How extreme drought events, introduced species, and disease interact to influence threatened amphibian populations

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Abstract: Over the past century, native amphibian species in California, USA, have declined in geographic range and population size, including precipitous declines of the California Red-legged Frog *Rana draytonii* Baird and Girard, 1852 and the California Tiger Salamander *Ambystoma californiense* Gray, 1853. Habitat loss, infectious disease (e.g., chytridiomycosis), and the introduction of invasive species are potential contributors to this decline, but it is unclear how these variables interact with extreme weather events, such as prolonged drought to affect amphibian population dynamics. We used a 7-y dataset (2013–2019) on amphibian breeding activity that encompassed a recent extreme drought event to assess 1) how breeding activities of these taxa differed during and after drought conditions and 2) the additive and interactive relationships of drought, chytrid fungal infection (Bd), and invasive species with the presence and abundance of larval amphibians. Based on 650 surveys of 110 lentic habitats in California, both amphibian species were highly sensitive to drought severity, such that the proportion of occupied sites during peak drought conditions decreased by 36% for *R. draytonii* and 87% for *A. californiense* relative to the initial year of the study. However, both taxa showed strong recovery, with increases in breeding occurrence as drought conditions eased in the following years (2016–2019). Invasive fishes and American Bullfrogs (*Rana catesbeiana* Shaw, 1802) also negatively predicted the occurrence and density of the 2 native taxa. Finally, we found that the relationship between Bd and *R. draytonii* larval density was influenced by drought conditions, suggesting that Bd's transmission rates or effects on host persistence are sensitive to climate. These findings illustrate the ability of native taxa to recover from recent drought conditions and reinforce the importance of controlling invasive species for improving breeding activities of threatened amphibians.

Key words: drought, invasive species, threatened amphibians, chytrid fungus, climate change, anthropogenic stressors

Freshwater ecosystems are considered highly vulnerable to both climate change and anthropogenic stressors (Woodward et al. 2010, Reid et al. 2019), with higher rates of species endangerment and extinctions relative to terrestrial and marine environments (Collen et al. 2009). Most threats to freshwater ecosystems are anthropogenic, including invasive species introductions, habitat modifications, and human-influenced disease outbreaks (Reid et al. 2019). Anthropogenic threats often occur alongside additional stressors imposed through changing climates, including more severe droughts (Lennox et al. 2019) and abnormal heat waves (Urrutia-Cordero et al. 2020). Freshwater ecosystems are essential for nearly all forms of life, so it is crucial to understand how these threats influence both the biological communities of freshwater habitats (Jackson et al. 2016) and the ecosystem functions they provide (McMahon et al. 2012). However, most studies focus on individual stressors in isolation such that the magnitude and direction of interactions among potential variables are often unknown—despite evidence that freshwater ecosystems are simultaneously

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threatened by changing climates, habitat modification, and introduced species and disease (Craig et al. 2017, Orr et al. 2020). Understanding the additive, as well as interactive, consequences of such threats is needed for effectively managing sensitive freshwater ecosystems.

Some of the most imperiled taxa in freshwater habitats are amphibians, whose populations suffer from habit alterations and deadly disease outbreaks (Lawler et al. 1999, Blaustein et al. 2012, Scheele et al. 2019). In California, USA, native amphibian species have declined precipitously because of habitat loss, invasive species introductions, and infectious disease (Fisher and Shaffer 1996, Davidson et al. 2001, Piovia-Scott et al. 2015). Currently, <20% of California's historical wetland habitat persists (Dahl 1990, Stein et al. 2014), and many of the remaining aquatic environments have been heavily modified. The California Red-legged Frog *Rana draytonii* Baird and Girard, 1852 and the California Tiger Salamander *Ambystoma californiense* Gray, 1853 have declined across an estimated 70% of their range and, as a result, are now under federal and state protection (Jennings and Hayes 1994, Fisher and Shaffer 1996, Davidson et al. 2001). Specifically, in the San Francisco Bay Area region (hereafter referred to as the Bay Area), livestock ponds and other constructed wetlands are important breeding sites for *A. californiense* and *R. draytonii* following the loss of natural wetland habitat (Bobzien and DiDonato 2007, Howell et al. 2019). Even in protected or well-managed habitats, however, occupancy and breeding success can be limited by local conditions. Invasive aquatic species such as the American Bullfrog *Rana catesbeiana* Shaw, 1802 and introduced fishes (e.g., bass, sunfish, and mosquitofish) often consume or compete with larvae of native amphibians (Jennings and Hayes 1994, Lawler et al. 1999). Furthermore, invasive species can function as reservoirs for pathogens (Daszak et al. 2004, Schloegel et al. 2010), such as the chytrid fungus (*Batrachochytrium dendrobatidis* [Bd]; but see Blaustein et al. 2020). Thus, alongside habitat preservation, active management of invasive species and pathogens may be necessary to maintain and promote populations of *A. californiense* and *R. draytonii* (USFWS 2002, 2017).

Globally, amphibian chytridiomycosis (a disease caused by infection by the fungus Bd) has been identified as a primary driver in the decline and extinction of >200 amphibian species (e.g., Scheele et al. 2019). This amphibian chytrid fungus has been detected in amphibian samples from California dating back to the 1910s (Huss et al. 2013, Adams et al. 2017, Vredenburg et al. 2019) and is known to infect a wide diversity of native amphibian taxa (Fellers et al. 2011, Wilber et al. 2020). However, the resulting fitness effects of infection can be divergent across species and populations (Joseph and Knapp 2018, Russell et al. 2019). For instance, although the introduction and spread of Bd throughout California's Sierra Nevada mountains has likely led to widespread declines in the Mountain Yellow-legged Frog complex, *Rana sierrae* Camp, 1917 and *Rana muscosa* Camp, 1917 (Fellers et al. 2001, Briggs et al. 2010, Vredenburg et al. 2019), there is little evidence for disease-driven declines in native species from lower elevation areas like the Bay Area (Fellers et al. 2011, Adams et al. 2020, Wilber et al. 2020). These differences could be attributable to several mechanisms, such as variation in amphibian immune response (e.g., associated with elevational temperature differences that influence the outcome of Bd–amphibian pathology [Cohen et al. 2017]), as well as the possibility of endemic host–pathogen dynamics, whereby moderate levels of infection have few effects on host fitness (Briggs et al. 2010). Bd infection is also sensitive to changes in temperature (Cohen et al. 2017), host density (Rachowicz and Briggs 2007), and habitat characteristics (Padgett-Flohr and Hopkins 2009). Understanding such variation in Bd pathology can provide land managers with additional insight into when and where to prioritize pathogen control relative to other threats (Scheele et al. 2014, Canessa et al. 2018).

