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Evidence-Based Conservation of Rare and Endangered Amphibians in California's Sierra Nevada

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ABSTRACT

Effective conservation and management of sensitive species requires knowledge of their ecology. For example, understanding habitat requirements can identify environmental constraints that govern persistence of a species, where on the landscape a species will occur, and habitat-related causes of declines. Understanding demography and vital rates can identify reasonable expectations for abundances, survival, and potential for population growth, and identify sensitive life stages. In addition, quantitative evaluations of management, conservation, and recovery actions can provide valuable insights into the success or failures of these programs and how to improve their effectiveness. In reality, these types of information typically are not available. Although assumptions about the ecology of a species may exist, quantitative data commonly is lacking, and management, conservation, and recovery actions are rarely evaluated for their success. Thus, management decisions are often based on incomplete information.

Amphibian populations have undergone severe declines and extinctions worldwide, including in relatively pristine areas. Yet, basic ecological data are often lacking for this taxon, which has been poorly studied relative to other vertebrates like birds and mammals. This dissertation addresses information gaps in the ecology of two federally listed anurans in the Sierra Nevada, CA, the threatened Yosemite toad (*Anaxyrus canorus*) and the endangered Sierra Nevada yellow-legged frog (SNYLF, *Rana sierrae*), which have both declined in distribution and abundance. These species occur in patchily distributed, high elevation, montane, aquatic systems. The wet meadows, lakes, ponds, and streams available to these species vary greatly in size, hydrology, topography, and isolation. Sierra Nevada weather is highly variable and unpredictable, spanning gradients from wet to drought and warm to cold years. The two species have different life history strategies that allow them to persist in these uncertain environments. In

Chapters 1 and 2, I addressed habitat selection, first investigating Yosemite toad habitat selection in wet meadows, where the species most commonly breeds (Chapter 1), and then investigating habitat selection of SNYLF in streams (Chapter 2), where the species has not been well-studied. In Chapter 3, I investigated the demography of SNYLF in streams. In addition to quantifying the ecology of SNYLF in streams, in Chapters 2 and 3 I investigated the success of a recovery action—population augmentation—by comparing habitat selection and demography of captive-reared frogs and wild frogs.

The Yosemite toad inhabits variable and unpredictable environments with short growing seasons. To investigate Yosemite toad breeding habitat selection in these environments, in Chapter 1 I quantified habitat characteristics in wet meadows at multiple scales including egg mass deposition microhabitats, tadpole microhabitats, breeding areas within wet meadows, and wet meadows. Using logistic regression, I quantified the relationship between breeding occupancy and habitat variables measuring surface water characteristics, water temperature, cover, habitat complexity, physical environment, and climate. I found that habitat selection by the Yosemite toad reflects choices that promote fast development which may be an adaptation to its short growing season and uncertain environment. Yosemite toads bred in very shallow, warm water with low gradient shorelines, and habitat characteristics associated with water and temperature were important at all scales. Overall, Yosemite toads selected for very small differences in these habitat components. For example, water depths where they laid eggs were consistently very shallow, averaging 0.04–0.05 m. Probability of occupancy increased with water temperature at all scales. Breeding areas within meadows ranged from potholes to flooded vegetation with standing or flowing water. Breeding meadows tended to have flatter slopes, west-facing aspects, and complex habitats in the form of springs, inlets, outlets, and breeding habitat type richness. The quantitative data describing important habitat components summarized in the chapter can be used to design appropriate management and conservation for the Yosemite toad across its range.

In Chapter 2, I addressed two information needs for SNYLF that inhabit streams in its northern range, habitat selection and the effectiveness of a recovery action. Because available habitats in streams can change across space and time, I investigated SNYLF habitat requirements across spatial and temporal scales. I quantified habitat use across diverse streams (third-order perennial streams to first-order intermittent headwater creeks), habitat types within streams (pool, riffle, cascade), and seasonal flows (high water at snowmelt to reduced flows late in the summer). Available habitat changed with flows. Perennial streams retained a variety of habitats across flows whereas intermittent streams dried to just a few pools. These results suggest that SNYLF is an aquatic generalist in streams, able to meet its ecological requirements in a variety of habitats, with some site-specific constraints affecting habitat selection. Frogs used all seasonal habitat types but selected deeper pools most often relative to available habitats in intermittent streams and selected riffles or showed no preference in perennial streams. Frogs avoided fast deep-water microhabitats, but otherwise, preferred flowing water or deeper water without flow. I also compared habitat selection between wild and captive-reared frogs that were released as a population augmentation in one intermittent stream. Captive-reared frogs selected similar habitats as wild frogs, suggesting captivity does not alter habitat selection behaviors post-release. This also suggests that reintroduction designs for this species can be based on wild frog preferences. Results in this chapter improve our understanding of how SNYLF habitat use varies among different types of streams and differs seasonally in response to changing habitat conditions, which in turn can inform designs of effective management, conservation, and recovery programs, including reintroductions of frogs in streams.

In Chapter 3, I conducted a three-year capture–mark–recapture study to investigate the demography of SNYLF in the same streams. The objectives were to quantify abundances and vital rates in diverse streams, evaluate a population augmentation recovery action in one of the streams, and use the demographic data to inform reintroduction designs. Population abundances were low in three streams supporting the need for recovery actions such as population augmentation. The two largest populations were in intermittent streams, possibly because fish are less common where water is not flowing year-round. Estimates of apparent survival rates of wild adults were relatively high (0.55 –0.90) whereas estimates of annual recruitment were relatively low (0.02–0.26). The oldest frog found in the streams was at least 13 years old whereas few tadpoles or subadults were seen in any of the streams. The high survival rates leading to long-lived adults may facilitate persistence of these stream populations, whereas low recruitment may limit population growth and recovery. The population augmentation was relatively successful with at least 52% of captive-reared frogs surviving their first summer after release and at least 36% surviving their first winter. These results offer promise for the use of reintroductions to augment depleted populations. The demography of wild frogs helps identify considerations for reintroduction designs. For example, the apparent low survival of wild eggs, tadpoles, and subadults, suggests releasing adults, as was done in this study, rather than younger life stages, may be more successful. The results also suggest that further research on factors affecting recruitment and survival of younger life stages is needed.

The Yosemite toad and SNYLF are examples of at-risk amphibians that require basic ecological knowledge to support effective management, conservation, and recovery. To date, these decisions mostly have been based on descriptive information and general field observations. This dissertation addresses key aspects of the ecology of these species as well as presents quantitative data to guide future actions.

CHAPTER 1

Identifying Key Breeding Habitat Features for the Federally Listed Yosemite Toad (*Anaxyrus canorus*)

***To be submitted as:**

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ABSTRACT

Understanding the habitat requirements of the endemic, federally Threatened Yosemite toad (*Anaxyrus canorus*) will facilitate development of effective conservation and management. We investigated breeding habitat relationships at multiple scales in wet meadows on National Forest lands across the species' range in the Sierra Nevada, CA. We quantified breeding habitat characteristics for egg mass deposition microhabitats, tadpole microhabitats, breeding areas within wet meadows, and wet meadows where the species was found. We used logistic regression to quantify the relationship between breeding occupancy and habitat variables, including surface water characteristics, water temperature, cover, habitat complexity, physical environment, and climate. We found that Yosemite toads bred in very shallow, warm water, and habitat characteristics associated with water and temperature were important at all scales. Overall, Yosemite toads selected for very small differences in these habitat components. Yosemite toads showed no preference for pothole breeding habitats versus flooded vegetation, or for standing versus flowing water. Probability of occupancy increased with water temperature. Eggs were laid in consistently shallow water depths, averaging 0.05 m ± 0.03 SD in potholes and 0.04 m ± 0.02 SD in flooded vegetation. In breeding areas within meadows, maximum water depths averaged 0.25 m ± 0.16 SD in potholes and 0.14 m ± 0.10 SD m in flooded vegetation habitats. Breeding areas tended to have low gradient shorelines and intermediate amounts of dense cover. Breeding meadows were as small as 0.1 ha, and the probability of occupancy increased with flatter slopes, west-facing aspects, and complex habitats in the form of springs, inlets, outlets, and breeding habitat type richness. These results provide a quantitative foundation for developing recovery actions for the Yosemite toad across its range.

INTRODUCTION

Quantitative data on a species' ecological requirements provide the foundation for effective conservation and management (Sutherland et al. 2004, Bull 2006, Cooke et al. 2017). Of these requirements, habitats are particularly important because they define the environmental constraints that govern a species' persistence. Habitat requirements determine where on the landscape a species will occur (Price et al. 2004, Liang and Stohlgren 2011, Blank and Blaustein 2012, Cuello et al. 2017), and habitat is often an aspect of a species' ecological needs that can most easily be manipulated and managed (Green et al. 2013, Lind et al. 2016). Given the widespread declines of amphibians (Stuart et al. 2004, Wake and Vredenburg 2008), understanding how habitats influence the persistence of at-risk frogs and salamanders is an important step toward developing conservation approaches for these imperiled taxa.

Ecological processes that affect habitats operate at multiple scales (Lewis et al. 1996, Van Buskirk 2005, Jedlikowski et al. 2016, Cecala et al. 2018). For amphibians, quantifying habitat relationships at multiple scales may be particularly important because of several aspects of their life history. Because they have permeable skin, amphibians require moist environments to avoid desiccation and, as ectotherms, they rely on their external environment for thermoregulation (Duellman and Trueb 1986). Temperature and moisture at the smallest scale (i.e., microclimate) are likely to serve as proximate cues for habitat selection, and amphibians actively regulate their body temperatures behaviorally (Mullally and Cunningham 1956, Brattstrom 1962, Bradford 1984). Larger scale processes in turn affect local conditions. Characteristics of habitat units like lakes, meadows, and streams, and their surrounding watersheds can affect the hydrology (Patterson and Cooper 2007, Lowry et al. 2010, Chandler et al. 2017, Cecala et al. 2018), water quality (Welsh and Ollivier 1998, Werner and Glennemeier

1999), and temperature regimes (Werner and Glennemeier 1999, Adams and Frissel 2001, Blomquist and Hunter 2009) of aquatic microhabitats. Finally, habitat complexity at multiple scales may provide important diversity of habitats to meet the full scope of life history needs such as reproduction, basking sites, cover, and food (Werner et al. 2009, McCaffery et al. 2014, Cuello et al. 2017, Browne and Paszkowski 2018).

