.07
Inhibition ~ oak + grass + scrub	145.79	0.01	8.20

FIGURE 3 Estimates of sample inhibition as a response to environmental and habitat variables for SCLTS, CTS, and CRLF. Top-competing model estimates of sample inhibition as a response to the proportion of floating vegetation per wetland site, where shading represents the 95% confidence interval (top left). An example of small, dense, floating vegetation on a filter (top right). The full model set, evaluated by QAICc, to estimate the probability of sample inhibition as a response to environmental and habitat variables (bottom). Variables hypothesized to influence sample inhibition included the proportion of floating vegetation (flv) and proportion of emergent vegetation (emv) covering the surface area of the wetland, as well as the proportion of oak woodland (oak), grassland (grass), scrubland (scrub), and the proportion of wetland perimeter with willows (willow). Photo credit: Mitchell J. Ralson.

to a webpage with more detail; this received 490 unique page views. We disseminated ~100 flyers within the range of the SCLTS (at local businesses, post offices, libraries, and community centers, and on telephone poles near SCLTS breeding sites). About 50 people attended our outreach webinar held in February 2022, with about 20 attending a subsequent field trip to wetlands on the Reserve. Following this outreach campaign, we were able to sample about 20 wetlands on private lands that had not previously been sampled.

After sample analyses were complete, we shared results with each property owner. For those with listed species present, we provided additional web-based resources on wetland management and offered additional consultation by staff from USFWS and the Resource Conservation District of Santa Cruz County. Numerous landowners accepted these invitations for additional guidance on pond management and/or enhancement opportunities. We also invited all landowners (those with and without listed species on their lands) to attend a final webinar sharing general findings of the project, attended by 67 people (private landowners as well as other stakeholders) in June 2023.

One private property near the Elkhorn Slough Reserve exemplifies the results achieved by this collaborative component of our project. The landowner invited us to collect eDNA samples in her two wetlands and accompanied us in the field. Sample analyses revealed CRLF in one wetland, SCLTS in another. The latter is of particular significance, since there were previously only 8 breeding sites known in Monterey County. The landowner was excited by the discoveries and invited our USFWS team member (CJM) to visit the property subsequently and provide advice on habitat management to support the listed species. Out of concern for predation on small larval amphibians, the landowner has also

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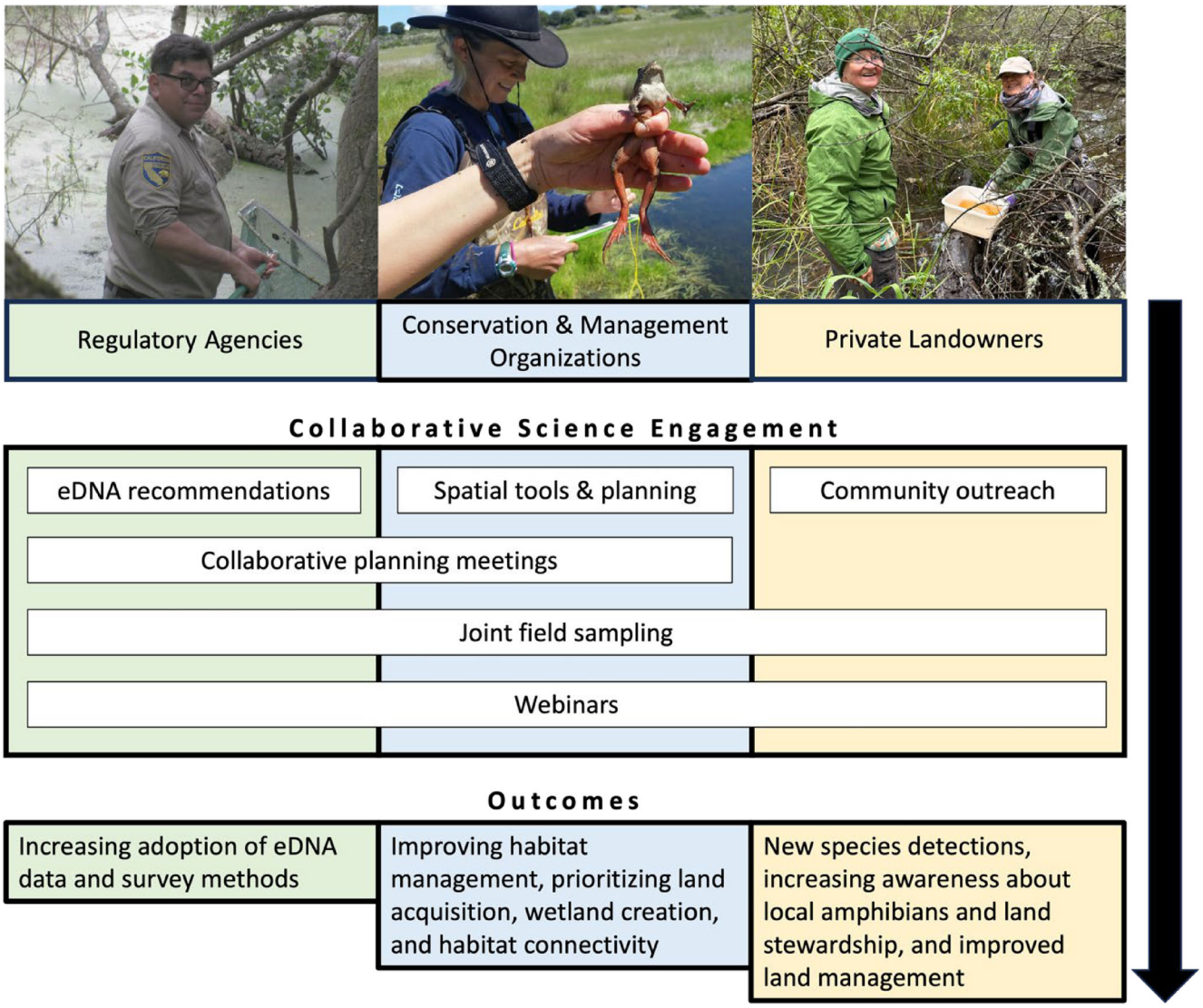

FIGURE 4 Process for engaging stakeholders in collaborative science to yield conservation action. Each colored column shows a different category of stakeholders, the ways in which they were engaged in the science, and the conservation outcomes that resulted for this category. Photo credit: Elkhorn Slough Reserve.

declined stocking of mosquitofish in their wetlands, which had previously occurred.

3.2.3 | Informing action by regional conservation organizations

We detected listed species in many wetlands owned by conservation organizations, and we shared the eDNA results with contacts at each organization as soon as they were available. In some cases, the results were translated directly into action. For example, a recent SCLTS detection at a coastal wetland (Zmudowski area) owned by CDFW resulted in staff assessments of the property, revealing evidence of trespassing, illegal mowing, and trash piles. This property is now receiving increased attention, with an immediate halt to the trespassing and property damage directly resulting from the recent eDNA detection.

In addition to working individually with conservation landowners, we provided spatial data management tools and a forum for brainstorming about priority conservation strategies in the Elkhorn Slough watershed. We generated a Google Earth (KMZ) file for the Elkhorn watershed that included past public records (from the California Native Biodiversity Database and from the monitoring database of the Elkhorn Slough Reserve) as well as the records from this project, for those landowners (public and private) that had given permission for the records to be shared publicly. We created various

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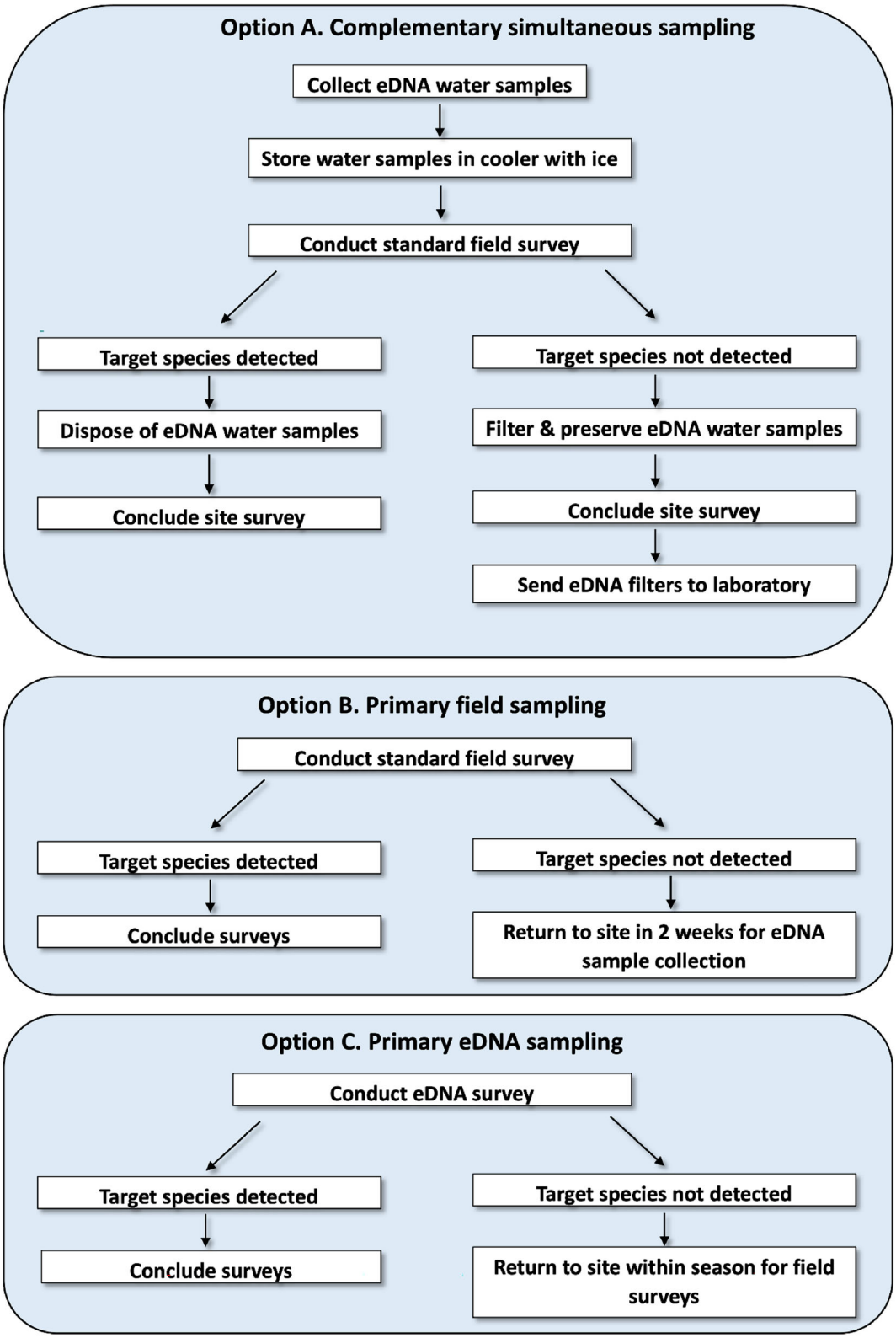

FIGURE 5 Three options for incorporating eDNA sampling into wetland amphibian survey plans. Option A involves applying both approaches regardless of findings, as we did in our paired surveys. Option B uses field sampling as the primary approach, and only incorporates eDNA if target species is not detected by field surveys. Conversely, Option C uses eDNA sampling as the primary approach, and only incorporates field surveys if the target species is not detected.