An important yet largely unaddressed question concerning freshwater ecosystems is how extreme weather events will interact with habitat availability, invasive species, and infectious disease to affect management of aquatic species. The recent and extreme drought in California (2013–2015)—which is considered to be among the most severe in the past 1200 y (Griffin and Anchukaitis 2014)—is likely to have both short- and long-term effects on aquatic communities. Because droughts in the southwestern USA are expected to become more frequent and more severe over the next century (Cook et al. 2004, Mann and Gleick 2015), it is essential to account for their direct and indirect effects on existing management plans. Amphibians are among the most sensitive taxa to the effects of drought (Foden et al. 2009, Walls et al. 2013), which can shorten wetland hydroperiod or eliminate habitats altogether (Daszak et al. 2004, McMenamin et al. 2008, Hossack 2017, Moss et al. 2021). The resultant increases in amphibian densities at wetlands with extant water can enhance pathogen transmission (e.g., Rohr and Palmer 2013), and associated increases in water temperatures and aridity can directly influence amphibian physiological processes, such as water uptake, development time, and susceptibility to infections (Hillyard 1999, McMenamin and Hadley 2010, Blaustein et al. 2012). At the landscape level, changes in the frequencies of drought or flooding may also influence the capacity of water-borne pathogens or invasive species to disperse among aquatic habitats (Padgett-Flohr and Hopkins 2009). However, episodic drought also has the potential to cause local extirpations of invasive predators, such as fishes and American Bullfrogs (Hecnar and M'Closkey 1997, Lennox et al. 2019), which both require permanent water. Because amphibians native to the western USA are more adapted to both intra- and interannual variation in water availability (Stebbins and Cohen 2021), the short-term costs of drought could be offset by longer-term gains, provided adult amphibians can persist until rains return.

The combination of such physical and biological threats highlights the complexity of understanding the net effects of extreme weather events on threatened amphibians in California and emphasizes the importance of long-term data collection to detect non-transient effects (Pechmann et al. 1991, Houlahan et al. 2000, Stuart et al. 2004). Because breeding dynamics unfold at annual time scales, longer-duration studies are necessary to overcome natural inter-annual variation. We sought to understand the complex and interacting relationships among variables structuring native amphibian populations in California. We used a 7-y dataset (2013–2019) on amphibian breeding activity that spans a recent extreme drought event to assess the direct effects of the drought on the breeding activities of these species and to assess the additive and interactive relationships of drought and other amphibian breeding stressors with the presence and abundance of larval amphibians. We expected to observe reduced breeding occurrence and density of the native species during the most severe drought years and to observe strong recovery during years with adequate rain fall. Because drought patterns likely influence additional amphibian breeding stressors, such as the distribution and behavior of invasive species and patterns of Bd spread, we expected to observe nuanced interactions of drought with other amphibian breeding stressors on amphibian breeding patterns.

METHODS

To address our research objectives, we intensively monitored 110 unique sites in the Bay Area of California, USA, to evaluate how *A. californiense* and *R. draytonii* breeding activity varied across 7 y of sampling, including 3 y of an extreme drought and 3 y post drought. By assembling information on the occurrence and density of larval/juvenile amphibians from 650 site-by-year visits with data on invasive taxa and Bd infection (5736 individuals), we assessed both the additive and interactive relationships of known amphibian stressors with the breeding patterns of 2 protected species. Specifically, we used generalized linear mixed-effects modeling (GLMM) with a model selection procedure to evaluate how invasive species, disease, and their interactions with drought were related to the occurrence and density of native amphibian taxa. We conducted all statistical analysis in the R statistical environment (version 4.2.1; R Project for Statistical Computing, Vienna Austria).

Study system

The study area was located within the Bay Area of California, including Santa Clara, Alameda, and Contra Costa counties. Study sites were ponds (i.e., small [<2 ha surface area] lentic waterbodies), many which were built or modified to support cattle grazing (Garone 2011). From 2013 through 2019, we surveyed between 80 and 101 sites in a given year (Table S1) during the late spring and summer, corresponding with the developmental season for amphibian larvae. We selected ponds from managed parks and protected areas that spanned a range of environments in the East and South Bay regions of California, roughly distributed between Berkeley in the north and San Jose in the south (Fig. 1). We prioritized ponds that, based on prior sampling by our team or park scientists (Johnson et al. 2013, Joseph et al. 2016, Moss et al. 2021), had strong potential to support lentic-breeding amphibians (i.e., with a hydroperiod sufficient in most years to allow metamorphosis). Within a given park or area of land, we sampled clusters of neighboring ponds—rather than all ponds on a property—to avoid selection bias while maintaining logistical feasibility. We visited each site twice within a single season: an early-season visit in early May to mid-June and a late-season visit in mid-June to early August (see Johnson et al. 2013 for more details on the sampling protocol). During each visit, we conducted visual encounter surveys by walking the perimeter and visually detecting the presence of amphibian species while also using a handheld GPS (with the tracking function) to measure pond perimeter. To assess amphibian larval densities, we conducted 3 to 5 seines/visit with a standard size seine (1 × 3-m net area, 4-mm mesh size). For each visit, seine locations were chosen