Habitat selection that affects persistence may be particularly complex for biphasic amphibians—those with an aquatic larval and terrestrial postmetamorphic stage—which must contend with temporally variable environments to persist in spatially dynamic landscapes. For example, successful reproduction depends on the presence of surface water for periods long enough for metamorphosis to occur (Semlitsch et al. 1996, Zylstra et al. 2015, Chandler et al. 2016). Many amphibians breed in ephemeral waters that can provide protection from predators (Snodgrass et al. 2000) and promote fast development which may be advantageous when conditions are unpredictable. However, ephemeral water poses risks when sites dry too soon desiccating eggs and larvae and leading to the loss of an individual's reproductive output for the year (Pechmann et al. 1991, Berven 1995, Semlitsch et al. 1996, Taylor et al. 2006). Long-term persistence of species that rely on these habitats requires hydroperiods sufficient for metamorphosis to occur frequently enough to replace reproductive adults (Taylor et al. 2006, Chandler et al. 2016). Hydroperiods are influenced by spatial dynamics such as local and watershed geomorphology (Lowry et al. 2010, Viers et al. 2013, Wolf and Cooper 2015) and temporal dynamics such as climate and variable weather (Semlitsch et al. 1996, Brooks 2004, Greenberg et al. 2015).

Challenges for long-term persistence may be particularly acute for amphibians that inhabit high elevation montane environments (Mullally and Cunningham 1956, McCaffery and

Maxell 2010). Active seasons may be as short as a few months, leaving small windows for reproduction, larval development and metamorphosis, and growth (Kagarise Sherman and Morton 1984, McCaffery and Maxell 2010). Aquatic habitats are patchily distributed and often surrounded by dry, steep terrain (Pilliod et al. 2002, Funk et al. 2005, Wang 2012). Weather patterns are highly variable and unpredictable, affecting water availability and temperatures, both of which are critical for amphibian survival, growth, and development (Kagarise Sherman and Morton 1984, Gould et al. 2019). Species have evolved varying life history strategies to ensure persistence in these uncertain and variable environments.

The federally threatened Yosemite toad, *Anaxyrus canorus*, endemic to high elevations of the central Sierra Nevada, CA, has evolved to persist in spatially and temporally variable, stochastic environments (Kagarise Sherman and Morton 1984). Although still distributed throughout much of its range, many populations are small and appear to have declined in recent years (Kagarise Sherman and Morton 1993, Brown et al. 2012). Causes of declines of the Yosemite toad are unknown, but may be related to the amphibian chytrid fungus, *Batrachochytrium dendrobatidis,* (Dodge 2013, Lindauer and Voyles 2019, Dodge et al. In Review) and the widespread drying of wet meadows in the Sierra Nevada (Menke et al. 1996, Loheide et al. 2009, Purdy et al. 2012). Spatially, wet meadows, where the Yosemite toad most commonly breeds (Karlstrom 1962, Kagarise Sherman 1980, Martin 2008, Brown et al. unpublished data), are patchily distributed throughout high elevations of the Sierra (Wang 2012, Maher et al. 2017). Temporally, Sierra Nevada weather is variable and unpredictable, spanning gradients from wet to dry years and warm to cold years (Howat and Tulaczyk 2005, Margulis et al. 2016). Snow covers the landscape for most of the year leaving only a 2–4 month growing season (Kagarise Sherman and Morton 1984, Howat and Tulaczyk 2005, Wolf and Cooper

2015). Given these environments, the Yosemite toad's general life history strategy is to breed at snowmelt in shallow, warm, ephemeral water that facilitates fast development within a single season (Karlstrom 1962, Kagarise Sherman 1980, Kagarise Sherman and Morton 1984). The Yosemite toad breeds over a short period from a few days to <2 weeks (Kagarise Sherman 1980, Brown et al. 2012). Eggs hatch into larvae in about 4–15 days and tadpoles metamorphose in an average of 6–8 weeks (Karlstrom 1962, Kagarise Sherman 1980, Sadinski 2004, Brown et al. unpublished data). After breeding, adults disperse into upland habitats, although they are occasionally found in wet meadows (Martin 2008, Morton and Pereyra 2010, Liang 2013). Even in normal water years, the shallow ephemeral breeding habitats may desiccate before tadpoles metamorphose, leading to the loss of all or a large proportion of recruitment for the year (Karlstrom 1962, Kagarise Sherman 1980, Brown et al. 2012).

Similar to many species, assumptions about the habitat requirements for the Yosemite toad are only beginning to be quantified in a systematic way (Martin 2008, Liang and Stohlgren 2011, Liang et al. 2017). To address this information need, we investigated Yosemite toad breeding habitat selection in wet meadows at three scales, including the whole meadow, breeding areas within meadows, and egg mass and tadpole microhabitats within breeding areas. Based on our knowledge of amphibian ecology and the species, we made predictions for the relationship of each variable with Yosemite toad habitat selection. First, we predicted that metrics describing water characteristics and temperature would emerge as important predictors of Yosemite toad occurrence, though the specific metrics would depend on the scale. At the meadow scale, we predicted that variables reflecting warm, wetter environments would be important. For example, we predicted occupancy would be greater in meadows with warmer air temperatures, south or west facing aspects, higher precipitation, and flatter slopes that tend to hold water. At the breeding area and microhabitat scales, we predicted eggs and tadpoles would be found most often in shallow, warm water with low gradient shorelines. Second, we predicted that cover variables would have some importance, but would not be primary drivers of habitat selection. We predicted occupancy would be associated with higher cover. Third, we predicted that meadows with a diversity of habitats, measured by variables reflecting habitat complexity, would be positively associated with occupancy. For example, some breeding areas have the potential for longer water retention in dry years but may be too deep and cold in wet years, whereas others may dry too early in drought years but be ideal warm water habitats in wet years. Finally, specific quantitative data on habitats commonly is not available when designing recovery actions. Thus, we include descriptive statistics for a subset of easily measurable habitat components selected by the Yosemite toad. Understanding how habitats influence population persistence for this threatened species can provide specific guidance for developing effective management and restoration plans at multiple scales.

METHODS

Study Area and Yosemite Toad Breeding Habitats

We studied wet meadows on National Forest lands across the historical range of the Yosemite toad in the Sierra Nevada, CA (Figure 1.1). This included meadows at elevations 1859–3703 m from the southern part of the Eldorado National Forest (Alpine County) to the northern part of Kings Canyon National Park (Fresno County). Sierra Nevada meadows are variable in their size, hydrology, topography, and vegetation. Similarly, Yosemite toad breeding areas within wet meadows are variable, spanning a gradient across two general types of habitat from flat, flooded, grassy areas to small potholes or ponds that have distinct shorelines and depressional topography (Figure 1.2). Spring snowmelt is the primary water source, and the size and hydroperiod of these surface water habitats depends on annual snowpack.

Study Design

We collected habitat data at three scales: meadows, breeding areas within meadows, and microhabitats of egg masses and tadpoles within breeding areas (Table 1.1, Figure 1.2). We collected data (described below) on physical environment, climate, habitat complexity, surface water characteristics, water temperature, cover, and for covariates describing conditions at the time of survey (Appendix 1). We refer to these as ecological functions.

Meadow and Breeding Area Scales

We collected habitat data at the meadow and breeding area scales from June–September 2003–2006 in 311 occupied and unoccupied wet meadows (Table 1.1). We selected meadows using a two-stage unequal probability design (Brown and Olsen 2013). We first selected a spatially distributed, unequal probability sample of small watersheds (2–4 km^2) based on historical locality data using a generalized random tessellation stratified survey design for a finite resource (Stevens and Olsen 1999, Stevens and Olsen 2004, Brown and Olsen 2013). Within each selected watershed, we surveyed all wet meadows. We used an augmented serially alternating panel design (Urquhart et al. 1998, Urquhart and Kincaid 1999) where, for this analysis, 115 meadows were visited annually and the remaining meadows were visited once (Brown and Olsen 2013).

Within each meadow, we delineated all contiguous wetted areas of similar habitat type and considered these potential breeding areas (Table 1.1). We categorized the habitat type of each breeding area into one of three categories, pothole (defined shoreline and depressional topography), flooded standing (flat topography flooded with standing water), or flooded flowing (flat topography flooded with flowing water) (Figure 1.2). We hypothesized that flowing water would be colder than standing water, and thus less likely to be occupied.

We used visual encounter surveys of all wadable water to determine breeding occupancy within each breeding area and respective meadow, indicated by the presence of egg masses, tadpoles, or newly metamorphosed toads (Crump and Scott 1994, Olson et al. 1997, Brown et al. 2012). To maximize detection of these life stages, we made an effort to survey watersheds early in the season before meadows dried and we conducted surveys from 0930–1900 after diurnal air temperatures warmed. We recorded beginning and ending air temperatures and time of day for each survey. We averaged these values to examine the importance of conditions at the time of survey for determining occupancy.

We compiled habitat metrics using a mix of remote and field collected data (Appendix 1).

Physical environment of meadows: We used ArcGIS to calculate meadow area from GIS layers created from orthophotographs and digital imagery. We calculated mean elevation for each meadow from a 10-m resolution digital elevation model (DEM) obtained from the National Elevation Database (NED) from Datadoors (www.datadoors.net). We created a slope aspect raster from this DEM, which was used to create aspect variables that compared the four cardinal directions (N, S, E, W), north versus south, east versus west, northeast versus southwest, and southwest versus all other aspects. We used a clinometer in the field to measure longitudinal (valley) and lateral slopes of each meadow.