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geospatial layers, including simplifications (if this species has ever been found at the wetland) and more detailed layers (data for each year). We shared this KMZ file and an associated README with about 40 people from regional conservation organizations and invited them to a workshop. We prepared a slideshow summarizing existing and new records for each of the three species and generated discussion questions about conservation strategies possibly appropriate given the new information. We hosted a workshop in February 2023 with participation by about 20 people from 10 regional conservation organizations.

The new eDNA detections and spatial planning tools were put to use by participating conservation organizations. For instance, the Elkhorn Slough Foundation (ESF) is exploring floodplain restoration in response to new SCLTS detections along Carneros Creek, and implementing a strategy of generating a network of newly restored or created wetlands that will serve as stepping stones to existing wetlands and high quality upland habitat on ESF lands (ESF Stewardship Director D. Dunkell, personal communication). The ESF has also contacted the private landowner described in the previous section (with new SCLTS and CRLF detection on their lands) to inquire about their interest in ESF eventually acquiring this property, which lies between existing protected lands in a drainage now recognized as valuable to SCLTS (ESF Land Acquisition Manager K. Contreras, personal communication).

4 | DISCUSSION

4.1 | Demonstrating reliability of the eDNA sampling approach

Despite the increasing adoption of eDNA methodology by academic scientists, regulatory agencies have been slower to incorporate eDNA into required survey protocols or use data for conservation planning (Kelly et al., 2023). Regulatory staff on our team identified the need for thorough, transparent comparisons between eDNA sampling and traditional methods as important for building trust in eDNA detection results. Our results showed eDNA sampling and traditional surveys yielded similar detection rates across all three species, and eDNA surveys had greater detection probabilities overall, as has been found in other studies (Beng & Corlett, 2020; Rees et al., 2014). The eDNA data were unlikely to include false positive detections because: we validated all assays extensively; we did not find contamination in any field or laboratory negatives; we followed protocols that have been demonstrated to lead to a very low probability of a false positive (Smith & Goldberg, 2020); and we did not observe species detections in locations where they would not be expected (e.g., wetlands with unsuitable habitat or wetlands outside of the range for SCLTS).

Occupancy modeling showed that eDNA detection probabilities were not particularly sensitive to common sources of environmental variation, and our spatially adaptive design was able to account for the spatial heterogeneity of eDNA across wetland sizes. However, we found that sample inhibition, a leading cause of false negative results in eDNA surveys, had a strong, negative influence on SCLTS detection. Further, we found that the proportion of surface water covered by floating vegetation at a site was positively related to the proportion of inhibited samples. These relationships suggest that sampling designs, and/or laboratory methods could potentially compensate for or avoid the complication of inhibition associated with vegetation for detecting this species.

Although our sampling protocol performed well, eDNA detection was still imperfect and had non-detections at wetlands where traditional surveys had detections, particularly when CTS and CRLF occurred at very low densities or at non-breeding CRLF sites with few adults. Additionally, we found that the timing of surveys was critical for detecting young SCLTS larvae at low densities; surveys within the same survey season could reduce the probability of missing detections of this species. Likewise, conducting multiple field surveys rather than a single one as we did in this study might have led to detection of target species in wetlands where we detected them with eDNA but not traditional surveys. A stratified combination of both traditional and eDNA sampling methods may provide the most complete information. This can be done by conducting both types of surveys on the same visit, which is most time-efficient for sites that are difficult to access; conducting traditional sampling first and considering following up with eDNA sampling if target species are not detected, which could be more efficient if preparing for eDNA sampling is time-limiting; or conducting eDNA surveys first, which only is efficient if results can be received quickly (Figure 5).

Overall, the combined technical and collaborative approach we implemented built trust and, according to feedback from key stakeholders, increased acceptance of the methodology by local regulatory agency and conservation organization staff, including those that had originally been highly skeptical. We followed the recommended process for science intended to have an impact on environmental policy or management—collecting sufficient new evidence and sharing it transparently (Fisher et al., 2020). The approach we took can be used in many systems: quantifying effectiveness of a well-designed and effective eDNA sampling and analysis

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strategy compared to traditional methods under a variety of conditions to generate partners' confidence in results.

We followed key steps recommended for successful applied environmental science, namely respectfully engaging partners early and often, having a plan for communicating and sharing outputs, and being transparent about uncertainties and limitations (Cooke et al., 2020). A deliberate approach to developing partnerships between scientists and stakeholders is critical for co-production of usable science that addresses urgent environmental challenges (Meadow et al., 2015). An important part of building trust was to include regulatory partners on our core team from the start. Unhurried time in the field together was particularly valuable for relationship-building and allowed team members to learn from each other about eDNA methods, local natural history, and discuss questions and challenges together. At the request of some team members, we generated recommendations specifically tailored for the locally relevant regulatory agencies (CDFW, USFWS), that included comparisons of the limitations of eDNA and traditional surveys, and guidance on how to use both to complement each other (Goldberg et al., 2023) (Figure 5). We repeatedly revised them with formative feedback from multiple staff from both agencies. The wetlands surveyed were a representative sample of central California wetlands, so the eDNA protocol and recommendations for regulatory agencies can be applied to them as well as other wetland amphibian species (e.g., spadefoot toads, bullfrogs, newts, western toads; Goldberg et al., 2018; CSG and MJR, unpublished data), although we recommend additional validation to ensure that the timing of surveys is tailored appropriately to the species. A challenge that remains is developing a standardized process for evaluating laboratories conducting analysis of these samples.

4.2 | Novel eDNA detections translated into conservation action

We detected new breeding sites for federally listed amphibians as a result of this study. Extensive outreach to landowners resulted in sampling of 187 wetlands in 2022 alone, which was facilitated by the ease and rapidity of eDNA sample collection relative to traditional surveys. We followed guidance for actionable science, ensuring key decision-makers were included and determining their needs for decision-making (Bisbal & Eaton, 2022). We engaged stakeholders who decide how to manage wetlands and adjacent uplands, what lands to acquire for conservation, and where to conduct restoration and wetland creation. As described above, the novel detections were rapidly translated into conservation action by some of the decision-makers, including improved wetland management by some private landowners, new land acquisition priorities for a land trust, and spatial planning to find optimal locations for new wetland creation sites to provide corridors and connectivity. Fundamental to our success was building relationships with conservation organizations and private landowners. One lesson learned was how slow the process of community engagement can be and how challenging it is to obtain access to wetlands on private property. While we received multiple responses since the beginning of our outreach effort 2 months prior to the start of our surveys, we continued to gradually hear from numerous community members over the subsequent years, hoping to have their wetlands sampled. We would thus recommend to others beginning the process of community engagement years in advance if possible.

Directly applying eDNA data to conservation action is still rare, although the potential has long been recognized (Goldberg et al., 2015; Lodge et al., 2012). We have described an integrated technical and collaborative approach that succeeded in almost immediate application of new eDNA results to conservation action. We heavily engaged key stakeholders on a core team from the start, and involved diverse decision-makers including regulatory agencies, private landowners, and conservation organizations, generating communication and products tailored to their needs. Together, we built a shared understanding of how traditional surveys and eDNA sampling compare and how they can complement each other, discovered new breeding sites for highly imperiled amphibians, and considered how decisions should be made as a result of the new findings. We developed a user-friendly technical report (Goldberg et al., 2023) of this validated eDNA protocol to support future implementation of these methods. This collaborative effort among scientists, agencies, conservation interests and the local community is a model for using eDNA for conservation across diverse landscapes.

AUTHOR CONTRIBUTIONS

MJR, CSG, and KW prepared the original manuscript draft. CSG and KW developed project conception, and KW and DF assisted with funding acquisition. MJR led data collection and analysis, and CSG and KW assisted with data interpretation. CJM, IML, SKF, CAW, KMC, MLA, and GHD provided substantial revisions to the manuscript, provided critical field work assistance, data collection, and wetland access.