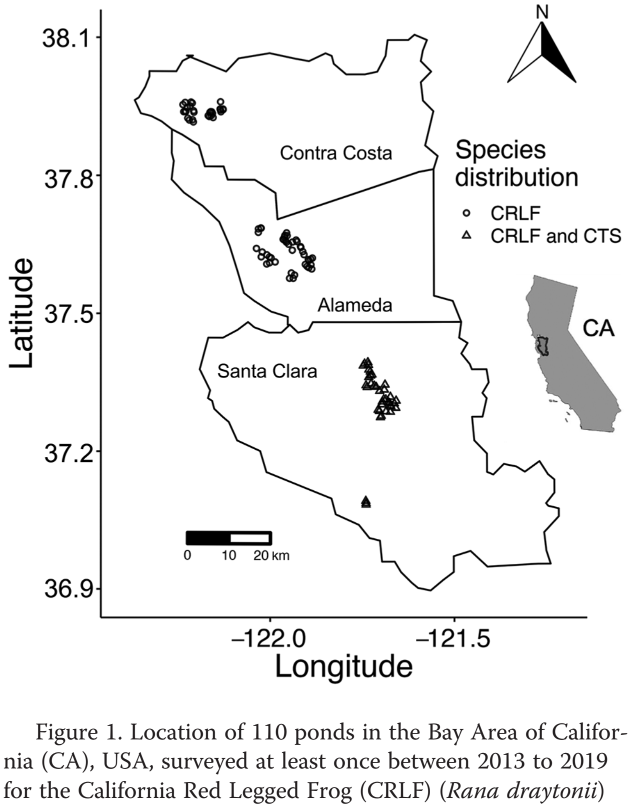

Figure 1. Location of 110 ponds in the Bay Area of California (CA), USA, surveyed at least once between 2013 to 2019 for the California Red Legged Frog (CRLF) (*Rana draytonii*) and the California Tiger Salamander (CTS) (*Ambystoma californiense*). Open circles indicate sites within only CRLF range, and open triangles points indicate sites within both CRLF and CTS ranges.

to cover different habitats in the pond (i.e., open water, shoreline, vegetated areas). For each seine, we recorded the distance seined and the count of all amphibians collected in the net (for more details see Johnson et al. 2012). We calculated density of each species as the number of individuals collected divided by the area seined and estimated larval density as the mean density across all seines at a given site visit. If we detected the presence of either larvae or recently metamorphosed *R. draytonii* or *A. californiense* with either visual survey or seine, we classified the site as supporting breeding in that year (Moss et al. 2021). Our methods were not designed to detect egg masses. All amphibians were released near the point of capture and the handling of the native amphibians followed proper protocols under approved permits (state of California sampling permit number: TE-181714-5 and TE-181714-6).

We also determined the presence and density of invasive species at each site visit based on a combination of visual encounter surveys and seine-net sampling as described above. Specifically, we quantified the presence and density of *R. catesbeiana* and invasive fishes, such as the Largemouth Bass *Micropterus salmoides* (Lacepède, 1802), the Bluegill *Lepomis macrochirus* Rafinesque, 1819, and the Western Mosquitofish *Gambusia affinis* (Baird and Girard, 1853), among others. All included species of invasive fishes are known to negatively affect native amphibian populations through mostly direct effects (e.g., consumption of larval amphibians and eggs) (Werner and McPeek 1994, Maezono and Miyashita 2003, Zeiber et al. 2008). Because of low sample sizes, we grouped all species of fish, and, thus, the density of fish was the sum total of all fish individuals caught in seines divided by the area seined. For American Bullfrogs, density estimates consisted of only larval individuals caught in seines, whereas presence included any post-hatching life stage (i.e., larvae, metamorph, or adult). We did not include invasive crayfishes because they occurred in <1% of site visits.

Infection testing for Bd

During the 2nd visit to each wetland in mid- to late summer, we swabbed late-stage larvae (*A. californiense*) or recently metamorphosed individuals (*R. draytonii* and the widespread Pacific Chorus Frog *Pseudacris regilla* [Baird and Girard, 1852], see Covariate development below) to determine Bd infection. We used a sterile synthetic swab to gently swab the chin, forelimbs, abdomen, hindlimbs, and cloaca for each taxon and life stage. Following a modified standard protocol (Briggs et al. 2010), we swabbed each location 10× for a total of 50 swabs/ind., after which the sampled individual was released in the area of capture. The swab was immediately placed in a sterile, pre-labeled vial and stored at −20°C before quantitative polymerase chain reaction testing. To test for Bd, we extracted DNA by using Prepman® (Thermo Fisher Scientific, Waltham, Massachusetts) with GeneReleaser® (Eurogentec, Seraing, Belguim) to reduce inhibition, followed by real-time quantitative polymerase chain reaction with a standardized TaqMan assay (Thermo Fisher Scientific) (Boyle et al. 2004, Briggs et al. 2010). We ran each sample in triplicate to determine infection status. A sample was deemed positive when 2/3 runs amplified with ≥1 zoospore/swab.

Statistical analysis

To assess the individual and interactive relationships of drought, invasive species, and infection with *A. californiense* and *R. draytonii* breeding patterns, we used GLMM to model 2 distinct metrics: the detection of juvenile life stages at a given site year (as indicated by the presence of larvae or recent metamorphs from across all survey methods and duplicate visits, henceforth larval occurrence) and the density of larval individuals (from seine samples). These metrics do not indicate the presence of egg masses or observations of breeding adults because our sampling window (May–August) occurred after most egg-laying and breeding events. Because of differences in geographic range sizes, we modeled *A. californiense* and *R. draytonii* larval occurrence and density patterns separately (*A. californiense* breeding only occurs in the southern portion of our sampling area; Moss et al. 2021). For the analysis of larval occurrence, our response variable was a binary variable indicating if larvae or juveniles were found at the site in a given year with either survey method (1 = yes, 0 = no). For analysis of larval densities, we only included sites at which larvae were detected, and we calculated a single pooled larval density measure site^{-1} y^{-1} (no. of ind. captured/m^2 of habitat seined). Total larval count from across the seine surveys, with the total distance covered by the seines as an offset, was the density response variable. Although mathematically equivalent to directly modeling density as a response variable, using an offset allowed us to adjust for variation in sampling effort. To determine that we met the assumptions of the GLMM, we visually inspected the distribution of the residuals to assure normal distribution.

Covariate development In models of both species occurrence and density, we included 4 distinct groups of covariates to assess relationships of different variables with *A. californiense* and *R. draytonii* breeding patterns: 1) habitat characteristics, 2) the presence and densities of invasive species (American Bullfrogs or invasive fishes), 3) infection by Bd, and 4) drought severity (Table 1). For habitat characteristics, we used a single covariate of pond perimeter, which is an important habitat characteristic in this system (Moss et al. 2021). We scaled pond perimeter by centering the values around the mean pond size across all sites and years and dividing by the standard deviation. We scaled this parameter to improve model fit. For invasive species, we included 4 variables indicating whether a site supported

Table 1. Model covariates for both breeding occurrence and breeding density models of California Red-legged Frog (*Rana draytonii*) and California Tiger Salamander (*Ambystoma californiense*) occurrences and densities in 110 ponds in the Bay Area of California, USA, between 2013 and 2019. NA = not applicable.