Climate of meadows: For each meadow, we obtained monthly precipitation and temperature data from Prism Climate Group (2010). For the summer months (June, July, and August), we determined maximum and minimum monthly temperatures. We also calculated mean summer temperature (mean of June, July, and August maximum and minimum temperatures). For each meadow, we calculated three measures of water input. We first calculated the total precipitation for the water year (October–September) to examine the amount of precipitation for the specific year. We then calculated the water year total as a percent of the 30-year normal for the year of survey to compare the specific year with the meadows' average. Finally, we calculated the 30-year normal annual precipitation (mean of 1971–2000) to determine whether the meadow was relatively wet or dry. We also calculated monthly precipitation for July plus August and for August only to examine the influence of summer rainfall. Finally, Yosemite toads generally take three to four years to reach sexual maturity (Kagarise Sherman 1980) and current occupancy may reflect conditions in prior years that were either conducive or detrimental to recruitment. Thus, to examine whether precipitation in prior years may affect occupancy, we calculated the water year total as a percent of the 30-year normal for the years 1,2,3,4 and 5 years prior to the survey year.

Habitat complexity: For each meadow during field surveys, we tallied the number of streams, tributaries, springs, and channels. We counted the number of breeding areas by breeding habitat type (potholes, flooded standing, flooded flowing). We used GIS data to calculate the number and perimeter length of lakes in the meadows. To quantify habitat complexity, we calculated Shannon diversity and richness metrics first using the three breeding habitat types, and second, using all aquatic habitats recorded in meadows (potholes, flooded standing, flooded flowing, springs, channels, large lakes).

Surface water: During field surveys for each breeding area, we estimated the length, width, and perimeter of water at the time of survey. We calculated area of water as length*width. We analyzed area and perimeter at the scale of the breeding area and summed them to one value combining all breeding areas within a meadow. We measured the maximum depth to the nearest

0.01 m and estimated the percent of each breeding area with flowing water. We analyzed these metrics at the scale of the breeding area, and averaged them to give one value at the meadow scale.

Water temperature, cover, and shoreline gradient: Within each meadow, we randomly selected three occupied and six unoccupied breeding areas for each breeding habitat type, when available, for a maximum possible sample of 27. We sampled every 20 m around the perimeter of breeding areas. For potholes, we sampled at the water's edge. For flooded standing and flooded flowing breeding areas, we sampled at a randomly selected interior point from the water's edge. Tadpoles were most often found at the edges of potholes where water is warmer, but edge habitats of flooded breeding areas were no different than the interior.

In each sample, we measured water temperature and depth of detritus 0.1 m from the water's edge for potholes or at the interior point within flooded habitats. We estimated the percent of herbaceous, shrub, woody debris, and total cover in a 1-m^2 plot extending from the water line into the water for pothole habitats or centered on the interior point within flooded habitats. Total cover included anything large enough to hide a tadpole or metamorphosed toad such as silt, vegetation, or woody debris. We recorded the dominant substrate type (silt/organics=<0.06mm, sand=0.06–2mm, fine gravel=2–32mm, coarse gravel=32–64mm, cobble=64–256mm, boulder= >256mm, bedrock). Most breeding areas had no shrubs, but because adult toads can be found at the base of shrubs, we tested whether breeding occupancy was associated with a small amount of shrubs. We created a binary variable, based on initial data exploration, for >0–10% shrub cover (i.e., small amount) compared with one category that included shrub cover = 0 or >10% (i.e., all other values). For each metric, we averaged the values from these samples to provide one value for the breeding area. For herbaceous and total cover, we also calculated the percent of samples with dense cover (≥80%). We then averaged breeding area values to provide one value for the meadow.

We used a clinometer to measure shoreline gradients including the littoral zone (from the water's edge extending 1 m into the water for potholes or at the interior point within flooded habitats) and high water (from the high water line extending 1 m towards the water, for pothole habitats only). Littoral gradients reflect habitat at the time of survey while high water gradients reflect habitat at snowmelt when eggs were laid. For both littoral and high water gradients, we calculated the average gradient, % undercut banks ($<90^{o}$), and % low gradient shoreline ($\geq 170^{o}$). We calculated values for each breeding area and then averaged the values for the meadow.

Egg Mass and Tadpole Microhabitat

We collected microhabitat data for egg masses and tadpoles in breeding areas in occupied meadows. For egg masses, we collected microhabitat data at the end of spring breeding in seven meadows in two watersheds from 2006–2016 (Table 1.1). For tadpoles, we collected microhabitat data at approximately 1–2 week intervals in seven meadows in two watersheds during the summer of 2006, and in eight meadows in eight watersheds during the summer of 2016 (Table 1.1). We selected occupied meadows that spanned a range of habitat types from potholes to flooded vegetation, and were accessible during snowmelt for egg masses and close to roads for tadpoles. Based on results for rangewide breeding areas, for the microhabitat scale, we simplified our breeding area categories and used potholes (defined shoreline and depressional topography) and flooded vegetation (flat topography flooded with standing or flowing water, Figure 1.2).

We collected microhabitat data at 0.5-m^2 occupied and randomly selected unoccupied plots. We determined locations of egg masses at the end of 5-day surveys for adults during the spring breeding period (Brown et al. 2012). We determined the locations of tadpoles within each breeding area at the time of survey. We divided breeding areas into 4-m^2 grids, and randomly selected 10% of the occupied and unoccupied cells, sampling a minimum of 8, when available, and a maximum of 16 cells. Because there usually were less than 8 occupied cells, we typically sampled all of them. We placed 0.5-m^2 circles of tubing over the largest cluster of eggs or tadpoles within occupied cells, in the center of unoccupied cells, or in the center of the largest patch of water if the unoccupied cell was drying.

For egg masses, we measured water temperature, water depth, depth of detritus, and dominant vegetation height in the center of each occupied and unoccupied 0.5-m^2 plot (Appendix 1). We recorded dominant substrate type using the same categories used for breeding areas described above. For the entire plot, we estimated the percent of water which reflects fragmentation, flowing water, herbaceous cover, shrub cover, woody debris cover, and total cover. We measured the littoral gradient with a clinometer. Littoral gradient was measured from the water's edge extending 1 m into the water for plots on the shoreline of potholes, and along the ground surface for interior plots or those in flooded vegetation breeding areas. We recorded the time of day the sample was collected since this can affect water temperature.

For tadpoles, we measured water temperature, water depth, and dominant vegetation height, and recorded dominant substrate type in the center of each occupied and unoccupied 0.5 m^2 plot (Appendix 1). For the entire plot, we estimated the percent of water reflecting fragmentation, herbaceous cover, and total cover. We also recorded the time of day the sample was collected.

Analysis

We conducted separate analyses for each of the three scales: meadows, breeding areas within meadows, and egg mass and tadpole microhabitats (Figure 1.2). We used logistic regression to quantify the relationship between the habitat-related predictor variables and the response variable. At the meadow and breeding area scales, the response variable was presence of breeding indicated by the presence of egg masses, tadpoles, or newly metamorphosed toads. At the microhabitat scale for egg masses and tadpoles, the response variable was presence of the respective life stage. All models included covariates that accounted for spatial and temporal correlation among sample units and the nested design (Table 1.1). We used central coordinates (x,y) of watersheds (the next larger scale) as fixed effects for the meadow scale and central coordinates of meadows as fixed effects for breeding areas (Table 1.1). We used breeding areas within meadows as random effects for egg mass microhabitat, and breeding areas within meadows and meadows within watersheds as random effects for tadpole microhabitat. Because of the long time series for the egg mass data, year was included as a random effect. At the other scales, year was included as a fixed effect.

As an exploratory analysis, we used descriptive graphics, generalized additive models (GAM), and model comparison with Akaike's Information Criterion (AIC, Burnham and Anderson 2002) to select the best functional parametric form for each habitat-related predictor variable and to develop a final set of uncorrelated variables. First, we log-transformed highly skewed variables, determined visually with boxplots or density plots. Second, we used GAMs to test for quadratic relationships of each predictor variable with occupancy. We added each variable to a baseline model with spatial and temporal covariates. We included squared terms for a variable when the GAM response curves showed a clear quadratic shape. Third, we selected among highly correlated variables (Pearson $r \geq \pm 0.8$) and among variables that measured similar characteristics (e.g., area versus perimeter of water) by adding each variable to a baseline model with spatial and temporal covariates. We selected the variable whose model had the lowest AIC. In general, collinearity among predictor variables was low. We excluded variables when the model had low AIC support (ΔAIC<2) compared to a model with only spatial and temporal covariates. Fourth, we included interaction terms between variables and breeding area habitat type (e.g., pothole, flooded vegetation) when these models were as parsimonious (ΔAIC<2) as models without the interaction term. We tested interactions among depth, gradient, temperature, and habitat type where indicated by the exploratory analysis. Finally, using these steps, we developed final sets of variables for further analysis for each scale (Appendix 2, 3, 4). Analyses were conducted using SAS® software (Proc Glimmix for regressions, SAS Institute Inc., Cary, North Carolina, USA) and the R software (R Development Core Team 2015).

For the meadow and breeding area scales, we developed models for each ecological function group (physical environment, climate, habitat complexity, surface water characteristics, water temperature, cover, conditions at the time of survey), developing additive models using all possible subsets of variables in the final set of analysis variables for each group (Symonds and Moussalli 2011). Climate variables were correlated with coordinates and year. Because we were interested in the effects of climate on occupancy, we ran these models without coordinates and year, recognizing that we may not be fully accounting for spatial and temporal correlation. We selected the most parsimonious model for each ecological function group using ΔAIC<2 as the criterion (details in Appendix 2, 3). In the presentation of results, we refer to these selected models as the parsimonious models. We present the covariate relationships for the selected models and assessed fit of the selected model using Receiver Operator Curves (ROC, Fielding and Bell 1997). We compared the top selected models from each ecological functional group against each other to investigate relative importance of these functional groups.