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Endangered Species Act. We are indebted to Michelle Bakker (California Department of Fish and Wildlife) and Gabriele Estill (Elkhorn Slough Foundation) for managing this grant on behalf of the team. This work was supported in part by the USDA National Institute of Food and Agriculture, McIntire-Stennis project 1018967. All co-author institutions provided additional support. J. Geller and M. Wheelock generously supported an early phase of this project. C. Endris generated the spatial analysis tools for conservation end users in the Elkhorn Slough watershed. We are very grateful to M. Sterling and C. Kiepe for assistance in the laboratory, the dozens of volunteers who assisted with fieldwork, Dr. Jesse Brunner for support with statistical modeling, and many landowners who invited our team to sample their wetlands (Table S15). We are appreciative of the Museum of Vertebrate Zoology, University of California, Berkeley, for providing specimen tissue samples. We are extremely grateful for thoughtful editorial suggestions provided by two reviewers, the senior associate editor and editor-in-chief, which improved the clarity of the paper.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

All trimmed DNA sequences have been uploaded to the NCBI GenBank database (accession numbers: PQ871355 - PQ871363) and all other data are located in the supplemental materials document.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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SUPPLEMENTAL MATERIALS

Translating eDNA data into conservation action: partnerships to support imperiled amphibians in coastal California wetlands

SUPPORTING TEXT A - eDNA Surveys
eDNA Sampling Strategy

We employed a site-level adaptive eDNA sampling strategy where the number of samples collected was dependent upon the estimated surface area (m^2) of the site (Table S1). We used a rangefinder to estimate the area at the time of the survey and before any eDNA samples were collected. For sites that were less than 5,000 m^2, we collected between two and five approximately evenly spaced water samples along the perimeter of the site. For large sites greater than 5,000 m^2, we collected one sample approximately every accessible 50 m along the perimeter of the entire site. If a stretch of the perimeter was inaccessible, then we collected a sample at the next accessible area. When it was not possible to survey the entire perimeter of a site, at least one 200 m transect was employed by collecting one eDNA sample every 50 m. We established locations for transects by visually selecting areas of the wetland that were considered to have the highest likelihood of species occupancy, and by selecting areas on the opposite side of a previously established transect.

Table S1. Site-level spatial sampling strategy for eDNA sample collection for the 2021 and 2022 surveys. The number of samples collected was based on the estimated surface area of the wetland at the time of the survey using a rangefinder. For sites >5,000 m^2, at least two 200 m transects were established along the perimeter of the site, with 5 samples collected per transect spaced approximately 50 m.

Area (m2)	<50	<1,000	1,000-3,000	3,000-5,000	>5,000
Samples Collected	2	3	4	5	5 samples per 200 m transect

Habitat and Environmental Variables

Table S2. Habitat and environmental data collected at each site where we conducted surveys for wetland amphibians in the Monterey Bay area of California, 2021-2022. Habitat variables were collected by visual estimation and environmental variables were collected using a handheld multimeter.

	Variable	Description
Habitat	Surrounding Landcover	Proportion landcover classes within an approximate 100 m around the perimeter of the site. (i.e. oak woodland, grassland, scrubland, wetland (freshwater or brackish), mixed forest, agriculture, developed, barren)
	Emergent Vegetation	Percent emergent vegetation across the entire site. (e.g. *Schoenoplectus sp.*, *Eleochris sp., Typha sp.*)
	Floating Vegetation	Percent floating vegetation across the entire site. (e.g. *Lemna minor, Azolla sp., Hydrocotyle sp.*)
	Sun Exposure	Percent exposure on the site, approximated by eye.

	Willow Perimeter	Percent willow (*Salix sp.*) around the perimeter of the site.
Environmental	Water Temperature	Water temperature at approximately 5 cm below surface at a single point along the site perimeter.
	Water pH	Water pH at approximately 5 cm below surface at a single point along the site perimeter.

Traditional Surveys

Dip netting occurred throughout all accessible areas of the site, particularly along perimeter areas with abundant emergent vegetation. We recorded the number of person-hours spent dip netting (Figure S1) to measure survey effort and the number of seine hauls performed. In addition, we recorded the number of each target species caught or observed and the presence of other native and introduced aquatic vertebrates (e.g., amphibians, fish, crustaceans).

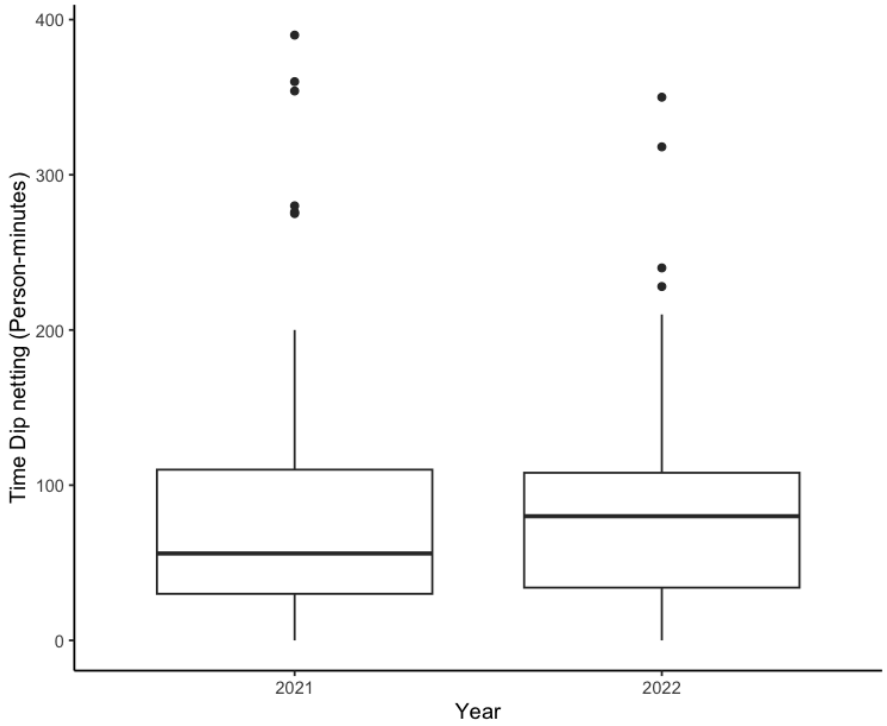


Figure S1. Time spent dip netting (person-minutes) for field surveys at wetlands across 2021 (N = 67) and 2022 (N = 59) in the Monterey Bay area, California. Dip netting occurred at most wetlands, while only seining occurred at others.

SUPPORTING TEXT B - eDNA Assays

eDNA Assays

Santa Cruz long-toed salamander

We obtained toe and tail clip samples from throughout the SCLTS range from each of the five recognized SCLTS metapopulations (USFWS 2009). We received tissue samples from the Museum of Vertebrate Zoology (Table S3), University of California, Santa Cruz, Elkhorn Slough National Estuarine Research Reserve, and U.S. Fish and Wildlife Service. We extracted DNA from these 49 tissue samples using the DNeasy Blood and Tissue Kit (Qiagen) within a designated high-quantity DNA laboratory at Washington State University.

Table S3. SCLTS tissue sub-sample loan from the Museum of Vertebrate Zoology, University of California, Berkeley.

Catalog Number	Sample Locality
MVZ:Herp:161822	Santa Cruz County
MVZ:Herp:161823	Santa Cruz County
MVZ:Herp:231245	Santa Cruz County
MVZ:Herp:180158	Monterey County
MVZ:Herp:180159	Monterey County

We amplified the mitochondrial cytochrome b gene from these samples using AmbPleth_cytb-F and AmbPleth_cytb-R primers (Lee-Yaw and Irwin 2012). The 48 samples that amplified were sequenced by the Arizona Genetics Core at the University of Arizona. We trimmed sequences by hand and aligned them using Sequencher version 5.3 (Gene Codes Corp.). All samples were the same haplotype and matched GenBank accession ID: JX650221.1. We then exported the inclusive consensus sequence. We developed a species-specific SCLTS quantitative polymerase chain reaction (qPCR) assay from this consensus sequence in Primer Express version 3.0.1 (Applied Biosystems) and validated the specificity of the primer set and probe *in silico* using Primer-BLAST (NCBI). We then validated the assay using extracted DNA from tissue samples of the target species and nine other amphibians (Table S4). The assay consisted of 1X NoROX QuantiTect Multiplex PCR Mix (Qiagen Inc.), 0.2 µM of each primer, 0.2 µM of the probe, and 3 µL of extracted DNA in a 15 µL reaction volume. All reactions included an internal positive control (Thermo Fisher Scientific Inc.) to monitor for qPCR inhibition. The reactions were run on a Bio-Rad CFX96 Touch Real-Time PCR Detection System with the following cycling protocol: activation at 95°C for 10 minutes, followed by 50 cycles at 95°C for 15 seconds, 60°C for 60 seconds. The assay amplified all SCLTS tissue samples and did not amplify any of the additional species (Table S4).

California and barred tiger salamander

We developed a competitive single nucleotide polymorphism mtDNA control region assay for the CTS and BTS using tissue sample sequences obtained for development of a previous assay with the same goal (Moss et al. 2022). This previous assay was designed for application in another part of the range and does not distinguish tiger salamanders from SCLTS. Therefore, to develop an assay that excluded SCLTS, we amplified the 48 SCLTS tissues using the THR and DL1 mtDNA d-loop primers from

Shaffer and McKnight (1996) and sequenced them at the Arizona Genetics Core at the University of Arizona to compare with existing tiger salamander sequences. We aligned the consensus sequence with the tiger salamander consensus sequence in Sequencher version 5.3 (Gene Codes Corp.). We found nine haplotypes, including five from the MVZ samples which were collected in 1977-8; four of those five haplotypes were also found in current samples (collected 2014-2020) (GenBank Accession numbers PQ871355 - PQ871363). We then identified primer and probe regions by hand that would not amplify for SCLTS and additionally validated the assay using Primer-BLAST (Ye et al. 2012).

After examining the success of this qPCR assay with multiple reagents, we decided that the final assay would consist of 15 µL reactions of 1x QuantiNova Pathogen Master Mix (Qiagen Inc.), 0.2 µM of each primer, 0.2 µM of each probe, and 3 µL of extracted DNA. All reactions included a QuantiNova internal control (Qiagen, Inc.) to monitor for qPCR inhibition. The reactions were run on a Bio-Rad CFX96 Touch Real-Time PCR Detection System with the following cycling protocol: activation at 95°C for 2 minutes followed by 50 cycles at 95°C for 5 seconds and 60°C for 30 seconds. The assay amplified all CTS and BTS tissue samples, respectively, and did not amplify any of the additional species (Table S4). We further validated the assay by testing water samples from ponds containing both CTS and CTS-BTS hybrids (R. Cooper pers. comm.). We successfully detected both CTS and BTS, respectively.

Table S4. The eDNA assays for Santa Cruz long-toed salamanders (SCLTS) and California and barred tiger salamanders (CTS-BTS-MONT).