Covariate	Covariate type	Description	Mean ± SD
Perimeter scaled m^2	Habitat	Pond perimeter (m; mean/site year)	134.2 ± 95.2
Fish presence	Invasive species	Fish of any species observed (1) or not (0)	0.17 ± 0.38
Fish density	Invasive species	Density of any fish species in the habitat (no. caught/m seined)	0.34 ± 1.25
Bullfrog presence	Invasive species	Bullfrog of any life stage observed (1) or not (0)	0.26 ± 0.44
Bullfrog density	Invasive species	Density of bullfrog larvae in the habitat (no. caught/m seined)	0.10 ± 0.75
Bd prevalence	Disease	Proportion of swabbed *Pseudacris regilla* individuals that tested positive for Bd from the current surveyed year	0.29 ± 0.33
Palmer's drought severity index (PDSI)	Drought severity	Regional drought severity metric that incorporates recent and long-term temperature and precipitation data	−2.74 ± 2.61
Within-pond perimeter	Drought severity	Relative pond perimeter compared with site-level mean pond size	NA

fishes or American Bullfrogs (binomial variables of 0 or 1 to indicate presence of any invasive fish or American Bullfrog life stage) in a given year, as well as their corresponding densities in a given year. To capture aspects of infection risk associated with Bd, we used a single covariate of prevalence of Bd in *P. regilla* in a given site-year (proportion of sampled *P. regilla* that tested positive for Bd). We elected to focus our analysis on infection prevalence in *P. regilla*, a common indicator species, rather than prevalence in the focal species to help standardize the estimate of infection risk, to offer a comparable metric across sites and years, and to provide information about Bd infection risk and potential influence on larval occurrence from sites where the 2 threatened taxa were not observed. For models of larval density at sites where the focal amphibian species were detected, we ran additional analyses using direct infection prevalence data from the 2 threatened species (Appendix S1).

For yearly estimates of mean drought severity during the amphibian breeding period (March–May), we used the Palmer Drought Severity Index (PDSI), which provides a robust estimate of long-term drought conditions by integrating precipitation and water balance metrics. Positive PDSI values indicate a wet year and negative values represent a dry year. We obtained drought metrics through the National Oceanic and Atmospheric Administration National Centers for Environmental Information for the California Central Coast Drainage division (https://www.ncei.noaa.gov). We also included a site-by-year-level variable (within-pond perimeter) that captured the drought-related variation in each pond's size across the years surveyed and provided site-specific information about how local water availability shifted across the study period. This variable was calculated by scaling a pond's perimeter value in a given year by its mean size across all years, then dividing by the SD.

Model construction and assessment We used a GLMM framework with a logit-link function to model breeding occurrence as a binomial response for each species at each site year. For larval densities, we used GLMMs with a Poisson distribution and a log-link function. We selected the GLMM approach because of its flexibility in incorporating multiple variables and their interactions while properly handling complex random effect structures. We included the identified covariates as fixed effects (Table 1), and to account for the nonindependence of repeated sampling of ponds over time, we added random intercept terms for pond identity and year of sampling. Although using multiple sampling methods likely improved our detection ability, as determined in previous work (Moss et al. 2021), we acknowledge that our detection of amphibians was likely imperfect. Thus, to ensure that variation in detection probability did not influence inferences from our GLMM approach, we also used the *unmarked* package in R (version 1.2.5; Fiske and Chandler 2011) to translate the best fitting models of larval occurrence to a single-season occupancy framework (see Appendix S2 for detailed occupancy modeling methods and results).

We assessed how drought interacted with Bd and invasive species by incorporating 2-way interaction terms between PDSI (which captured variation in drought intensity) and invasive species presence or density and Bd infection prevalence. Because we had 2 alternative metrics for American Bullfrog taxa (presence or density), we built separate models using each of the distinct parameters to avoid issues of collinearity. To improve model convergence and facilitate parameter comparisons, we scaled all continuous variables by subtracting the mean and dividing by the SD (Gelman and Hill 2007). After building the initial full models, we subsequently removed non-informative terms one by one, which we identified as having associated $p > 0.05$, reran the models, and compared fit using likelihood-ratio tests. If removing the term resulted in a poorer fit (defined as a likelihood ratio test $p < 0.05$) we retained the removed term (Bolker et al. 2009).

RESULTS

Drought impacts on habitat availability

The aquatic habitats used by amphibians were substantially affected by drought conditions during the survey period. As drought severity peaked in 2014 to 2015, a high fraction of the ponds either failed to fill with water or dried during the summer (prior to the start of August). The percentage of ponds drying peaked in 2014, with 40% of ponds drying before August, compared with <10% in the years following drought (2016–2019). The number of sites drying in our study area correlated with drought severity (Pearson correlation coefficient: $-0.87, p = 0.01$), with the most severe drought conditions occurring from 2014 to 2015 (Fig. 2). During the most severe drought conditions, 38 and 21% of sites went dry in 2014 and 2015, respectively, compared with only 13% going dry in 2013.

Patterns of chytrid fungus infection

Between 2013 and 2019, we tested 5736 individual amphibians from 538 unique site-by-year visits that had detected amphibian breeding activity (out of 650 total unique site-by-year visits) for the presence and load of the chytrid fungus (Bd). Of the 2 focal species, we quantified Bd status in 385 adults and recently metamorphic juveniles of *R. draytonii* and 265 larval *A. californiense*, with the remaining surveys consisting of 5086 surveys of late-stage larvae or recently metamorphosed *P. regilla.* Across all years, the mean annual infection prevalence for *R. draytonii* was 45 ± 5%, with the highest value in 2017 (59%; 30/51 individuals swabbed) and the lowest in 2018 (27%; 3/11) (Fig. S1). For *A. californiense*, the mean annual infection prevalence was 5 ± 2%, with the highest value in 2015 (10% of 52 individuals tested) and the lowest in 2013 and 2018, for which no infections were detected (2013: 0/7; 2018: 0/26). For *P. regilla,* the overall infection prevalence was 28% (1412/5086) and ranged from 24% in 2014 to 34% in 2016.