For the microhabitat scale, we followed the same approach except that, because of the small number of variables, we developed one model rather than a model for each functional group. The model was developed using all possible subsets of the final set of habitat variables and we identified the parsimonious model using $\Delta AIC<2$ as the criterion (details in Appendix 4). For the parsimonious model, we present the covariate relationships with estimated occupancy and assessed its fit using ROC (Fielding and Bell 1997). As a last step at this microhabitat scale, we use descriptive statistics to quantify the temperatures, depths, and gradients of microhabitats used for egg mass deposition and by tadpoles (Appendix 5).

RESULTS

In general, we found similar habitat use patterns across the different scales. Temperature and water, for example, appeared to be important factors associated with Yosemite toad breeding habitats at all scales, though how this manifested varied among the scales.

Meadow

Breeding occupancy (presence of eggs, tadpoles, or metamorphosing toads) in meadows varied by year and by survey day. Annually, the estimated probability of occupancy was highest in 2005 (Figure 1.3). Seasonally, the estimated probability of occupancy was highest early to mid-summer, and then declined as tadpoles metamorphosed and sites dried over the summer.

Physical Environment: Under the most parsimonious model, estimated probability of occupancy increased with meadow area, decreased with longitudinal slope, and increased with west-facing aspects (Table 1.2, Figure 1.3, Appendix 2). The median occupied meadow in the data was 3.4 ha, but occupied meadows were as small as 0.1 ha (Table 1.3).

Habitat Complexity: In the exploratory analysis, models with richness metrics performed better than models with Shannon diversity metrics (Appendix 2). Under the most parsimonious model, the estimated probability of occupancy increased with the number of springs, inlets, outlets, and channels, and with breeding habitat type richness (Table 1.2, Figure 1.3).

Surface water: In the exploratory analysis, the model with ln(perimeter) had the most support of the variables measuring amount of water, though most of these variables were highly correlated (Appendix 2). This was also the most parsimonious model for this group (Table 1.2, Figure 1.3). The estimated probability of occupancy increased with water perimeter, most steeply up to about 1100 m.

Cover: In the exploratory analysis of the herbaceous cover metrics, the model with %plots with dense herbaceous cover (≥80%) had the most support (Appendix 2). Similarly, of the total cover metrics, the model with %plots with dense total cover (≥80%) had the most support. Under the parsimonious model, the estimated probability of occupancy was highest at intermediate amounts of dense herbaceous cover, intermediate amounts of dense total cover, and a small amount of shrub cover (1–10%, Table 1.2, Figure 1.3). Further, occupancy was positively associated with detritus depth, and most detritus depths were less than a few centimeters (median=0.01 m, Figure 1.3).

Water temperature: The estimated probability of occupancy in meadows increased with average water temperature (Table 1.2, Figure 1.3, Appendix 2).

Shoreline Gradient: In the exploratory analysis, the model with percent of low gradient ($\geq 170^{o}$) littoral zone had more support than the other measures of shoreline gradient (Appendix 2). Under the parsimonious model, the estimated probability of occupancy increased with the percent of low gradient shoreline (Table 1.2, Figure 1.3).

Climate: In the exploratory analysis, the temperature variables were highly correlated with each other and the precipitation variables were highly correlated with each other (Appendix 2). Under the most parsimonious model for this group, the estimated probability of occupancy decreased with warmer air temperatures and with %precipitation (relative to the 30-year normal) four years prior to the survey year (Table 1.2, Figure 1.3).

Survey Covariates: In the parsimonious model examining conditions at the time of survey, estimated probability of occupancy was highest in the afternoon from approximately 1145 to 1815 and at air temperatures of approximately 13–24 ^{o}C (Table 1.2, Figure 1.3, Appendix 2).

Ecological Function Group Comparison: The physical environment model had the most support among the ecological function group models at the meadow scale (Table 1.2). The other models were >2 ΔAIC from the top model and all models except the climate model ranked higher than the baseline model by >2 ΔAIC.

Breeding Area

At the breeding area scale, breeding occupancy (presence of eggs, tadpoles, or metamorphosed toads) was not associated with habitat type (Figure 1.4a). Yosemite toads bred in all three types of breeding habitats equally; models with habitat type generally were not more supported than those without this variable, though interactions with habitat type mattered for some variables. Data did not support our prediction that habitats flooded with flowing water were colder; if anything, these habitats were warmer than flooded standing or pothole habitats (Figure 1.4b).

Surface water: In the exploratory analysis, the model with ln(perimeter) had the most support among the variables measuring amount of water (area, perimeter, Appendix 3). Models with depth were not supported at this scale, likely because there was not enough variation in the available habitat; the majority of breeding area depths were shallow (medians < 0.2 m, Table 1.4, Appendix Figure 1.5-1).

The most parsimonious model in this ecological function group was ln(perimeter)*habitat type interaction (Table 1.5). Perimeter length had a greater influence on estimated probability of occupancy for flooded standing and flooded flowing breeding areas than for potholes (Figure 1.5). Perimeter lengths were smallest in potholes and largest in flooded vegetation with flowing water (Figure 1.5).

Cover: In the exploratory analysis, the model with %plots with dense herbaceous cover (≥80%) had the most support among the herbaceous cover metrics, and the model with %plots with dense total cover (≥80%) had the most support among the total cover metrics (Appendix 3). Under the parsimonious model in this group, the estimated probability of occupancy was highest at intermediate amounts of dense herbaceous cover and dense total cover (Table 1.5, Figure 1.5). Breeding occupancy was positively associated with detritus depth, and most detritus depths were < 0.02 m.

Water Temperature: In the exploratory analysis, the model with maximum water temperature had the most support (Appendix 3). Occupancy was positively associated with maximum water temperature (Table 1.5, Figure 1.5, Table 1.4, Appendix Figure 1.5-1).

Shoreline Gradient: Shoreline gradient has different meanings in the different habitat types; flooded vegetation habitats are all low gradient and do not have shorelines in the same sense as potholes. Shoreline gradients were generally low overall (Table 1.4, Appendix Figure 1.5-1). In the exploratory analysis, the percent of low gradient littoral zone ($\geq 170^{o}$) was the most supported shoreline gradient variable, and the squared form had the most support (Table 1.5,

Appendix 3). The estimated probability of occupancy was highest in breeding areas with intermediate percentages of low gradient littoral zones (Figure 1.5).

Survey Covariates: Under the parsimonious model examining conditions at the time of survey, the estimated probability of occupancy was highest early to mid-afternoon, and at temperatures similar to those for the meadow scale (Table 1.5, Figure 1.5, Appendix 3).

Ecological Function Group Comparison: Surface water had the most support among the different ecological function groups at the breeding area scale (Table 1.5). The other models were >2 ΔAIC from the top model and all ranked higher than the baseline model by >2 ΔAIC.

Egg Mass Microhabitat

Egg mass microhabitats had shallow, warm water. Based on descriptive data, occupied plots tended to be warmer than unoccupied plots (Table 1.6, Appendix Figure 1.5-2). Water depths appeared to be very specific and were similar between pothole and flooded vegetation habitat types (Table 1.6, Appendix Figure 1.5-2). In potholes, occupied plots were shallower than unoccupied plots whereas in flooded vegetation, they were deeper. Further, the exploratory analyses indicated that water depth may interact with temperature; higher temperatures were found in shallower water (Figure 1.6). In potholes, most littoral zones were low gradient (Table 1.6, Appendix Figure 1.5-2).

Temperature, surface water, and cover variables were associated with egg mass microhabitats. The most parsimonious model included water depth*temperature*habitat type interaction, %water, %flow*habitat type interaction, %herbaceous cover^2, and dominant vegetation height (Appendix 4). Water temperature and depth influenced the selection of egg mass microhabitat differently between the habitat types. The estimated probability of occupancy was positively associated with water temperature, but the association was stronger in potholes than in flooded vegetation (Figure 1.7). The probability of occupancy was negatively associated with water depths in potholes and positively associated with water depths in flooded vegetation. Potholes tended to have colder temperatures and a wider range of available water depths, whereas temperatures and depths were more uniform in flooded vegetation breeding areas (Figure 1.6, Table 1.6).

In addition, the estimated probability of occupancy was positively associated with %water, negatively associated with %flow and vegetation height, and the probability of occupancy was highest at intermediate levels of herbaceous cover (Figure 1.7). Thus, Yosemite toads tended to lay their eggs in warm, shallow water, in areas with more contiguous water and less flow, and in areas with intermediate amounts of herbaceous vegetation cover and shorter vegetation height.

Tadpole Microhabitat

Similar to egg mass deposition sites, tadpole microhabitats were generally warm and shallow, though there were fewer differences between occupied and unoccupied tadpole plots than observed in egg mass plots (Table 1.6, Appendix Figure 1.5-3). The majority of littoral gradients measured were very low (Table 1.6, Appendix Figure 1.5-3).

The most parsimonious model included %water, temperature, and the quadratic dominant vegetation height (Appendix 4). Surprisingly, depth did not appear consistently in this group of models. Shoreline gradients for tadpole plots were only collected in 2006. There was little support for models with these variables, likely because most littoral gradients were low.

The estimated probability of tadpole occupancy was positively correlated with water temperature and %water in the plot, and occupancy was highest at intermediate levels of vegetation height (Figure 1.7). The estimated probability of occupancy was slightly negatively correlated with depth in pothole habitats and slightly positively correlated with depth in flooded vegetation habitats.