Santa Cruz long-toed salamander	
SCLTS-F Primer	TGAGGAGCGACAGTCATTACAAA
SCLTS-R Primer	CCGCCTCAAATTCATTGAACA
SCLTS-FAM Probe	6FAM-CCGCAATTCCATATATAGGTGA-MGB
California and barred tiger salamander	
CTS-BTS-MONT-F Primer	TTCCCTTGAGGCGCCA
CTS-BTS-MONT-R Primer	TGYTTWGAGGAGGCTGAGGG
CTS-FAM Probe	6FAM-CGGCTTGAAGACTCATTCATC-BHQ+
BTS-CAL610 Probe	CAL610-CGACTCGAAGATTCATTCATCA-BHQ+
Validated Species	
Ambystoma californiense *Ambystoma macrodactylum croceum* *Ambystoma mavortium* *Anaxyrus boreas* *Ensatina escholtszii*	*Rana catesbeianus* *Pseudacris sierra* *Rana draytonii* *Taricha granulosa* *Taricha torosa*

California red-legged frog

We validated a SCLTS-CRLF multiplex assay using the CRLF mtDNA assay from Halstead et al. (2018) with tissue samples from both species and using the same thermoprofile from both single species assays to confirm no loss of sensitivity.

SUPPORTING TEXT C - qPCR Standards
Laboratory Analysis

qPCR Standards

We ran gBlocks Gene Fragment standards (Integrated DNA Technologies) in duplicate wells for each copy number for the SCLTS assay, with the lowest level at the limit of detection (sensu Klymus et al. 2020) for the assay. These consisted of 10-fold dilutions from 5,000 to 5 copies. The standard curve ranged from 10,000 to 10 copies for the CRLF and the CTS and BTS assays. We determined whether a sample was positive using the criteria in Figure S2.

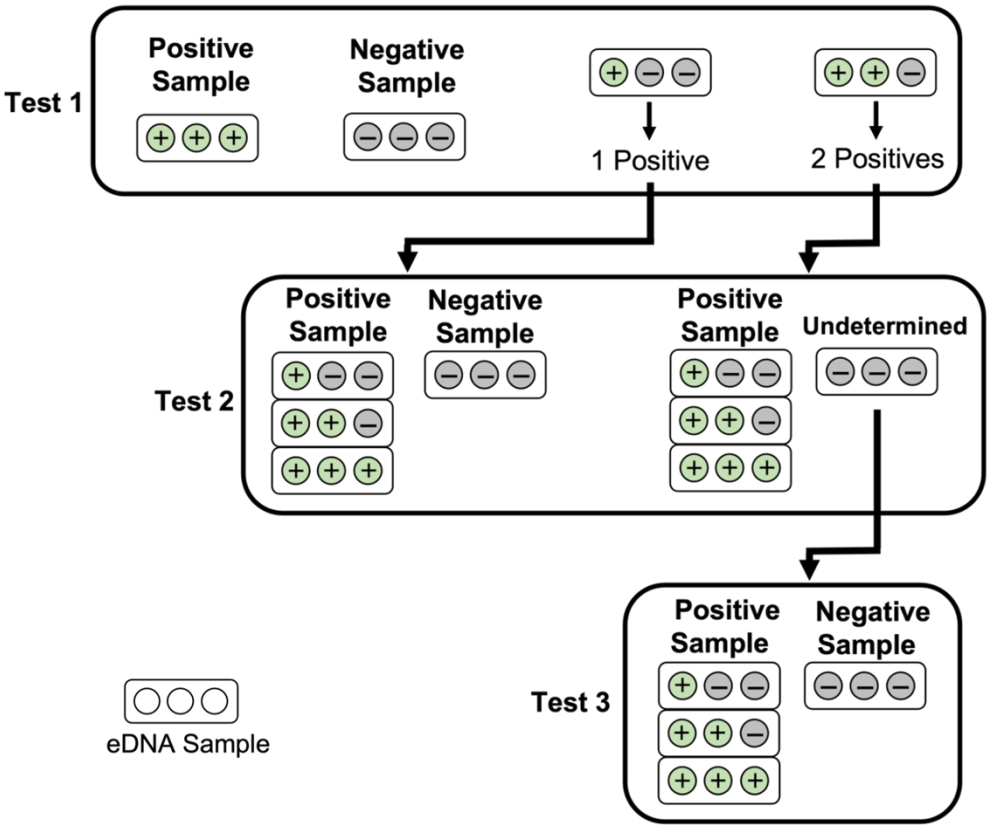


Figure S2. Environmental DNA workflow of filter sample extract analysis through quantitative PCR (qPCR).

SUPPORTING TEXT D - Multi-species Occupancy Model Framework

Multi-species Occupancy Model

We analyzed the 2022 eDNA-only data using a Bayesian multi-species occupancy model across the study area for each species. Because the SCLTS has a smaller range relative to the other species, we restricted wetland sites for the SCLTS to within its range. The multi-species occupancy model was fit in JAGS (Plummer 2009) using the jagsUI package (v. 1.5.2, Kellner 2016) in R (v. 4.2.2, R Core Team 2022). All continuous, non-proportional environmental and habitat variables were standardized by subtracting the mean and dividing by the standard deviation of the data.

We modeled species as random effects, where α and β were species-specific effects on detection probability (p) and hyperpriors μ and τ were the community mean and precision for detection probability, respectively. We constructed the multi-species occupancy model with hyperprior distributions so that the partial pooling of detection parameters between each species could occur. Partial pooling allowed species with many wetland detections to share detection data with species with few wetland detections. The

model did not share occupancy parameters between species, as each species has unique habitat requirements. The prior distributions were as follows:

$$\alpha \sim Normal(0, 1)$$
$$\beta \sim Normal(\mu, \tau)$$

$$\mu \sim Normal(0, 1)$$
$$\tau \sim Uniform(0, 1)$$

The model described the binary latent occupancy state variable $z_{i,k}$, where Ψ was the probability of occupancy for each wetland site i, and for species k, and the observed detection variable $y_{i,j,k}$ represented the detection probability p for sampling occasion j for each wetland site and species. The probability of detection was conditional on whether the species was present or absent at the wetland site.

$$z_{i,k} \sim Bernoulli(\Psi_{i,k})$$

$$y_{i,j,k} \sim Bernoulli(z_{i,k} * p_{i,j,k})$$

The probability of species occupancy and detection was modeled using the logit link function with intercept α_0 and β_0, and covariates $\alpha_1, \alpha_2, \ldots \alpha_7$ and $\beta_1, \beta_2, \ldots \beta_7$, which are described in Table S5.

$$logit(\Psi_{i,k}) = \alpha_{0,k} + \alpha_{1k} * emv.psi_{i,k} * emv_{i,k} + \alpha_{2k} * flv_{i,k} + \alpha_{3k} * sun_{i,k} + \alpha_{4k} * oak_{i,k} + \alpha_{5k} * grass_{i,k} + \alpha_{6k} * scrub_{i,k} + \alpha_{7k} * willow_{i,k}$$

$$logit(p_{i,k}) = \beta_{0,k} + \beta_{1k} * ph_{i,k} + \beta_{2k} * temp_{i,k} \beta_{3k} * area_{i,k} + \beta_{4k} * tfilter_{i,k} + \beta_{5k} * prop.inhib_{i,k} + \beta_{6k} * flv_{i,k} + \beta_{7k} * emv_{i,k}$$

We fit the model with 3 separate Monte Carlo – Markov chains (MCMC) of 100,000 samples each, with a burn in of 10,000 samples. This resulted in 240,000 draws from the posterior distribution for each parameter. We diagnosed model convergence using the Gelman-Rubin statistic (Gelman and Rubin, 1992) and assessed model goodness-of-fit using the Bayes p-value as a posterior predictive check (Gelman et al. 2013).

Table S5. Multi-species occupancy model covariates and descriptions. All covariate data were scaled except for categorical variables and proportions.

Model Covariate	**Description**
Oak, scrub, and grass	Surrounding landcover (proportion)
emv	Emergent vegetation
flv	Floating vegetation
sun	Sun exposure
willow	Willow perimeter (proportion)
temp	Water temperature
ph	Water pH
area	Wetland area (m^2)
tfilter	Time from sample collection to filtration (for each sample)
prop.inhib	Proportion of samples per wetland site that were inhibited

Covariate	Occupancy Influence			Detection Influence	Description	Source
	SCLTS	CRLF	CTS	All Species		
Oak Woodland	+	+/-	+/-		SCLTS are associated with oak woodlands.	Anderson (1967)
Grassland	+	+/-	+		SCLTS and CTS are associated with grasslands.	Trenham (2000)
Scrubland	+/-	+/-	+		CTS can be associated with scrubland.	Trenham (2000)
Sun Exposure	+/-	+	+/-		CRLF adults bask.	Fellers and Kleeman (2007)
Willow Perimeter	+	+	+/-		SCLTS larvae can be found in partially submerged willows and CRLF adults reside in willows.	Chad Mitcham (pers. comm.) Fellers and Kleeman (2007)
Emergent Vegetation	+	+	+	High (-)	SCLTS and CTS deposit eggs on emergent vegetation, and all 3 species larvae utilize emergent vegetation for shelter; plant matter may limit eDNA dispersion and increase sample inhibition.	Anderson (1967) Alvarez et al. (2013) Lance and Guan (2020) Loredo et al. (1996)
Floating Vegetation	+	+	+	High (-)	All 3 species utilize vegetation for shelter; plant matter may increase sample inhibition.	Anderson (1967) Lance and Guan (2020) Trenham (2000)
Water Temperature	+/-	+/-	+/-	Low (+) High (-)	Mild water temperatures are ideal for all 3 species; cold water temperatures slow eDNA degradation while warm water temperatures accelerate degradation.	Eichmiller et al. (2016)
Water pH	+/-	+/-	+/-	Low (-) High (+)	Neutral water pH is ideal for all 3 species; low pH accelerates eDNA degradation while high water pH slows degradation.	Strickler et al. (2015)
Inhibition				High (-)	Sample inhibition can mask eDNA signals and may result in false negative detections.	Jane et al. (2015)

Table S6. Expected environmental and habitat variable relationships between occupancy and detection for the Santa Cruz long-toed salamander (SCLTS), California tiger salamander (CTS), and California red-legged frog (CRLF).