Patterns of *R. draytonii* larval occurrence and density

We detected larval or juvenile stages of *R. draytonii* in 57/110 sites (52%) at least once during the 7-y study. Among years, an average (±1 SE) of 31 ± 2% of sites supported *R. draytonii* larvae or metamorphic juveniles. The lowest *R. draytonii* breeding activity was in 2014 during the most severe point of the drought, with only 18% (18/101) of sites with evidence of breeding occurrence (Fig. 3A), in part because a high number of ponds failed to adequately fill with water (38%). Despite having the lowest occupancy value, 2014 had the highest larval density among occupied sites (0.47 ind./m^2; $n = 18$), consistent with drought-mediated crowding effects, whereas 2016 had the lowest larval density (0.07 ind./m^2; $n = 29$ sites) (Fig. 3C).

There was high agreement between the GLMM and occupancy modeling frameworks for *R. draytonii* breeding occurrence (Table S2). Here, we present only the GLMM results. Based on the results of the occupancy analysis we had a detection probability of 0.76 ± 0.03 for *R. draytonii* breeding activity (see Appendix S2 for full occupancy modeling results). *Rana draytonii* breeding occurrence was predicted by a combination of invasive species (fish presence), pond size (pond perimeter), and drought intensity

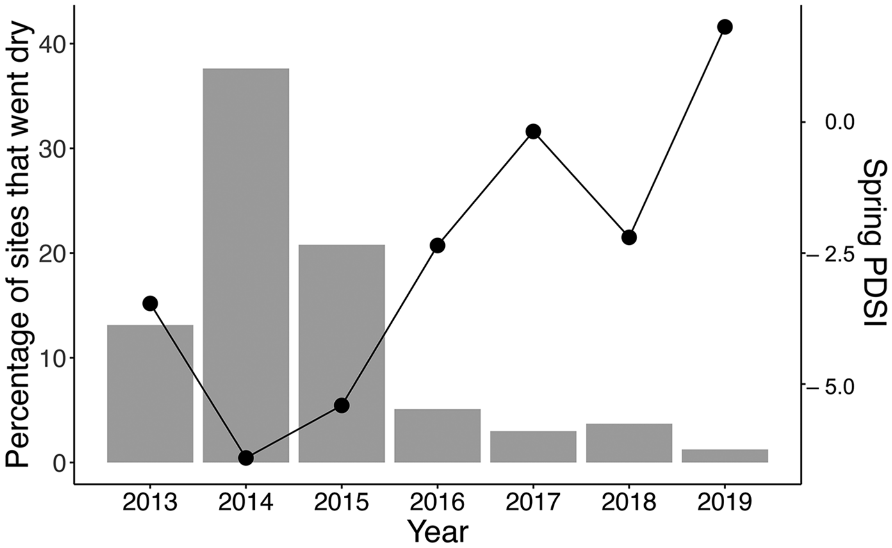

Figure 2. Drought condition intensity was correlated with hydroperiods of pond breeding habitats in the Bay Area of California, USA, over a 7-y sampling period (Pearson's $r = -0.87, p = 0.01$). Peak drought conditions occurred between years 2013 to 2015, where mean springtime Palmer's drought severity index (PDSI) values (dots with connecting lines) were <-2.5, indicating an extreme drought. Correspondingly, we observed a relatively high proportion of ponds going dry, with a peak of 38% (38/101) of sites drying in 2014.

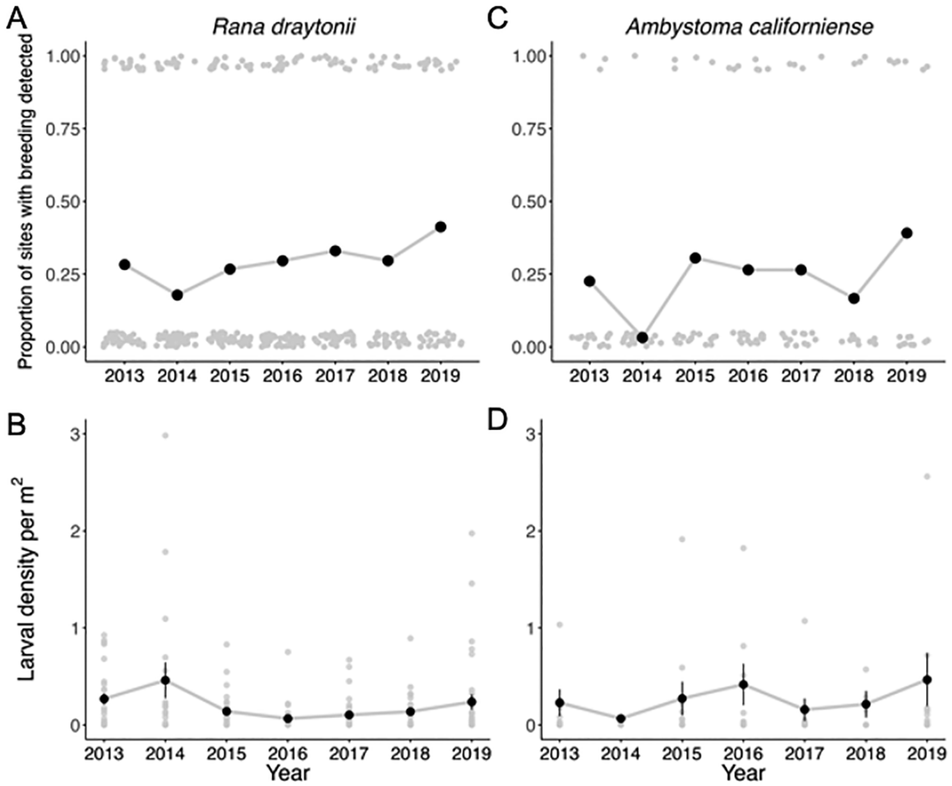

Figure 3. Breeding occurrence (A, B) and larval density (C, D) patterns of California Red-legged Frog (*Rana draytonii*) and California Tiger Salamander (*Ambystoma californiense*) in 110 ponds in the Bay Area of California, USA, from 2013 to 2019. Gray dots represent site-by-year observations and black dots represent annual means.