DISCUSSION

The Yosemite toad must contend with a variable and unpredictable environment and a short growing season (Kagarise Sherman and Morton 1984, Wolf and Cooper 2015). Its habitat selection reflects choices that promote fast development, likely an adaptation to these uncertain conditions (Brattstrom 1962, Karlstrom 1962, Kagarise Sherman 1980, Sadinski 2004). Our results provide quantitative evidence characterizing Yosemite toad breeding habitat and support previous descriptions that they breed in very shallow, warm water, with low gradient shorelines (Mullally 1953, Karlstrom and Livezey 1955, Karlstrom 1962, Cunningham 1963, Kagarise Sherman 1980). Water and temperature were important drivers of Yosemite toad breeding habitat selection at each of the three scales—meadows, breeding areas, and microhabitats for eggs and tadpoles. Yosemite toads appeared to find their preferred microhabitats in a variety of broader breeding area habitat types, showing no preference for whether a particular breeding area was a pothole or flooded vegetation with standing or flowing water. However, potholes and flooded vegetation habitats differed in structural features that may influence other more important characteristics. Similar to other studies, our results found that Yosemite toads select for very small differences in habitat structure (Liang et al. 2017).

Water variables, although not the most supported ecological function group at the meadow and breeding area scales, were important at all scales and generally reflected the need for hydroperiods long enough for metamorphosis to occur (Semlitsch et al. 1996, Green et al. 2013, Chandler et al. 2016). Egg mass and tadpole microhabitats tended to be in less fragmented water, measured by %water in a plot, and Yosemite toads bred less often in the smallest breeding areas and in meadows with the smallest amounts of water. This agrees with a study of 19 meadows where tadpoles were found more often in pools with more surface water (Liang et al. 2017). Yosemite toads will, however, lay eggs in very small pockets of water and small meadows; the smallest occupied meadow was 0.1 ha. Finally, Yosemite toads bred more often in low gradient meadows, which may retain higher water tables longer, leading to longer hydroperiods in breeding area surface water (Wolf and Cooper 2015, Baccei et al. 2020). Species distribution modeling with only occupied meadows also found higher Yosemite toad occupancy in low gradient meadows, but in contrast with our results, did not find an association with meadow area (Liang and Stohlgren 2011). Larger, flatter meadows had higher Yosemite toad genetic diversity and more genetic inflow (Maier et al. 2022).

Water temperature was a primary driver of Yosemite toad habitat selection at all scales. Although the temperature ecological function group models were not the most supported at the meadow and breeding area scales, temperature or habitat characteristics that affect temperature were consistently strong predictors across scales. Similar to Liang et al. (2017), we found that breeding occupancy was associated with warmer water temperatures. Occupied water was usually quite warm, even at snowmelt, with microhabitat temperatures averaging 22.3–25.2 ^{o}C and maximums of 32.0–35.6 ^{o}C. The range of water temperatures in this study was similar to values reported by other studies including older descriptive papers (Mullally 1953, Karlstrom 1962, Liang et al. 2017). Maximum temperatures in this study approached the estimated critical maximum temperatures of 36–38 ^{o}C for tadpoles (Karlstrom 1962). Contrary to our expectations, flowing water over vegetation was no cooler than shallow standing water, and model results showed no preference for breeding areas with standing versus flowing water. The importance of temperature was further shown by other variables at the different scales in interesting ways. For example, while all the highly correlated hydrology metrics (e.g., area) had substantial support, the length of the water perimeter, habitat that tends to be shallow and warm, was the most important. In addition, meadows with west-facing aspects, which receive more heat from the sun than east or north-facing aspects, were more likely to be occupied (Liang and Stohlgren 2011).

When considering options for managing and restoring habitats, it is helpful to think in terms of structures that can be modified (Hamer et al. 2002, Green et al. 2013, Lind et al. 2016). Our results suggest that the amount of water, which reflects hydroperiods (Semlitsch et al. 1996, Green et al. 2013, Chandler et al. 2017), and water temperature (Karlstrom 1962, Liang et al. 2017) are likely the two most important breeding habitat characteristics for the Yosemite toad. Structures that can be modified to influence these characteristics include water depth and shoreline gradient which may singly or interactively influence water temperature. Shallow water tends to be warmer and gradual sloping shorelines have shallow zones which tend to have warmer water (Mullally 1953, Karlstrom 1962, Pereyra et al. 2011). Conversely, deeper water is more likely to have the six to eight week hydroperiods needed for metamorphosis (Green et al. 2013, Lee et al. 2015, Chandler et al. 2016, Gould et al. 2019). Thus, we predicted egg mass and tadpole microhabitats would have relatively shallow water depths while maximum depths of occupied breeding areas would be relatively deep. Our results suggested that water depths are very important for egg mass microhabitat selection, but models with water depth had less support at the other scales.

For egg mass deposition, Yosemite toads showed a clear preference for very shallow water of a specific depth (0.04–0.05 m); there was a negative relationship between water depth and egg mass deposition in potholes which tend to have deeper water, whereas there was a positive relationship with water depth in flooded vegetation areas where water tends to be shallow and of more uniform depth. These preferred depths are most likely related to optimal temperatures for egg development (Karlstrom 1962, Kagarise Sherman 1980). Egg masses are vulnerable at these depths and it is quite common for eggs to desiccate with daily water level fluctuations or die from overnight freezing (Karlstrom 1962, Kagarise Sherman 1980, Kagarise Sherman and Morton 1984, Sadinski et al. 2020, pers. obs.).

For tadpoles, temperature may be the main driver of habitat selection, with depth contributing little extra explanatory power. Almost all water in Sierra Nevada meadows, excluding lakes, tends to be shallow, with maximum depths in the study meadows averaging 0.11–0.25 m depending on the habitat type. Water depths in the meadows were so shallow that water temperatures tended to fluctuate diurnally, becoming relatively consistently warm by mid-day. Tadpoles behaviorally select temperatures (Mullally 1953, Karlstrom 1962), and in the cooler early morning, we have observed tadpoles clustered up against the edges of potholes where water was warmer, and then swimming throughout the same pothole later in the day when all the water had warmed. In contrast with Liang et al. (2017), who found occupied pools to be slightly, but significantly, deeper than unoccupied pools, our models found little evidence that toads selected breeding areas with deeper maximum depths. Liang et al. (2017) did not distinguish between habitat types and their average depths were more similar to our flooded vegetation habitats. Both studies, however, found that Yosemite toads use very shallow water and may cue into very small differences in habitat.

Shorelines were predominately low gradient, likely explaining the low support for this variable. We have, however, observed that most egg masses in potholes are laid in gently sloping areas which may provide a way for eggs to be laid in the preferred shallow water and still allow a path for larvae to swim to deeper water upon hatching (Karlstrom 1962). Flooded vegetation habitats do not have true shorelines. We also have observed that Yosemite toads tend to breed less often in the steep-sided tarns common in the Sierra Nevada (also Sadinski 2004). At snowmelt in these tarns, the shoreline water is deeper than typical egg mass sites, though water depths and temperatures available to tadpoles may be similar during the rest of the summer.

In general, Yosemite toad tadpoles preferred intermediate levels of dense herbaceous cover, which may provide refuge from predators, shelter from the environment, and open areas for basking. Most potential breeding areas had very high cover of some sort consisting of silt substrate, detritus, and herbaceous vegetation. Yosemite toad adults commonly hide at the base of willows in meadows (Mullally 1953, Karlstrom 1962, Martin 2008), and during breeding, we often find individuals in these areas (pers. obs.). However, Yosemite toads tend not to breed in meadows dominated by shrubs or in water with high shrub cover, likely because the shading prevents solar input resulting in cooler water. The positive association between probability of occupancy and meadows with a small percent of shrubs at breeding areas supports these observations. Although all life stages of Yosemite toads are toxic to many predators similar to other bufonid species (Grasso et al. 2010), they are still prey to garter snakes, some birds, and some aquatic insects and thus may select areas that provide refuge (Kagarise Sherman 1980, Kagarise Sherman and Morton 1993).

Although spatial structure as a habitat component has been studied (Sjogren et al. 1991, Funk et al. 2005, Browne et al. 2009, Cuello et al. 2017), there have been few empirical studies on the importance of habitat complexity as a habitat component, particularly when taking into account temporal variability. For example, species subject to stochastic weather may switch to different habitats depending on current conditions (Fryxell et al. 2005, Roe and Georges 2007, Oliver et al. 2010). For species like the Yosemite toad that are exposed to variable, unpredictable weather, a diversity of available habitats in breeding meadows may be crucial for their long-term persistence (McCaffery et al. 2014). Habitats such as potholes may provide sufficient hydroperiods during low snowpack years when the flooded vegetation areas dry early, whereas the flooded vegetation habitats may be preferred during high snowpack years when potholes may be too deep and cold. Meadows that contain both types of habitats may confer resilience by the presence of suitable habitat during a variety of weather. Supporting this hypothesis, we found a positive association of occupancy with meadows that have a variety of breeding area habitat types. Additionally, the diversity of habitats as well as the presence of springs, inlets, outlets, and channels may indicate an underlying hydrology that supports longer hydroperiods in breeding areas. Springs and channels also may provide other non-breeding wet habitats for adults and subadults. Overall, our results suggest Yosemite toads prefer meadows with habitat complexity.