SUPPORTING TEXT E - Sample Inhibition Models
Sample Inhibition Generalized Linear Models

We investigated the influence of habitat variables on the proportion of inhibited eDNA samples per wetland site using generalized linear models with a quasibinomial link function. Prior to model fitting, we calculated Pearson's correlations for each continuous predictor variable and visually assessed correlations for categorical predictor variables by plotting. We did not find any correlations between predictor variables ($|r| < 0.7$). We constructed six competing models using the lme4 package (v. 1.1-31) in R and evaluated evidence for them using QAICc.

Collaborative Project Partners

Table S7. Core collaborative team members and stakeholders who significantly contributed to the project by providing feedback throughout all steps of the project, and assistance with conducting surveys and obtaining wetland survey permissions. Some team members are affiliated with multiple organizations; only their primary affiliation is shown.

Organization	Regulatory authority	Conservation practice	Conservation Science	Core team members
Elkhorn Slough Reserve		X	X	Susanne Fork, Inger Marie Laursen, Kerstin Wasson
California Department of Fish and Wildlife	X	X		Dave Feliz
Washington State University			X	Caren Goldberg, Mitchell Ralson
U.S. Fish and Wildlife Service	X	X		Chad Mitcham
Santa Lucia Conservancy		X	X	Christy Wyckoff
Biosearch Environmental Consulting			X	Mark Allaback
U.S. Army - Fort Ord Base Realignment and Closure (Chenega Tri-services)		X	X	Bart Kowalski

University of California - Natural Reserves		X	X	Gage Dayton
Resource Conservation District of Santa Cruz County		X		Kelli Camara

Table S8. List of regional organizations that assisted with conducting field surveys or provided access for wetland surveys.

Organizations
Bureau of Land Management
California State Parks
California Department of Fish and Wildlife
Center for Natural Lands Management
Elkhorn Slough Foundation
Elkhorn Slough National Estuarine Research Reserve
Land Trust of Santa Cruz County
Monterey Peninsula Regional Park District
Northern Monterey County High School
Elkhorn Slough Farm
Palo Corona Regional Park
Resource Conservation District of Santa Cruz County
Santa Lucia Conservancy
United States Fish and Wildlife Service
Watsonville Wetlands Watch

Table S9. All wetlands surveyed in 2021 and 2022 for SCLTS, CRLF, and CTS in Santa Cruz County and northern Monterey County. Detections marked with an asterisk (*) represent new detections.

	TRAD 2021			eDNA 2021			TRAD 2022			eDNA 2022		
Wetland I.D.	**SCLTS**	**CRLF**	**CTS**	**SCLTS**	**CRLF**	**CTS**	**SCLTS**	**CRLF**	**CTS**	**SCLTS**	**CRLF**	**CTS**
# Dets. ->	**14**	**22**	**7**	**18**	**27**	**11**	**13**	**19**	**11**	**17**	**21**	**10**
APT.01	X			X			X	X				
APT.02							X			X		
APT.03	X			X						X		
APT.04	X			X			X			X		
APT.05	X	X	X	X	X	X	X	X	X	X	X	X
APT.06	X	X	X	X	X	X	X		X	X		
APT.07	X			X	X	X		X		X	X	
APT.08				X			X			X		

APT.09				X						X		
APT.10												
APT.11	X			X			X			X		
APT.12							X			X		
APT.13	X			X			X			X		
APT.14	X	X		X	X					X		
APT.15	X	X		X	X		Did not survey in 2022			Did not survey in 2022		
APT.16	X						Did not survey in 2022			Did not survey in 2022		
ES.01					X							
ES.02						X			X			X
ES.03												
ES.04		X			X			X			X	
ES.05		X			X			X			X	
ES.06		X			X			X			X	
ES.07		X			X			X			X	
ES.08												
ES.09	X			X			X			X		
ES.10	X			X			X			X		
ES.11				X								
ES.12			X			X			X			X
ES.13	X			X			X			X		
ES.14				X						X		
ES.15							X	X		X	X	
ES.16												
ES.17												
ES.18			X			X			X			X
ES.19												
ES.20							Did not survey in 2022			Did not survey in 2022		
ES.21					X		Did not survey in 2022			Did not survey in 2022		
FO.01												
FO.02												
FO.03												
FO.04						X						X
FO.05			X						X			
FO.06						X			X			X
FO.07									X			X
FO.08							Did not survey in 2022			Did not survey in 2022		
FO.09							Did not survey in 2022			Did not survey in 2022		
FO.10							Did not survey in 2022			Did not survey in 2022		
PAC.01		X			X			X			X	
PAC.02					X			X			X	

PAC.03		X			X						X	
PAC.04												
PAC.05			X*		X	X*		X				
PAC.06		X			X			X			X	
PAC.07		X			X			X			X	
PAC.08		X			X						X	
SLC.01		X			X			X			X	
SLC.02		X			X			X			X	
SLC.03		X			X			X			X	
SLC.04					X							
SLC.05		X			X	X			X		X	X
SLC.06		X							X			X
SLC.07			X			X			X			X
SLC.08		X			X			X			X	
SLC.09		X			X			X			X	
SLC.10		X			X						X	
SLC.11		X			X			X			X	
HA.01							Did not survey in 2022			Did not survey in 2022		

	eDNA-only 2022		
	SCLTS	**CRLF**	**CTS**
APT.17			
APT.18			
APT.19	**X**		
APT.20	**X**		
APT.21			
APT.22			
APT.23			
APT.24			
APT.25			
APT.26			
APT.27			
APT.28			
APT.29			
APT.30	**X**		
APT.31			
APT.32			
APT.33			
APT.34			
APT.35		**X**	
APT.36		**X**	

APT.37			
DR.01		**X**	
DR.02		**X**	
DR.03		**X**	
DR.04		**X**	
DR.05			**X**
DR.06		**X**	
DR.07		**X**	**X**
DR.08		**X**	
DR.09		**X**	**X**
DR.10			**X**
DR.11		**X**	
DR.12			
DR.13		**X**	**X**
DR.14		**X**	
DR.15		**X**	
DR.16		**X**	
DR.17		**X**	
DR.18		**X**	
ES.22		**X**	
ES.23			
ES.24			
ES.25		**X***	
ES.26			
ES.27			
ES.28			
ES.29			
ES.30			
ES.31			
ES.32			
ES.33	**X***		
ES.34	**X***		
ES.35			
ES.36			
ES.37			
ES.38		**X**	
ES.39			
ES.40			
ES.41			

ES.42	**X***		
ES.43	**X**		
ES.44			
ES.45			
ES.46		**X**	
FO.11			
PAC.08		**X**	
PAC.09		**X**	
PAC.10		**X**	
PAC.11		**X**	
PAC.12		**X**	
PAC.13		**X**	
PAC.14			
PC.01		**X**	
PC.02		**X**	
PC.03			
SC.01			
SC.02			
SC.03		**X**	
SC.04		**X**	
SC.05			
SC.06			
SC.07			
SC.08			
SC.09			
SC.10			
SC.11		**X**	
SLC.12			
SLC.13		**X**	
SLC.14		**X**	
SLC.15			
SLC.16			
SLC.17			
SLC.18			
SLC.19			
SLC.20			
SLC.21		**X**	
SLC.22		**X**	
SLC.23			

SLC.24		X	
SLC.25		X	
SLC.26		X	
SLC.27		X	
SLC.28		X	
SLC.29		X	
SLC.30			
SLC.31			
SLC.32		X	
SLC.33		X	
SLC.34		X	
UC.01			
UC.02			
UC.03			
UC.04			
WAT.01			
WAT.02		X	
WAT.03			
WAT.04			
WAT.05			
WAT.06			
WAT.07			
WAT.08			
WAT.09			
WAT.10			
WAT.11			
WAT.12			
WAT.13			
WAT.14			
WAT.15			

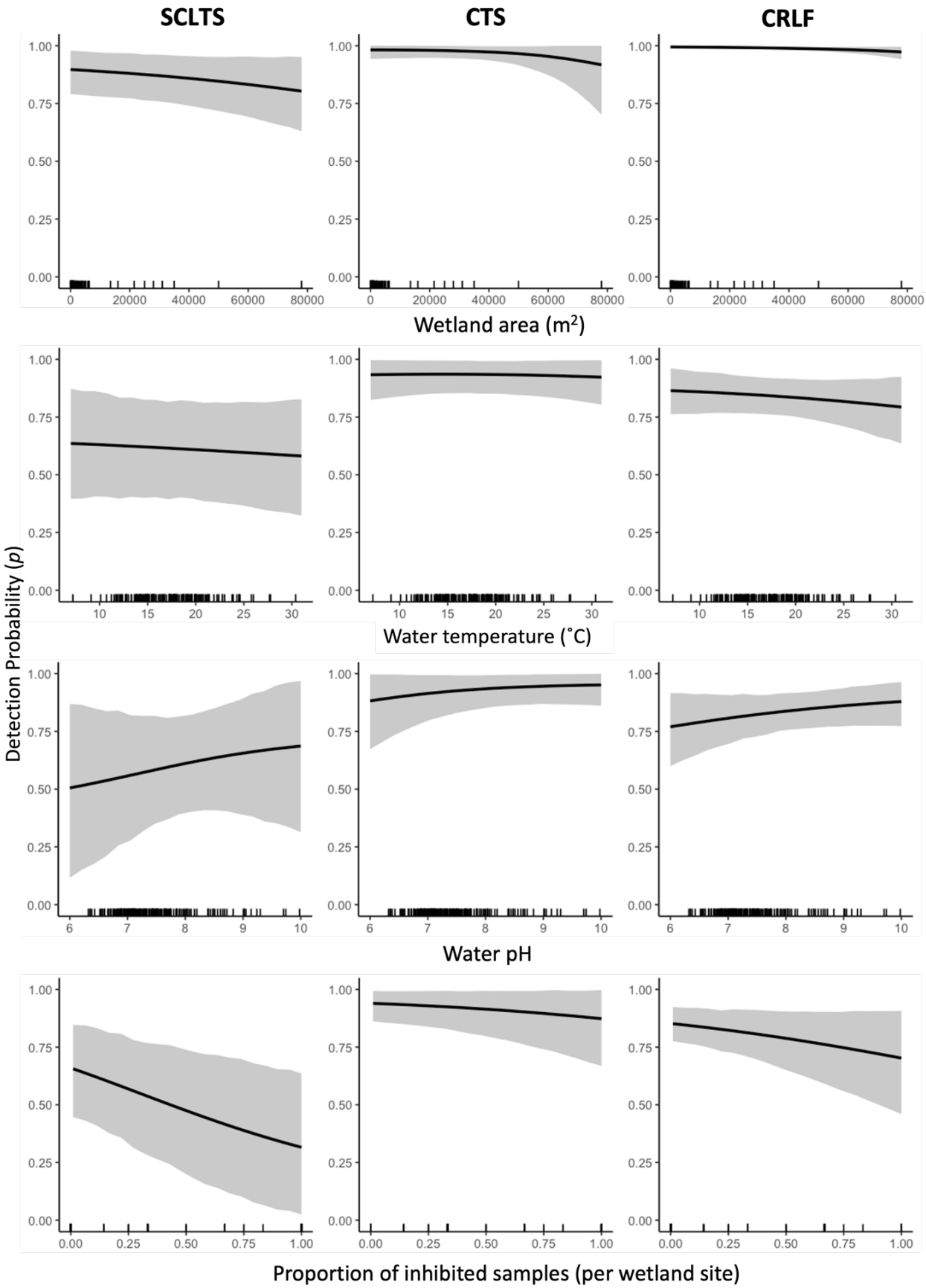
SUPPORTING TEXT F - Occupancy Model Results
Model Diagnostics