(PDSI value) (Table 2, Fig. 4A). Neither American Bullfrog density nor presence influenced *R. draytonii* occurrence, whereas the presence of fish reduced the likelihood of detectable breeding by ~45% (estimated fish coefficient: GLMM: -1.76 ± 0.83, $p = 0.03$; Fig. S2). Drought (negative PDSI) decreased breeding occurrence likelihood (GLMM: 0.15 ± 0.05, $p = 0.007$; Fig. S3), whereas overall pond perimeter positively predicted the likelihood of *R. draytonii* larvae (GLMM: 0.52 ± 0.23, $p = 0.03$), such that larger ponds had a higher likelihood of detectable breeding. Bd infection levels did not have a detectible effect on *R. draytonii* breeding occurrence nor did we detect interactions between drought and either invasive species presence or density.

For the density of larval *R. draytonii* among sites where they were detected, the final model included American Bullfrog density, pond perimeter, Bd prevalence, and drought intensity. Increased American Bullfrog larvae density was associated with lower *R. draytonii* larvae density (GLMM: -0.64 ± 0.27, $p = 0.05$; Figs 4B, S4), whereas fish presence did not affect larvae density. The lack of association between fish presence and *R. draytonii* larvae density was likely due to the limited number of sites that supported both fish and larval *R. draytonii* ($n = 19/110$ sites). Pond perimeter negatively influenced larval density (GLMM: -0.17 ± 0.06, $p < 0.00001$), such that larger ponds supported lower densities of *R. draytonii* (Fig. 4B). Interestingly, Bd infection prevalence in *P. regilla* was negatively associated with larval *R. draytonii* density, and the strength of this effect varied across drought conditions (estimated Bd coefficient: -0.38 ± 0.07, $p = 0.0001$, estimated Bd × PDSI interaction coefficient: -0.10 ± 0.03, $p < 0.0001$). Thus, in non-drought years (PDSI > 0), Bd infection was negatively correlated with *R. draytonii* density, whereas this effect was reversed in severe drought years.

Patterns of *A. californiense* breeding occurrence and density

Among the 36 southernmost (see Fig. 1) sites in our surveys, which were within the distributional range of *A. californiense*, we detected *A. californiense* larvae in an average (±1 SE) of 24 ± 4% of ponds. The year with the highest percentage of sites with *A. californiense* was 2019 (39%; 9/23), and the lowest year was 2014 (3%; 1/31) (Fig. 3B). In sites where *A. californiense* was detected, mean densities from seine sampling ranged from 0 (larvae detected though through visual observation) to 2.56 larvae/m^2 (Fig. 3D).

There was high congruency between the GLMM and occupancy modeling frameworks for *A. californiense* breeding occurrence (Table S2). Here, as above, we present only

Table 2. Output table of generalized linear mixed-effects modeling results for the best-fit models for California Red-legged Frog (*Rana draytonii*) and California Tiger Salamander (*Ambystoma californiense*) occurrences and densities in 110 ponds in the Bay Area of California, USA between 2013 and 2019. Estimated coefficients ±SE are given, along with the *p*-values in parentheses, for variables included in the best-fit models. * indicates perfect separation and thus we cannot provide an estimated coefficient. If the model parameter was not included in the final model, it was identified as –. PDSI = Palmer drought severity index, Bd = chytrid fungal infection, O = occurrence, D = density, R^2_{m} = marginal R^2, which relates to the proportion of variance explained by the fixed factors alone, R^2_{c} = conditional R^2, which relates to the proportion of variance explained by both the fixed and random (pond identity, year of sampling) variables.

				Model parameters						
				Invasive species		Disease	Habitat	Drought severity		Interactions
Focal species	Model type	R^2m	R^2_{c}	Bullfrog density	Fish presence	Bd	Perimeter scaled m^2	Within-pond perimeter	PDSI	Bd:PDSI interaction
Rana draytonii	O	0.07	0.70	–	−1.76 ± 0.83 (0.03)	–	0.52 ± 0.23 (0.03)	–	0.15 ± 0.05 (0.01)	–
Rana draytonii	D	0.22	0.55	−0.64 ± 0.27 (0.05)	–	−0.38 ± 0.07 (0.0001)	−0.17 ± 0.06 (<0.0001)	–	0.07 ± 0.09 (0.72)	−0.10 ± 0.03 (<0.0001)
Ambystoma californiense	O	0.10	0.55	–	*	–	–	0.96 ± 0.30 (0.001)	–	–
Ambystoma californiense	D	0.22	0.72	–	*	0.70 ± 0.27 (0.01)	−1.53 ± 0.54 (0.005)	0.93 ± 0.35 (0.007)	–	–

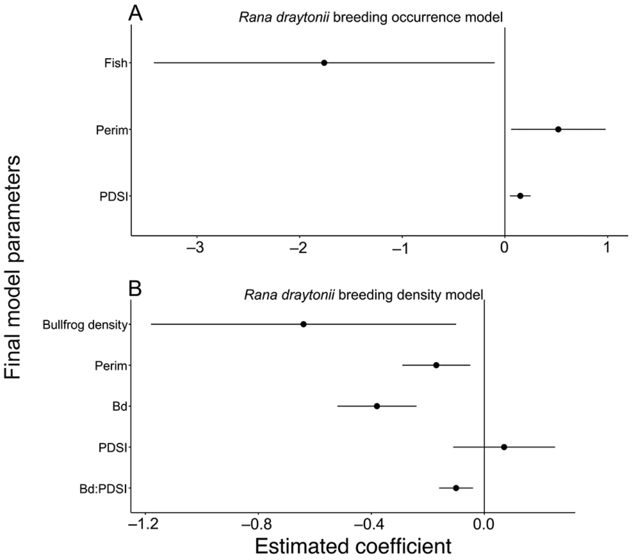

Figure 4. Coefficient plot for breeding occurrence (A) and density (B) models for *Rana draytonii*. The parameters retained in the final model are on the *y*-axis with the estimated coefficient values ±1 SE on the *x*-axis. Coefficient values ±1 SE that do not intersect with 0 had $p < 0.05$ in our models. Perim = pond perimeter scaled m^2, Within perim = within-pond perimeter, PDSI = Palmer drought severity index, Bd = chytrid fungal infection.

the GLMM results. Based on the results of the occupancy analysis, we had a detection probability of 0.60 ± 0.07 for *A. californiense* breeding activity (Appendix S2). *Ambystoma californiense* breeding occurrence was influenced by the presence of invasive fish and the within-pond scaled perimeter. In fact, *A. californiense* larvae were never observed in sites with fish, suggesting either that the presence of fish prevents breeding or that the types of ponds suitable for fish and tiger salamanders are fundamentally different (Fig. 5A). We were not able to obtain an estimated coefficient for fish presence because of issues of perfect separation in our model. Neither American Bullfrog presence nor density was correlated with *A. californiense* occurrence, either as main effects or interactively with drought and, thus, neither coefficient was retained in the model (Fig. S5). Although drought intensity (PDSI) was not directly correlated with breeding occurrence, there was a positive association of within-pond size variation, such that a pond had a higher probability of supporting *A. californiense* in years in which the pond was large relative to its mean size (estimated within-pond scaled perimeter coefficient: GLMM: 0.96 ± 0.30, $p = 0.001$). Site-level Bd infection prevalence in *P. regilla* was not a predictor of *A. californiense* larval occurrence.