Given the Yosemite toad's reliance on warm ephemeral water, we predicted that occupancy would be higher in wetter and warmer conditions. Contrary to our predictions, occupancy was associated with drier and colder years. In contrast, Wang (2012) found a positive association between genetic effective population size (N_e) and precipitation, but not with air temperature, whereas Liang and Stohlgren (2011) concluded Yosemite toads tolerated a wide range of air temperatures and precipitation. Our results suggest breeding may be more successful in drier years. Yosemite toads take 3–4 years to mature (Kagarise Sherman 1980), so the amount of precipitation four years prior to surveys may reflect weather conditions when breeding adults were tadpoles. Demographic data spanning a historic drought (2012–2016) support both the counter-intuitive positive association with drier years and the lag effect; we are currently exploring this in more detail (Brown et al. unpublished data, also McCaffery and Maxell 2010). Colder air temperatures may lead to slower snowmelt or drying of surface water resulting in longer hydroperiods. Alternatively, air temperature was highly correlated with location (easting, elevation), making it difficult to separate the effects of these factors. Yosemite toads occur at high elevations which tend to be colder. Overall, support was weak for the climate models, which may be partly due to the coarse resolution (4 km^2) of the climate data relative to the scale of our meadows. Topography and microclimates of mountain environments are highly variable and the effects of climate on the hydrology of mountain meadows and wetlands can be complex (Margulis et al. 2016, Barton et al. 2018, Gould et al. 2019). Matching the spatial resolution of climate data with the study system of interest, which often is not possible currently, can improve accuracy (Barton et al. 2018). The climatic characteristics of Yosemite toad breeding meadows warrants further study as finer resolution weather data becomes available.

Understanding how habitat associations influence a species' persistence is fundamental to developing effective conservation and management. The Yosemite toad's selection of breeding habitats may be an adaptation that facilitates fast development in short growing seasons in a variable and unpredictable environment. Their selection of very shallow warm water suggests they prioritize fast development over hydroperiods that would increase the likelihood of metamorphosis. This choice seems somewhat paradoxical given the high mortality of eggs and tadpoles (pers. obs., Kagarise Sherman 1980, Kagarise Sherman and Morton 1984, Sadinski et al. 2020). Water depths of only a few centimeters provide little protection from freezing temperatures or desiccation. The trade-off may be more time for metamorphosed toads to grow before entering hibernation for the winter. Larger metamorphs may have a greater chance of surviving long, cold winters (Berven 1990, Wilbur 1997). It also is possible that current conditions differ from previous conditions. Meadows generally may be drier than historically, resulting in increased tadpole desiccation. Climates may have been colder with even shorter growing seasons. High recruitment failure is common in ephemeral water-breeding amphibians and population persistence may rely on long-lived adults (Biek et al. 2002, Taylor et al. 2006). The oldest known Yosemite toads are at least 12 years old (Kagarise Sherman and Morton 1984, Brown unpublished data), and conditions leading to successful recruitment must occur often enough to replace adults for long-term persistent of the species.

This study provides insights on opportunities and risks of habitat management to the Yosemite toad. Wet meadows are sensitive to small changes in their hydrology (Patterson and Cooper 2007), which may alter hydroperiods of shallow surface water and lead to large effects on Yosemite toad breeding success. Wet meadows are among the most impacted habitats in the Sierra Nevada (Menke et al. 1996, Purdy et al. 2012), and many common management activities can affect their hydrology (Purdy et al. 2012, Viers et al. 2013, Baccei et al. 2020). Restoration of degraded meadows is a growing priority in the Sierra Nevada (NFWF 2010), and while this provides an opportunity to benefit the Yosemite toad, it also poses a risk if the fine-scale habitat requirements of the species are not incorporated into restoration designs. For example, surface water that is too deep may not be suitable breeding habitat. Finally, climate change has altered temperature and precipitation patterns in the Sierra Nevada, as well as increased the frequency and duration of droughts. These broad weather patterns will likely affect the hydrology, climates, and growing season of Yosemite toad breeding meadows.

Finally, our results provide specific quantitative guidance for management and conservation of Yosemite toad breeding habitat at multiple scales. Management to benefit the species includes protection of the very shallow, warm surface waters used by the species for breeding and the fine-scale microtopography that influences the presence and hydroperiods of this surface water. Developing habitat manipulations that increase hydroperiods without substantially deepening surface water would increase the resilience of breeding areas to risks like climate change. Appropriate meadow restoration designed to benefit or even avoid harm to the toad would incorporate design criteria at very fine resolutions of a few centimeters. Water depth and shoreline gradients, which affect water temperatures, are habitat structures that can be manipulated and incorporated into meadow restoration designs or other management actions. Finally, our meadow scale results could inform prioritization of meadows to protect, manage, or restore. For example, meadow scale characteristics such as low slope and habitat complexity may indicate an underlying hydrology that supports shallow warm water habitats.

This study, which is based on a large portion of the Yosemite toad's range, quantifies many of the descriptive general assumptions about the species habitat requirements. Important habitat components were similar across scales, and were reflected in habitat structures, such as meadow slope, specific to a given scale. Analyzing by ecological function allowed exploration of habitat components that might have been obscured by other habitat components had we combined all variables. The Yosemite toads clearly preferred breeding areas with very specific structure, and research is warranted on the hydrologic and geomorphic processes that create, maintain, and regulate the very shallow surface water used for breeding. Habitat selection and habitat management occurs at multiple scales, and ecological processes that affect habitats vary across scales (Wiens 1981, McGarigal et al. 2016, Cecala et al. 2018). Understanding preferred habitats provides insights into how best to conserve and manage habitats that promote Yosemite toad persistence and recovery in their variable and unpredictable environments.

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Table 1.1. Design for regression analysis examining Yosemite toad breeding habitat relationships at the meadow, breeding area, and egg and tadpole microhabitat scales on National Forest lands across the species' range in the Sierra Nevada, CA.

			Microhabitat	
	Meadow	Breeding Area within Meadow	Egg Deposition	Tadpole
Sample Size (sample unit*survey)	512	1468	1688	1796
Number Occupied (sample unit*survey)	101	191	364	488
Spatial Sample Unit	311 wet meadows within 62 watersheds	Contiguous areas of water within 313 meadows within 62 watersheds	0.5-m^2 plots within 40 breeding areas within 7 occupied meadows in 2 watersheds	0.5-m^2 plots within 47 breeding areas within 13 occupied meadows within 8 watersheds
Temporal Coverage	1 survey/year from 2003-2006, 115 meadows surveyed annually	1 survey/year from 2003-2006, 115 meadows surveyed annually	1 survey/year for 11 years (2006 to 2016)	Surveys approximately every 2 weeks in 2006 and 2016
Controlling for Design	Fixed effects for watershed coordinates, survey day, and year	Fixed effects for meadow coordinates, survey day, and year	Random effects for breeding area within meadow, year	Random effects for breeding area within meadow and meadow within watershed, fixed effect for year

Table 1.2. Comparison of Yosemite toad habitat selection models by ecological function group at the scale of meadows. Meadows were surveyed from 2003-2006 on National Forest lands across the Yosemite toad's range in the Sierra Nevada, CA. All models, except for the climate model, included watershed coordinates ($x*y+x^2$), survey day^2, and year. Models with climate variables included survey day^2 and not coordinates and year. N=512. K=number of parameters in the model, AIC=Akaike's Information Criterion, ΔAIC=change in AIC value of model from top model, AUC=Area under the curve value.

Function Group	Model	K	Deviance	AIC	ΔAIC	AUC
Physical Environment	ln(site area) + longitudinal slope + EW aspect	13	316.0	342.0	0.0	0.90
Habitat Complexity	ln(#springs, inlets, outlets, channels) + breeding area habitat type richness	12	349.3	373.3	31.3	0.86
Surface Water	ln(perimeter)	11	352.4	374.4	32.4	0.86
Cover	%plots with dense (≥80%) herbaceous cover^2 + %plots with dense (≥80%) total cover^2 + shrubs(1-10%) + ln(detritus)	16	408.5	440.5	98.5	0.80
Water Temperature	average water temperature	11	438.6	460.6	118.6	0.75
Survey Conditions	air temperature^2 + survey time^2	14	447.2	475.2	133.2	0.74
Gradient	%littoral gradient ≥ 170^o	12	455.4	479.4	137.4	0.73
Climate	average air temperature + %precipitation 4 years prior to year of survey	5	470.3	480.3	138.3	0.69
Baseline Model	xcoord*ycoord+xcoord^2+survey day^2+year	10	462.0	482.0	140.0	0.70

Table 1.3. Descriptive statistics for area and slope of Yosemite toad breeding meadows (N=512) surveyed from 2003-2006 on National Forest lands across the species' range in the Sierra Nevada, CA. These variables can be easily determined from GIS and may be useful for designing management and conservation actions. N=number of meadows.

	N	Median	Mean	SD	Min	Max
Site area (ha)						
Unoccupied	411	0.5	2.1	4.6	0.0	46.1
Occupied	101	3.4	6.4	7.9	0.1	37.2
Longitudinal slope						
Unoccupied	411	5.0	7.7	7.0	0.0	42.0
Occupied	101	3.0	4.8	4.3	0.0	22.0

Table 1.4. Descriptive statistics for water temperature, water depth, and shoreline gradient in Yosemite toad breeding areas within meadows (N=1468) surveyed from 2003-2006 on National Forest lands across the species' range in the Sierra Nevada, CA. These variables can be measured in the field and may be useful for designing and evaluating management and conservation actions. Shoreline gradients were not measured in flooded vegetation habitats. N=number of breeding areas.