All MCMC chains converged in the multi-species occupancy model, as indicated by the Gelman-Rubin statistic. There was no evidence of a lack of model fit from the Bayes *p*-value ($p = 0.50$); a *p*-value near 0.50 indicates that the simulated data is well-fit and distributed around the observed data (Gelman et al., 2013).

Mean Occupancy Model Probabilities

Table S10. Mean occupancy model posterior probabilities and 95% credible intervals for occupancy (Ψ) and detection (p) for the Santa Cruz long-toed salamander (SCLTS), California tiger salamander (CTS), and California red-legged frog (CRLF), from the 2022 eDNA surveys.

	Occupancy (*Ψ*)		**Detection (*p*)**	
Species	**Mean**	**95% CRI**	**Mean**	**95% CRI**
SCLTS	0.21	0.08, 0.46	0.75	0.54, 0.88
CTS	0.14	0.04, 0.35	0.96	0.87, 0.99
CRLF	0.37	0.17, 0.49	0.89	0.81, 0.94


SCLTS
CTS
CRLF
Detection Probability (p)
Wetland area (m²)
Water temperature (˚C)
Water pH
Proportion of inhibited samples (per wetland site)

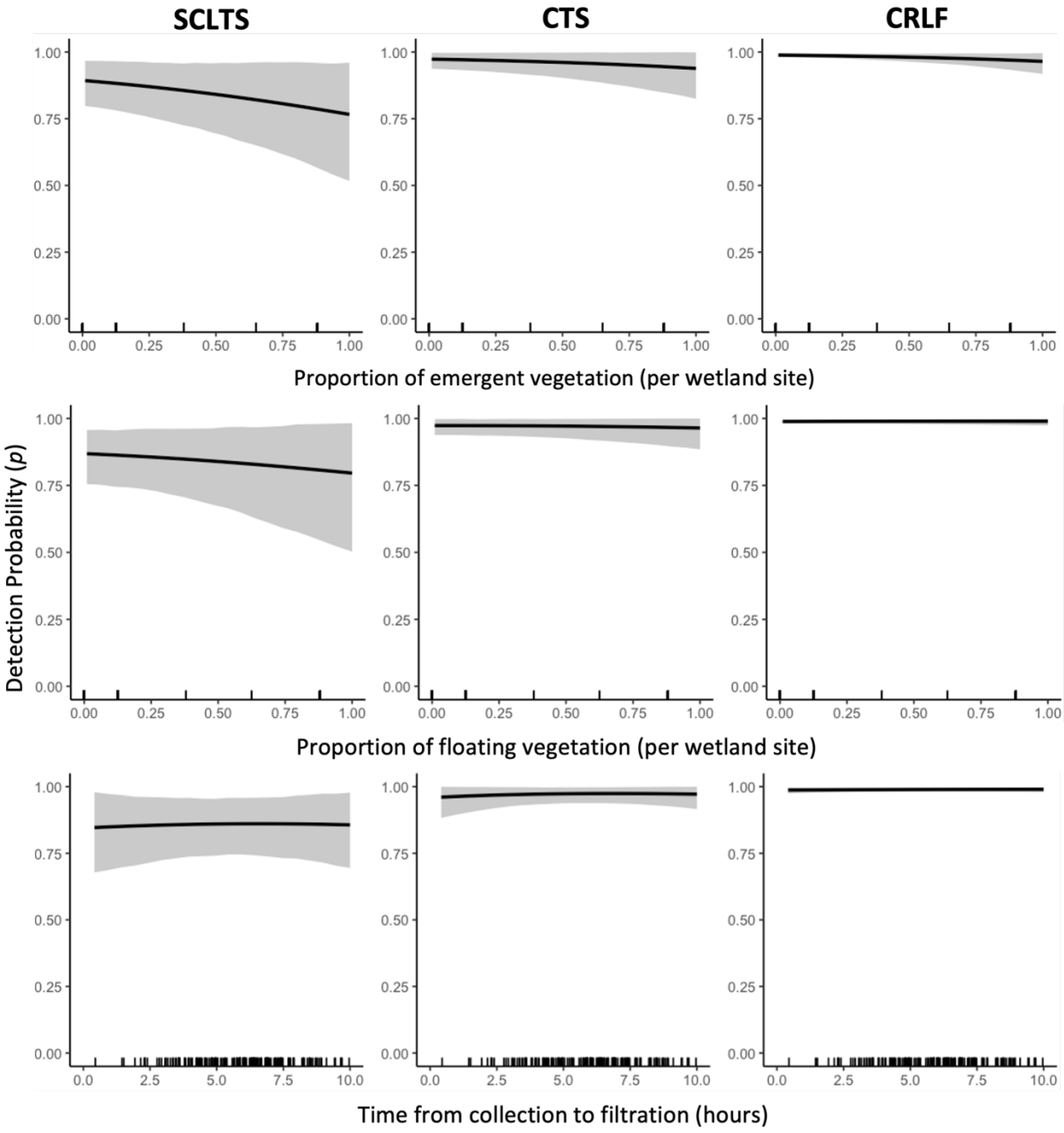
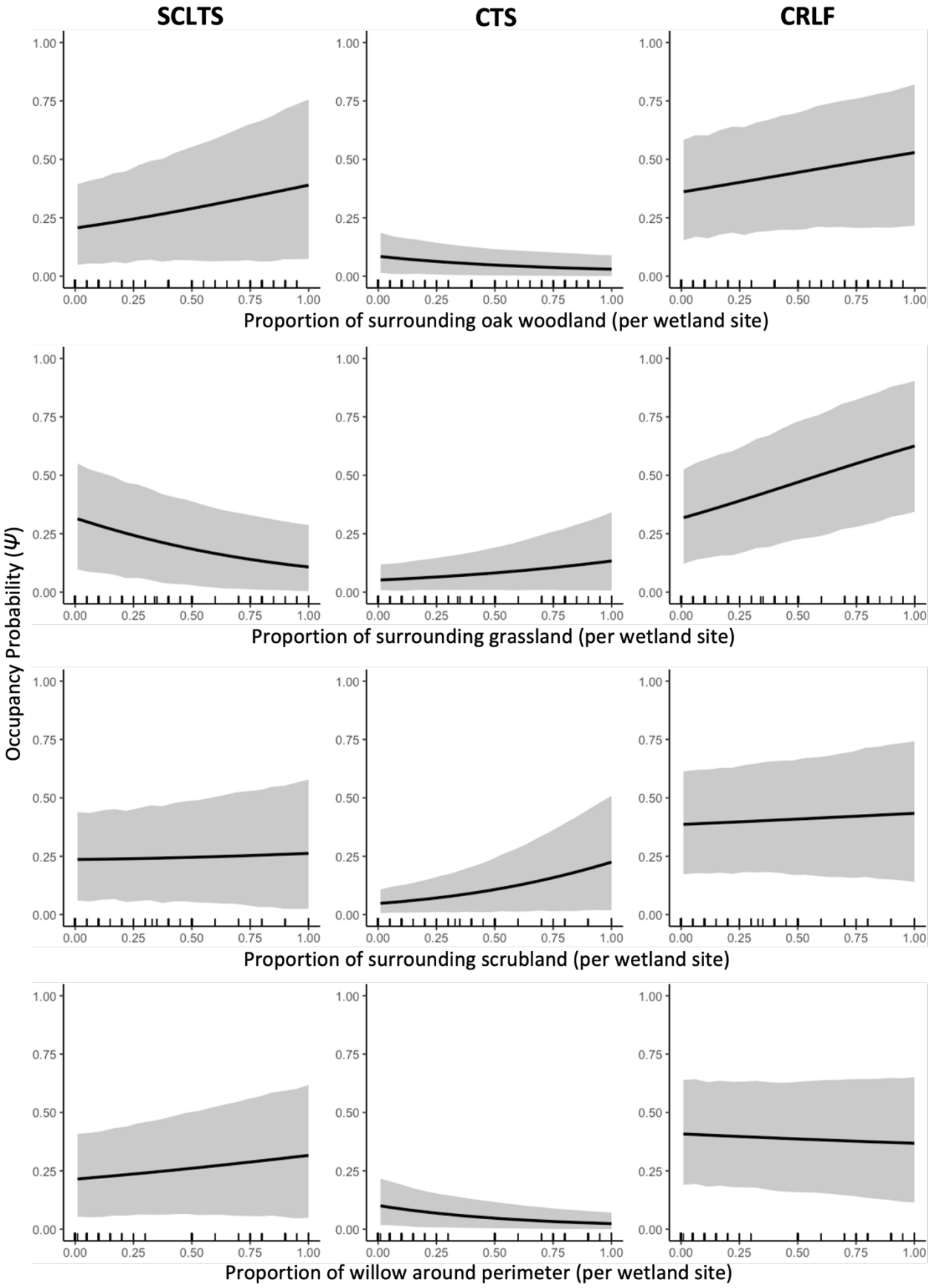


Figure S3. Model predicted Santa Cruz long-toed (SCLTS), California tiger salamander (CTS), and California red-legged frog (CRLF) detection probabilities for wetland area (m^2), water temperature (°C), water pH, the proportion of inhibited samples per wetland site, proportion of emergent vegetation, proportion of floating vegetation, and the time from sample collection to filtration (hours). Solid lines represent the mean predicted estimate and shading represents the 95% credible intervals. Model predictions were estimated by randomly sampling 10,000 draws from the posterior probability distributions, while holding all other parameters at their mean posterior probability distribution value. Observed data ranges for each variable represent the rug plot along the x-axes.