Ambystoma californiense larval density was influenced by pond perimeter (both scaled values and the within-pond scaled value), as well as Bd infection prevalence in *P. regilla*. Larval density was negatively associated with total scaled pond perimeter (estimated scaled perimeter coefficient: −1.53 ± 0.54, $p = 0.004$; Fig. S6) such that smaller ponds across the landscape tended to support higher larval densities. However, larval densities were positively associated with the within-pond scaled perimeter, such that when ponds were large relative to their normal size, there were higher densities (estimated within-pond scaled perimeter coefficient: 0.93 ± 0.35, $p = 0.008$; Fig. 5B). Bd infection prevalence in *P. regilla* was positively related to *A. californiense* density (estimated Bd infection scaled coefficient: 0.70 ± 0.27, $p = 0.01$), the effect of which was insensitive to drought severity. We did not detect any negative effects of American Bullfrog presence or density on *A. californiense* larval densities, nor did we observe detectable interactions between drought and either Bd prevalence or American Bullfrogs.

DISCUSSION

In the current study, we analyzed the breeding patterns of 2 threatened amphibian species across 650 unique site visits to assess the additive and interactive relationships of drought, invasive species, and disease with breeding activity. Specifically, we related breeding patterns to the presence and density of invasive species (invasive fishes and American Bullfrogs) and to the prevalence of a pathogen (Bd). Across the study's 7-y duration, which included

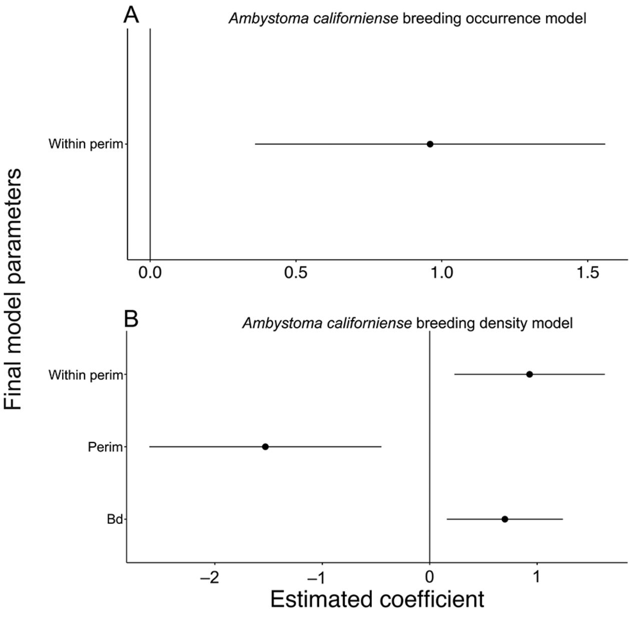

Figure 5. Coefficient plot for breeding occurrence (A) and density (B) models for *Ambystoma californiense* with the estimated coefficient values ±1 SE on the *x*-axis. Coefficient values ±1 SE that do not intersect with 0 had $p < 0.05$ in our models. Perim = pond perimeter scaled m^2, Within perim = within-pond perimeter, PDSI = Palmer drought severity index, Bd = chytrid fungal infection.

an exceptional drought event, both *R. draytonii* and *A. californiense* larval occurrence patterns and density were sensitive to extreme drought. We observed low occurrence distributions during the most severe stretches of the drought (2014–2015), which were driven by loss of available breeding habitats. Importantly, however, both taxa showed evidence of recovery, with recolonization in the years following drought as water levels increased. Additionally, the presence of invasive fish and, in the case of *R. draytonii* larvae density, the density of American Bullfrogs was negatively associated with native amphibian breeding patterns. However, the infection prevalence of Bd exhibited contrasting relationships with the larval densities of the threatened species: *A. californiense* larval densities associated positively with Bd prevalence, whereas *R. draytonii* larval densities associated negatively, but only during wetter years. Our study highlights the complex response of native amphibians to strong drought events and reinforces the need to evaluate multiple stressors in assessing factors shaping amphibian breeding patterns.

The observed direct and indirect effects of drought events on amphibian breeding patterns is consistent with previous studies highlighting how drought reduces breeding success and is likely to become a major stressor as it continues to increase in frequency (Walls et al. 2013, Cayuela et al. 2016, Zylstra et al. 2019, Moss et al. 2021). However, our study demonstrates the nuanced response of amphibians to extreme drought and, in particular, the ability for these taxa to rebound after extreme drought events. Both focal species were less likely to breed during peak drought conditions, but impacts of drought did not appear to be long lasting, as evidenced by a rapid increase in the number of breeding sites once the drought subsided. This rebound was likely facilitated by a combination of the presence of large permanent waterbodies that act as refugia during extreme drought events and associated amphibian behaviors such as adult overwintering locations and mobility. Adult *R. draytonii* are known to move between breeding habitats during dry-down periods to wait out drought periods in permanent water bodies (Bulger et al. 2003, Tatarian 2008). Adult *A. californiense* occupy small mammal burrows and other underground habitats during the dry months, likely allowing them to persist through some degree of drought (Shaffer et al. 1993). Thus, in managed landscapes it is important to consider the availability and maintenance of permanent waterbodies, as well as their landscape connectivity, for supporting native species (Hossack et al. 2013, 2015). However, permanent waterbodies can also facilitate the spread of invasive species, such as American Bullfrogs, that compete with and consume native amphibians (Snow and Witmer 2010, Novoa et al. 2020), underscoring important conservation tradeoffs that require system-specific assessments.