	Pothole						Standing						Flowing					
	N	Median	Mean	SD	Min	Max	N	Median	Mean	SD	Min	Max	N	Median	Mean	SD	Min	Max
Maximum water temperature (^{o}C)																		
Unoccupied	511	18.0	18.1	5.2	3.0	31.0	417	19.0	19.2	5.7	4.0	36.0	349	18.0	18.7	6.2	5.0	34.0
Occupied	65	21.0	21.2	4.6	13.0	35.0	67	23.0	22.6	6.3	7.0	36.0	59	25.0	24.6	4.8	7.0	37.0
Maximum water depth (m)																		
Unoccupied	510	0.19	0.25	0.23	0.01	1.70	417	0.08	0.12	0.14	0.01	1.30	348	0.09	0.11	0.10	0.01	1.00
Occupied	64	0.20	0.25	0.16	0.01	0.90	67	0.10	0.14	0.10	0.03	0.45	59	0.10	0.15	0.15	0.01	0.70
%Low gradient shoreline (≥170^{o}) (littoral)																		
Unoccupied	511	20.0	42.3	45.9	0.0	100.0	417	100.0	84.4	33.8	0.0	100.0	349	100.0	94.6	18.3	0.0	100.0
Occupied	65	66.7	57.0	45.0	0.0	100.0	67	100.0	90.2	19.5	0.0	100.0	59	100.0	93.2	19.0	0.0	100.0
%Low gradient shoreline (≥170^{o}) (high water)																		
Unoccupied	508	0.0	24.9	39.8	0.0	100.0	-	-	-	-	-	-	-	-	-	-	-	-
Occupied	62	0.0	33.0	41.8	0.0	100.0	-	-	-	-	-	-	-	-	-	-	-	-

Table 1.5. Comparison of Yosemite toad habitat selection models by ecological function group at the scale of breeding areas within meadows. Surveys were conducted from 2003-2006 on National Forest lands across the Yosemite toad's range in the Sierra Nevada, CA. All models included meadow coordinates ($x*y+x^2$), survey day^2, and year. N=1468. K=number of parameters in the model, AIC=Akaike's Information Criterion, ΔAIC=change in AIC value of model from top model, AUC=Area under the curve value.

Function Group	Model	K	Deviance	AIC	ΔAIC	AUC
Surface Water	ln(perimeter)*habitat type	13	928.7	958.7	0.0	0.80
Cover	%plots dense(≥80%) herbaceous cover^2 + %plots dense (≥80%) total cover^2 + ln(detritus)	15	944.1	974.1	15.5	0.79
Water Temperature	maximum water temperature	11	971.6	993.6	35.0	0.77
Shoreline Gradient	littoral gradient^2*habitat type	18	1004.4	1040.4	81.8	0.75
Survey Conditions	air temperature^2 + survey time^2	14	1015.0	1043.0	84.4	0.73
Baseline Model	xcoord*ycoord+xcoord^2+survey day^2+year	10	1034.2	1054.2	95.6	0.71

Table 1.6. Descriptive statistics for water temperature, water depth, and littoral gradient at the microhabitat scale for Yosemite toad egg masses (N=1688 collected 2006–2016 in seven meadows) and tadpoles (N=1796 collected in 2006 in seven meadows and 2016 in eight meadows) in the Sierra Nevada, CA. These variables can be measured in the field and may be useful for designing and evaluating management and conservation actions. N=number of habitat plots.

	Pothole						Flooded					
	N	Median	Mean	SD	Min	Max	N	Median	Mean	SD	Min	Max
Egg Mass												
Water temperature (°C)												
Unoccupied	668	22.0	20.2	5.6	1.0	30.0	656	21.0	20.7	5.7	3.0	32.0
Occupied	187	24.0	23.4	4.9	6.0	32.0	177	22.0	22.5	5.9	6.9	33.0
Water depth (m)												
Unoccupied	668	0.08	0.09	0.07	0.01	0.40	656	0.03	0.03	0.03	0.00	0.40
Occupied	187	0.05	0.05	0.03	0.00	0.35	177	0.04	0.04	0.02	0.00	0.09
Littoral gradient												
Unoccupied	668	175.0	170.8	16.6	70.0	190.0	656	175.0	173.7	18.4	0.0	185.0
Occupied	187	175.0	174.0	8.6	110.0	180.0	177	175.0	175.2	13.7	0.0	185.0
Tadpole												
Water temperature (°C)												
Unoccupied	423	20.1	20.8	5.2	9.3	34.3	885	24.0	23.4	5.8	5.0	35.3
Occupied	217	22.3	22.3	5.0	10.3	32.7	271	25.0	25.2	5.4	10.8	35.6
Water depth (m)												
Unoccupied	423	0.08	0.09	0.06	0.01	0.38	885	0.03	0.04	0.02	0.00	0.31
Occupied	217	0.07	0.08	0.05	0.01	0.27	271	0.04	0.05	0.03	0.01	0.30
Littoral gradient[1]												
Unoccupied	132	175.0	173.6	12.9	90.0	185.0	323	175.0	174.5	6.7	120.0	190.0
Occupied	121	175.0	171.6	20.7	50.0	210.0	151	175.0	176.6	5.3	150.0	185.0

[1] Gradients for tadpole microhabitat were only collected in 2006.

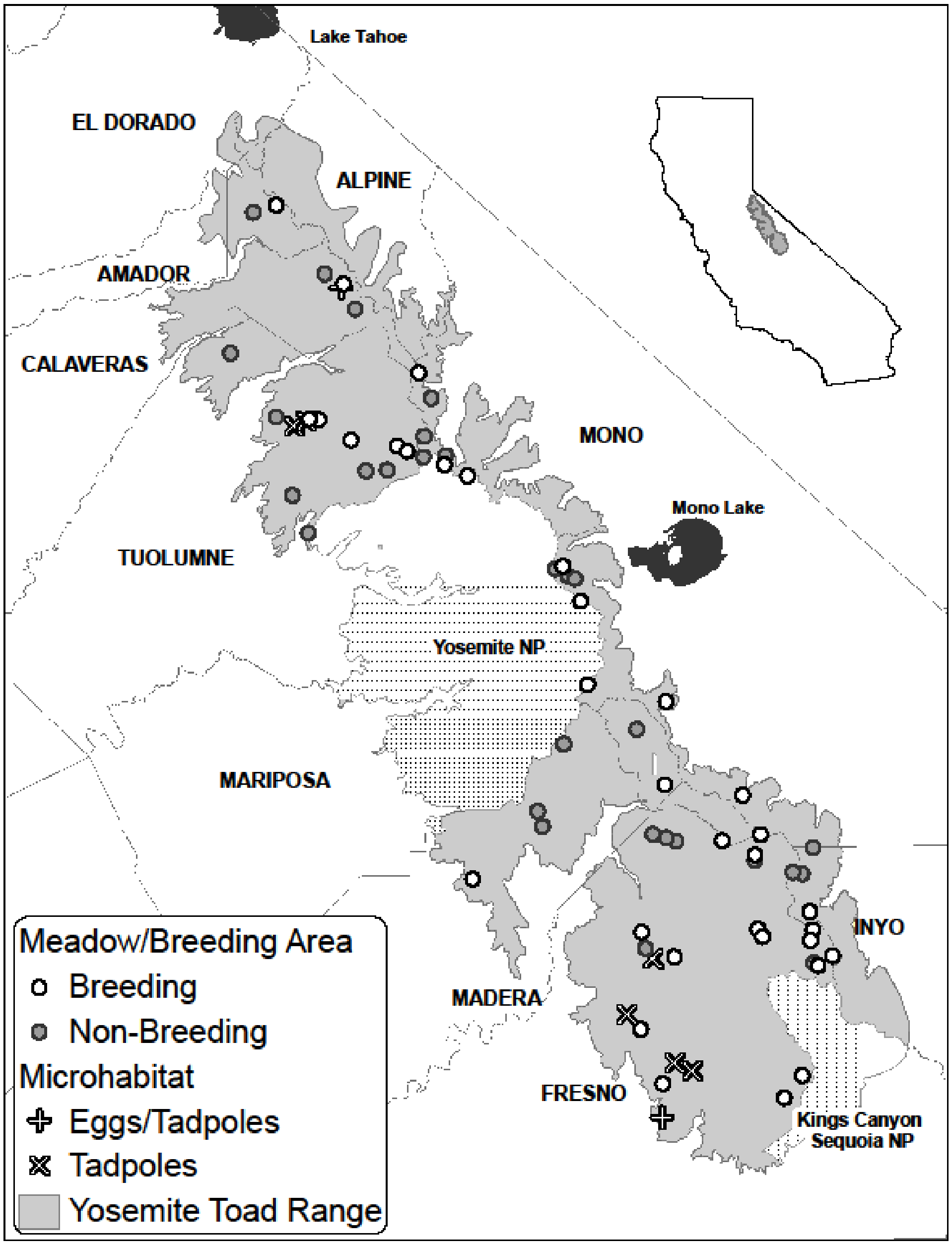


Figure 1.1. Monitoring area which included National Forest lands with the range of the Yosemite toad (*Anaxyrus canorus*) in the Sierra Nevada, California. National Park lands within the range (shown by hatch marks) were not surveyed. Circles show locations of surveyed watersheds for meadow and breeding area scales (2003-2006). Open circles indicate presence of at least one occupied meadow. Crosses and Xs show locations where microhabitat data for eggs (2006-2016) and tadpoles (2006, 2016) were collected.