SCLTS
CTS
CRLF
Occupancy Probability (Ψ)
Proportion of surrounding oak woodland (per wetland site)
Proportion of surrounding grassland (per wetland site)
Proportion of surrounding scrubland (per wetland site)
Proportion of willow around perimeter (per wetland site)
0.00
0.25
0.50
0.75
1.00

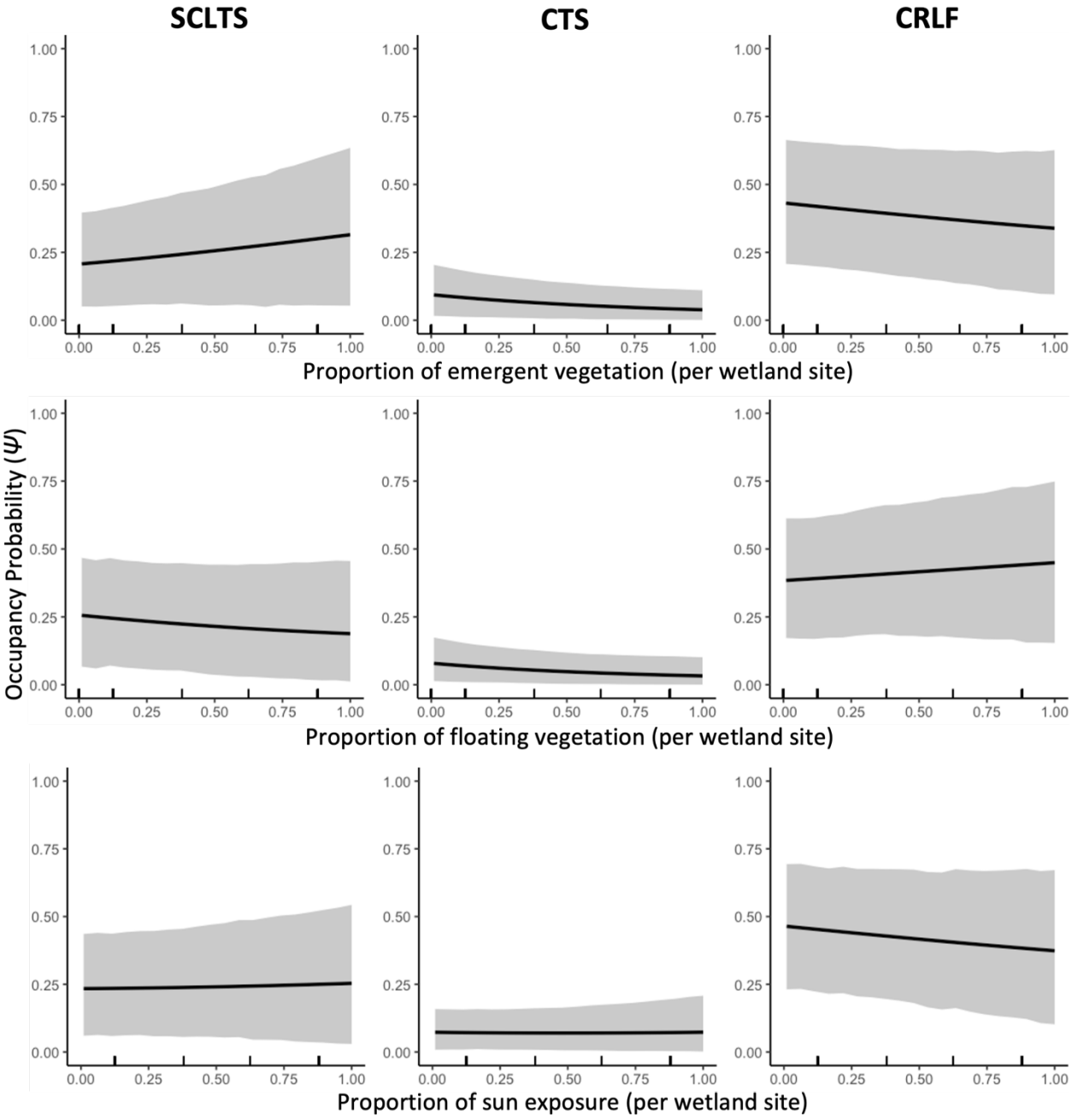


Figure S4. Model predicted Santa Cruz long-toed salamander (SCLTS), California tiger salamander (CTS), and California red-legged frog (CRLF) occupancy probabilities for the proportion of surrounding oak woodland, grassland, scrubland and the proportion of wetland perimeter covered with willows, proportion of emergent vegetation, floating vegetation, and sun exposure per wetland site. Solid lines represent the mean predicted estimate and shading represents the 95% credible intervals. Model predictions were estimated by randomly sampling 10,000 draws from the posterior probability distributions, while holding all other parameters at their mean posterior probability distribution value. Observed data for each variable are represented by the rug plot along the x-axes.

		SCLTS				CRLF				CTS			
	Parameter	Est.	S.D.	2.50%	97.50%	Est.	S.D.	2.50%	97.50%	Est.	S.D.	2.50%	97.50%
Occupancy	Int.	-1.298	0.589	-2.457	-0.146	-0.549	0.521	-1.575	0.468	-1.845	0.642	-3.118	-0.607
	emv	0.541	0.597	-0.626	1.715	-0.451	0.468	-1.371	0.464	-1.172	0.677	-2.524	0.128
	flv	-0.565	0.691	-1.931	0.783	0.278	0.495	-0.694	1.245	-1.211	0.795	-2.82	0.289
	sun	0.035	0.612	-1.168	1.233	-0.425	0.51	-1.423	0.573	-0.181	0.699	-1.539	1.203
	oak	0.915	0.702	-0.465	2.287	0.738	0.496	-0.232	1.711	-1.401	0.79	-2.991	0.110
	grass	-1.586	0.710	-3.007	-0.224	1.389	0.479	0.456	2.332	0.931	0.631	-0.312	2.167
	scrub	0.06	0.676	-1.278	1.372	0.205	0.517	-0.821	1.211	1.738	0.623	0.515	2.961
	willow	0.521	0.528	-0.522	1.548	-0.207	0.44	-1.073	0.651	-1.82	0.741	-3.339	-0.43
Detection	Int	1.097	0.478	0.147	2.027	2.085	0.318	1.482	2.729	3.189	0.690	1.909	4.609
	ph	0.284	0.521	-0.815	1.344	0.244	0.234	-0.21	0.71	0.307	0.427	-0.483	1.254
	temp	-0.080	0.201	-0.473	0.319	-0.163	0.226	-0.629	0.266	-0.054	0.314	-0.649	0.644
	area	-0.245	0.198	-0.648	0.130	-0.509	0.302	-1.118	0.072	-0.509	0.64	-2.035	0.678
	tfilter	0.025	0.292	-0.578	0.584	0.072	0.217	-0.351	0.499	0.12	0.465	-0.8	1.161
	prop.inhib	-1.617	0.809	-3.337	-0.199	-0.859	0.533	-1.884	0.21	-0.688	0.716	-2.007	0.828
	flv	-0.409	0.778	-2.031	1.039	0.266	0.647	-0.952	1.584	0.012	0.881	-1.762	1.801
	emv	-0.903	0.563	-2.018	0.201	-0.998	0.561	-2.135	0.072	-0.696	0.724	-2.03	0.879

Table S11. The mean occupancy model posterior distribution parameter estimates, standard deviation, and the 2.50% and 97.50% credible intervals (logit scale) for species occupancy and detection, for the Santa Cruz long-toed salamander (SCLTS), California tiger salamander (CTS), and California red-legged frog (CRLF).