The presence of aquatic invasive species, specifically American Bullfrogs and nonnative fishes, was a major stressor for *R. draytonii* and *A. californiense* during the study period. The presence of these invasive species tended to correlate negatively with the breeding occurrence and larval densities of threatened amphibian species but varied in relation to type of invasive species. The presence of fish tended to exclude breeding from occurring for both species, whereas American Bullfrogs had limited impacts on breeding. This difference in how the 2 types of invasive species affected the distributional occurrence of protected species could be a result of the distinct effects they have on native amphibians. Fish tend to have more predatory interactions with native amphibians, whereas American Bullfrogs tend to primarily have strong competitive interactions with amphibians (Pearl et al. 2004, Preston et al. 2012). Our findings are consistent with previous studies showing that many fish species actively consume eggs and developing amphibian larvae (Barry and Shaffer 1994, Lawler et al. 1999, Joseph et al. 2016). For instance, Amburgey et al. (2014) showed that pond-breeding chorus frogs in Colorado largely occur in fishless habitats because fish actively consume eggs and developing larvae. In contrast, the competitive interactions between American Bullfrogs and amphibians might not be strong enough to prevent breeding from occurring but can reduce larval abundance in cases where the American Bullfrog is the superior competitor (Anderson et al. 2019).

Although infection by Bd remains a major concern for numerous amphibian taxa (Rödder et al. 2009, Scheele et al. 2019), few studies have assessed how the population effects of Bd infection vary across drought conditions (Terrell et al. 2014, Beyer et al. 2015). In wet years (PDSI > 0) higher levels of Bd infection prevalence were associated with lower *R. draytonii* densities, but in drier years (PDSI < 0) the opposite was true. These contrasting effects of Bd infection on the densities of *R. draytonii* larvae could potentially be attributable to numerous mechanisms that alter both the ability for Bd to infect and grow on a host (Terrell et al. 2014) and the host's ability to tolerate the infection (Bradley et al. 2015). For instance, increased Bd growth rates in wet years could result in higher host pathology and lower larval density. During drought, ponds that provided refugia held extremely high densities of amphibians, which could further facilitate rapid spread of Bd and explain the positive relationship between density and Bd prevalence during dry years. Importantly, however, these findings represent a correlative relationship, and it is possible that Bd levels were responding to changes in amphibian density or other unmeasured variables. In light of recent evidence linking historical patterns of Bd infection with localized declines of *R. draytonii* (Adams et al. 2020), understanding the contemporary impacts of Bd infection on *R. draytonii* breeding sites and larval densities warrants continued monitoring efforts.

For *A. californiense,* we observed consistently low levels of Bd infection throughout our study, with a maximum prevalence of 17% observed in 2017. Although *A. californiense* can support Bd infection (Padgett-Flohr 2008), there have been few studies of infection variation in natural populations of *A. californiense* (Padgett-Flohr and Hopkins 2009). Our finding of positive correlation between *A. californiense* larvae density and Bd infection, coupled with the overall low Bd infection values in this species, does not support a strong role for Bd in limiting the distribution of *A. californiense* (see also Wilber et al. 2020). However, the recently emerged salamander chytrid fungus *Batrachochytrium salamandrivorans* (Bsal), which has yet to be detected in North America (Waddle et al. 2020), is known to be highly pathogenetic in Caudata hosts (Martel et al. 2014). Thus, it is crucial to continuously monitor these populations to ensure early detection if and when Bsal spreads to this area.

Globally, amphibian declines are driven by numerous abiotic and biotic factors, and to effectively manage for these concurrent threats, practitioners need to understand whether combinations of stressors act additively or have unexpected synergistic effects (Blaustein and Kiesecker 2002, Buck et al. 2016, Grant et al. 2016, Knapp et al. 2016). We did not find evidence that drought and invasive species acted interactively to affect breeding activity of our focal species. However, drought likely played a strong role in allowing the colonization of the focal species into novel habitats by disproportionally influencing the invasive species' ability to persist in drying ponds (Moss et al. 2021). We did not quantify the effects of drought on invasive species populations, but previous studies have demonstrated strong negative effects and subsequent positive impacts on native communities in numerous systems (Knorp and Dorn 2014, McDevitt-Galles and Johnson 2018). Aquatic invasive species, particularly fish and American Bullfrogs, may be more sensitive to drought than native amphibian species, owing to their reliance on permanent waterbodies. This drought sensitivity in introduced species is especially relevant in regions where native amphibians are more drought-adapted (Walls et al. 2013), such as the western USA, where all native species can develop to metamorphosis within a single developmental season (Stebbins and McGinnis 2012). We observed drought to have only transient effects on native amphibian breeding populations, whereas the presence of invasive species was a more consistent negative influence across time. Thus, increased drought event frequency could result in enhancing these protected species' breeding activities by attenuating the persistent negative effect of invasive species' presence across the landscape. This outcome will depend, however, on whether native species can withstand the effects of even more severe and prolonged droughts that may be on the horizon. For instance, extreme drought conditions, as observed in California in 2021, could limit opportunities for native species to rebound, particularly if droughts continue for multiple years.

By monitoring breeding activity of 2 threatened amphibian species during and after an extreme drought, we assessed how these taxa responded to and recovered from severe drought conditions while facing concurrent threats of invasive species and disease. Overall, both *R. draytonii* and *A. californiense* breeding activities were sensitive to direct or indirect effects of drought intensity and the presence and density of invasive species. The positive correlations between Bd infection and *A. californiense* larval densities, and the interactive effect of Bd and drought severity for *R. draytonii* densities, revealed intriguing patterns that would benefit from further investigation. However, we caution against drawing strong conclusions from correlative findings, especially when applied to infectious disease patterns, which could result from multiple underlying mechanisms. We recommend further experimental work to follow up on these, which could better quantify how the fitness effects of Bd infection vary across a gradient of drought stress. In addition, our findings reinforce the importance of reducing and removing invasive species from amphibian breeding habitats and suggest that, although the native taxa may be sensitive to drought conditions, they can withstand even historic levels of drought, provided that suitable refugia exist. Drought may even indirectly enhance these taxa's breeding activity by reducing habitat availability for invasive species through periodic drying of more permanent ponds, although this outcome will likely depend on the severity and duration of the drought. Our study underscores the need for continued management to prevent recolonization by invasive species and promote long-term fitness of native amphibian populations.

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