OCCUPANCY MODEL JAGS CODE

```
sink("model1.txt")
cat("
  model {
  ####Hyperparameters for detection only
  mu.p0 ~ dnorm(0,1)
  sd.p0 ~ dunif(0,1)
  tau.p0 <- 1/sd.p0^2

  mu.ph.p ~ dnorm(0,1)
  sd.ph.p ~ dunif(0,1)
  tau.ph.p <- 1/sd.ph.p^2

  mu.temp.p ~ dnorm(0,1)
  sd.temp.p ~ dunif(0,1)
  tau.temp.p <- 1/sd.temp.p^2

  mu.area.p ~ dnorm(0,1)
  sd.area.p ~ dunif(0,1)
  tau.area.p <- 1/sd.area.p^2

  mu.tfilter.p ~ dnorm(0,1)
  sd.tfilter.p ~ dunif(0,1)
  tau.tfilter.p <- 1/sd.tfilter.p^2

  mu.prop.inhib.p ~ dnorm(0,1)
  sd.prop.inhib.p ~ dunif(0,1)
  tau.prop.inhib.p <- 1/sd.prop.inhib.p^2

  mu.flv.p ~ dnorm(0,1)
  sd.flv.p ~ dunif(0,1)
  tau.flv.p <- 1/sd.flv.p^2

  mu.emv.p ~ dnorm(0,1)
  sd.emv.p ~ dunif(0,1)
  tau.emv.p <- 1/sd.emv.p^2

  #####Priors
    for(k in 1:nspec){
   p0[k] ~ dnorm(mu.p0, tau.p0)
   ph.p[k] ~ dnorm(mu.ph.p, tau.ph.p)
   temp.p[k] ~ dnorm(mu.temp.p, tau.temp.p)
   area.p[k] ~ dnorm(mu.area.p, tau.area.p)
   tfilter.p[k] ~ dnorm(mu.tfilter.p, tau.tfilter.p)
   prop.inhib.p[k] ~ dnorm(mu.prop.inhib.p, tau.prop.inhib.p)
   flv.p[k] ~ dnorm(mu.flv.p, tau.flv.p)
   emv.p[k] ~ dnorm(mu.emv.p, tau.emv.p)

   psi0[k] ~ dnorm(0,1)
   emv.psi[k] ~ dnorm(0,1)
```

```
    flv.psi[k] ~ dnorm(0,1)
    sun.psi[k] ~ dnorm(0,1)
    oak.psi[k] ~ dnorm(0,1)
    grass.psi[k] ~ dnorm(0,1)
    scrub.psi[k] ~ dnorm(0,1)
    willow.psi[k] ~ dnorm(0,1)
  }

 ##### State model
  for(k in 1:nspec){
    for(i in 1:nsite){
      logit(psi[i,k]) <- psi0[k] + emv.psi[k] * emv[i,k] +
        flv.psi[k] * flv[i,k] +
        sun.psi[k] * sun[i,k] +
        oak.psi[k] * oak[i,k] +
        grass.psi[k] * grass[i,k] +
        scrub.psi[k] * scrub[i,k] +
        willow.psi[k] * willow[i,k]
      z[i,k] ~ dbern(psi[i,k]) #latent state z
    }
  }

  ##### Observation model
  for(k in 1:nspec){
    for(i in 1:nsite){
      for(j in 1:nrep){
        logit(p[i,j,k]) <- p0[k] + ph.p[k] * ph[i,k] +
          temp.p[k] * temp[i,k] +
          area.p[k] * area[i,k] +
          tfilter.p[k] * tfilter[i,k] +
          prop.inhib.p[k] * prop.inhib[i,k] +
          flv.p[k] * flv[i,k] +
          emv.p[k] * emv[i,k]
        mu.p[i,j,k] <- z[i,k] * p[i,j,k] #probability of detection
        Y.all[i,j,k] ~ dbern(mu.p[i,j,k]) #detection, non-detection
```

Traditional Survey Naïve Occupancy Rates

Table S12. Naïve occupancy rates for the Santa Cruz long-toed salamander (SCLTS), California tiger salamander (CTS), and California red-legged frog (CRLF) from the 2021 and 2022 standard field surveys. We surveyed 46 wetlands within the SCLTS range, and 67 wetlands for CTS and CRLF in 2021. In 2022, we surveyed 41 wetlands within the SCLTS range, and 59 wetlands for CTS and CRLF.

Species	2021	2022
SCLTS	0.30	0.32
CTS	0.10	0.19
CRLF	0.33	0.32

Habitat and Environmental Variables Measured

Habitat Variables

Table S13. Habitat variables measured at wetlands throughout the Monterey Bay area, California in 2022. Floating and emergent vegetation, and sun exposure classes (0–4) correspond to the range of the proportion of the wetland surface area containing vegetation and exposed to sunlight, respectively. Wetland counts are recorded under each class.

Variable	0 (0%)	1 (1 - 25%)	2 (26 – 50%)	3 (51 – 75%)	4 (76 – 100%)
Floating Veg.	84	54	8	6	21
Emergent Veg.	20	76	23	18	36
Sun Exposure	0	27	11	13	122

Environmental Variables

Table S14. Habitat and environmental variables measured at wetlands throughout the Monterey Bay area, California in 2022. Water temperature and pH measurements are recorded from a single eDNA water sample collection location along the perimeter of a wetland. Wetland area (m^2) is estimated using a range finder at the time of the survey.

Variable	Median	Minimum	Maximum
Water Temperature (°C)	17.28	7.22	30.39
Water pH	7.29	6.32	9.98
Area (m^2)	376	12	78,000

Table S15. List of the many volunteers and other helpers who assisted with field surveys, wetland access, and survey logistics.

Name	Affiliation	Geographic area	Field assistance	Site access and logistics
Amy Kennedy	Private Landowner	Monterey Co.	X	
Amy Palkovics	California State Parks	Monterey Co.		X
Ashley Baillie	Central Coast Wetlands Group	Monterey Co.		
Bart Kowalski	Chenega Tri-Services, LLC (U.S. Army/Fort Ord)	Monterey Co.	X	X
Bonny and Colin Fulton	Private Landowner	Monterey Co.		X
Brian Woodward	Santa Lucia Conservancy	Monterey Co.	X	X
Bruce Delgado	Bureau of Land Management	Monterey Co.	X	
Bryan Largay	Land Trust of Santa Cruz County	Santa Cruz Co.		X
Bryan Mori	Bryan Mori Biological Consulting Services	Santa Cruz and Monterey Cos.		X
Carly White	California Department of Fish and Wildlife	Santa Cruz Co.	X	
Celeste Stanik	Elkhorn Slough Reserve Volunteer	Elkhorn Slough	X	

Chad Mitcham	U.S. Fish and Wildlife Service	Santa Cruz Co.	X	X
Chad Steiner	Biosearch Environmental Consulting	Santa Cruz Co.	X	
Chris Caris	U.S. Fish and Wildlife Service	Santa Cruz Co.	X	X
Christy Wyckhoff	Santa Lucia Conservancy	Monterey Co.	X	X
Connor O'Hara-Baker	Elkhorn Slough Foundation	Elkhorn Slough	X	
Dan Hermstad	Santa Cruz Resource Conservation District	Santa Cruz Co.		
Dannie Daniels	Private Landowner	Monterey Co.	X	X
Dash Dunkell	Elkhorn Slough Foundation	Elkhorn Slough	X	X
Dave Feliz	Elkhorn Slough Reserve	Monterey Co.	X	
Dave Feliz	California Department of Fish and Wildlife	Elkhorn Slough	X	X
Debie Chirco-Macdonald	Community Volunteer	Santa Cruz Co.	X	X
Diane Kodama	U.S. Fish and Wildlife Service	Santa Cruz Co.		X
Eric Tynan	Castroville Community Services District	Monterey Co.		X
Erika Knudsen	Community Member	Santa Cruz Co.		X
Freedom Greenhouse	Jacobs Farm	Santa Cruz Co.		X
Gabi Estill	Elkhorn Slough Foundation	Elkhorn Slough	X	
Gage Dayton	University of California Santa Cruz Natural Reserves	Santa Cruz/Monterey Cos.	X	X
Garrett Smart	Santa Cruz County	Santa Cruz Co.		X
Greg Feaster	Pajaro Dunes North	Santa Cruz Co.		
Helen Carr	Private Landowner	Santa Cruz Co.		X
Jackie Nelson	Monterey Peninsula Regional Park District	Monterey Co.		X
Jake Smith	Monterey Peninsula Regional Park District	Monterey Co.		X
James Oakden	Coastal Conservation and Research	Monterey Co.		X
Javier Zamora	JSM Organics	Monterey Co.		X
Jay Ryan	Central Coast Wetlands Group	Monterey Co.		
Jeff Cann	California Department of Fish and Wildlife	Monterey Co.		X
Jill McKee	Private Landowner	Santa Cruz Co.		X
Jonathan Pilch	Watsonville Wetlands Watch	Santa Cruz Co.		X

Josie Moss	Community Volunteer	Santa Cruz Co.	X	
Kelli Camara	Santa Cruz Resource Conservation District	Santa Cruz Co.		X
Ken Collins	Elkhorn Slough Foundation	Elkhorn Slough	X	X
Ken Pollak	Elkhorn Slough Reserve Volunteer	Monterey Co.	X	
Kevin Contreras	Elkhorn Slough Foundation	Elkhorn Slough		X
Kevin O'Connor	Central Coast Wetlands Group	Monterey Co.		X
Kim Glinka	Community Volunteer	Santa Cruz Co.	X	
Lauren Sullivan	U.S. Department of Agriculture		X	
Lawrence Erickson	Community Volunteer	Santa Cruz Co.	X	
Mark Allaback	Biosearch Environmental Consulting	Santa Cruz Co.	X	X
Mark Harris	North Monterey County High School	Monterey Co.		X
Mark Silberstein	Elkhorn Slough Foundation	Elkhorn Slough	X	X
Mason Cole	Coastal Conservation and Research	Monterey Co.		X
Mollie Dorrance Lambert	Dorrance Ranches	Monterey Co.	X	X
Nancy Burnett	Packard Ranch	Monterey Co.	X	X
Nancy Scarborough	Community Volunteer	Santa Cruz Co.	X	
Patrice Kinion	Private Landowner	Santa Cruz Co.		X
Patrick Furtado	Community Volunteer	Monterey Co.	X	
Pedro Rodriguez	Packard Ranch	Monterey Co.		X
Peter & Helen Carr	Private Landowner	Santa Cruz Co.		X
Rachel Anderson	Biological Consultant	Monterey Co.		X
Rebecca Hurley	Santa Cruz County	Santa Cruz Co.		X
Robert Stephens	Packard Ranch	Monterey Co.		X
Ron Goodman	Community Volunteer	Santa Cruz Co.	X	
Ross Clark	Central Coast Wetlands Group	Monterey Co.		X
Shawn Wagoner	Burleson Consulting	Monterey Co.	X	
Sheryl Gaebelein	Elkhorn Slough Reserve Volunteer	Monterey Co.	X	
Steve Dorrance	Dorrance Ranches	Monterey Co.		X
Steve & Cindy Engebretson	Private Landowner	Santa Cruz Co.		X
Tanya and Jess Atkinson	Private Landowner	Monterey Co.		X

Terris Kasteen	California Department of Fish and Wildlife	Santa Cruz Co.	X	X
Thor Anderson	Burleson Consulting	Monterey Co.	X	
Tony Vastola	Elkhorn Slough Reserve Volunteer	Monterey Co.	X	
Traci Filous	Land Trust of Santa Cruz County	Santa Cruz Co.		X

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