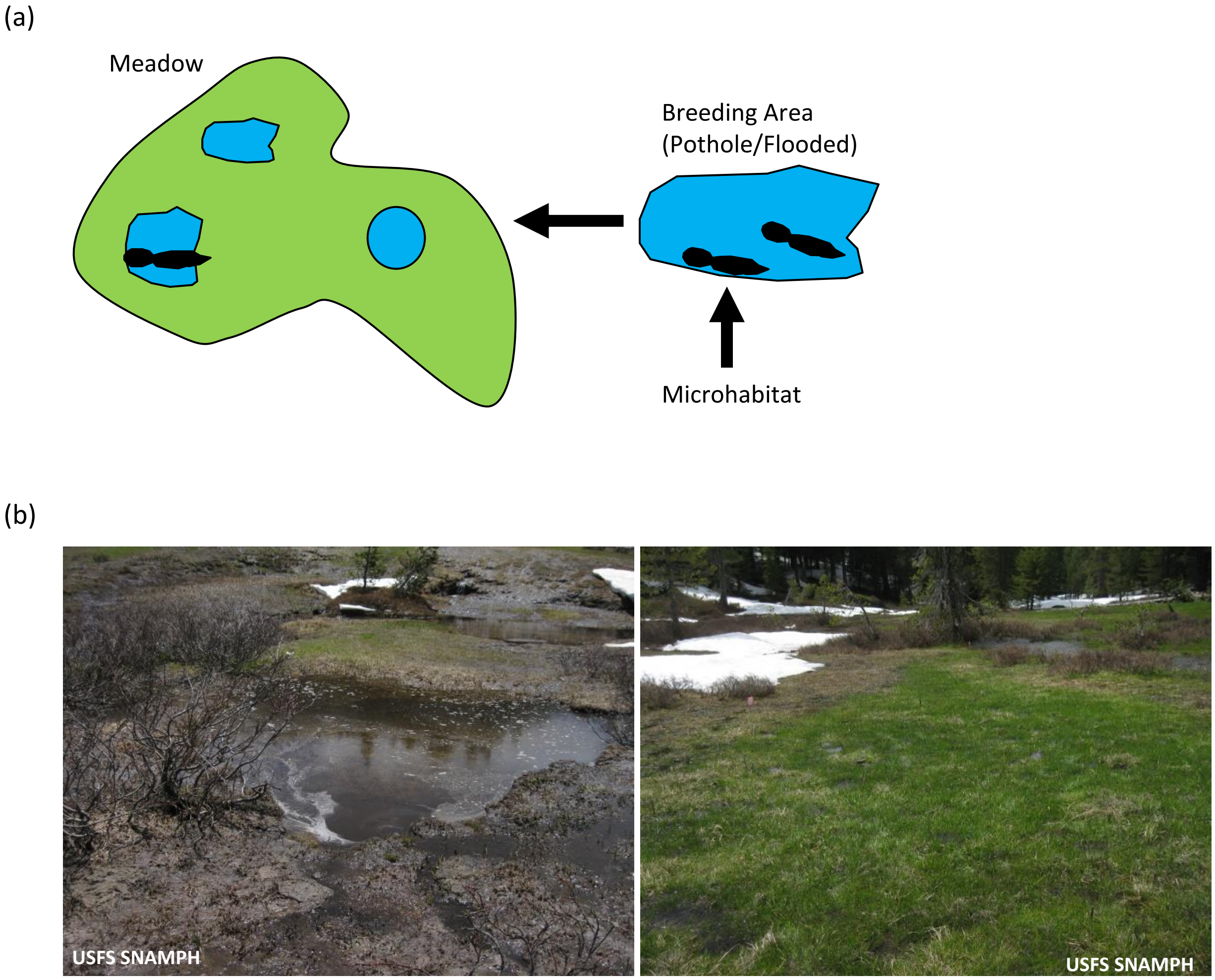
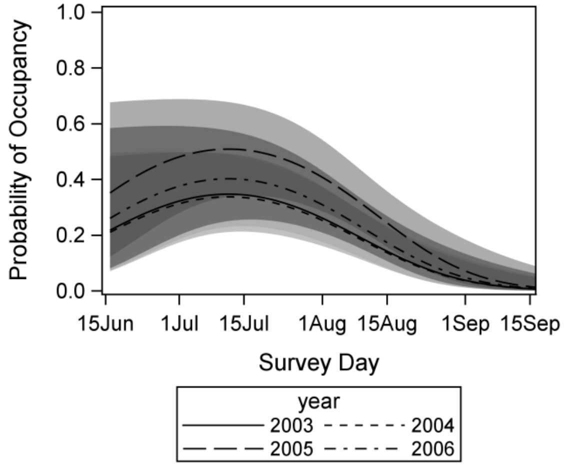


Figure 1.2. Scales of habitat data collection for the Yosemite toad including (a) meadow, breeding area, and microhabitats for eggs and tadpoles, and (b) examples of pothole and flooded vegetation breeding areas. Water in flooded vegetation habitats can be standing or flowing. Pictures from USDA Forest Service Sierra Nevada Amphibian Monitoring Program (SNAMPH).

Survey Day From Baseline Model



Physical Environment

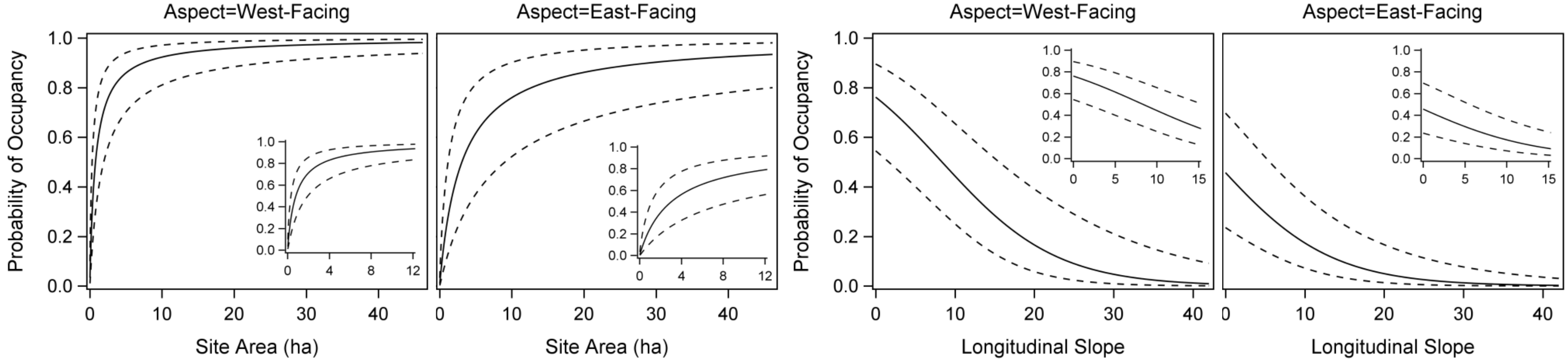


Figure 1.3. Estimated logistic regression relationships between breeding occupancy and habitat variables by ecological function group in wet meadows used by Yosemite toads. Meadows were surveyed from 2003-2006 on National Forest lands across the Yosemite toad's range in the Sierra Nevada, CA. Differences among years are shown for survey day. Differences in occupancy for the categorical variable, shrub cover, is only shown only for %dense herbaceous vegetation. Insets show low value end of curves for some variables. All models, except for the climate model, include watershed coordinates ($x*y+x^2$), survey day^2, and year. The climate model includes survey day^2.

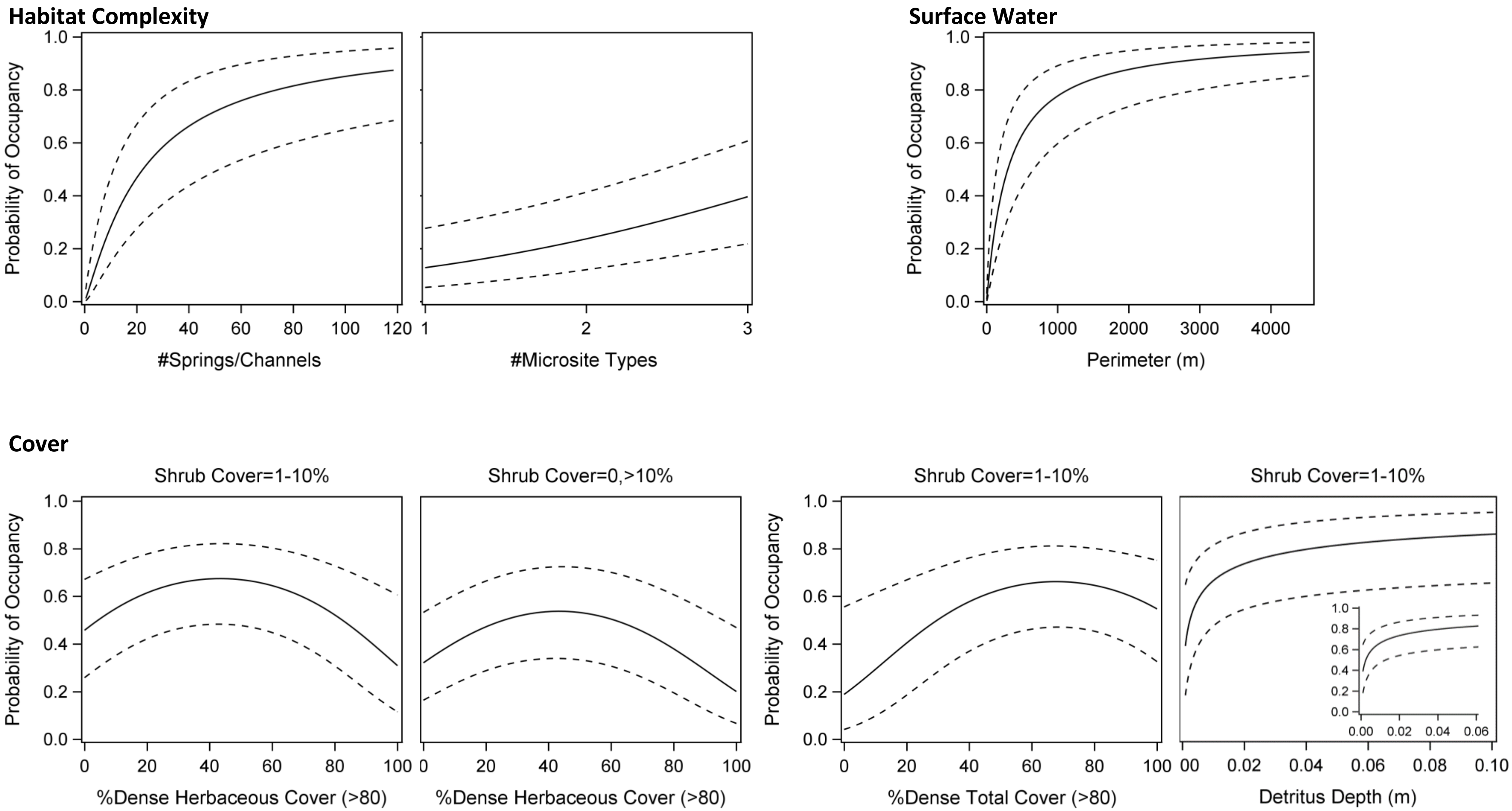
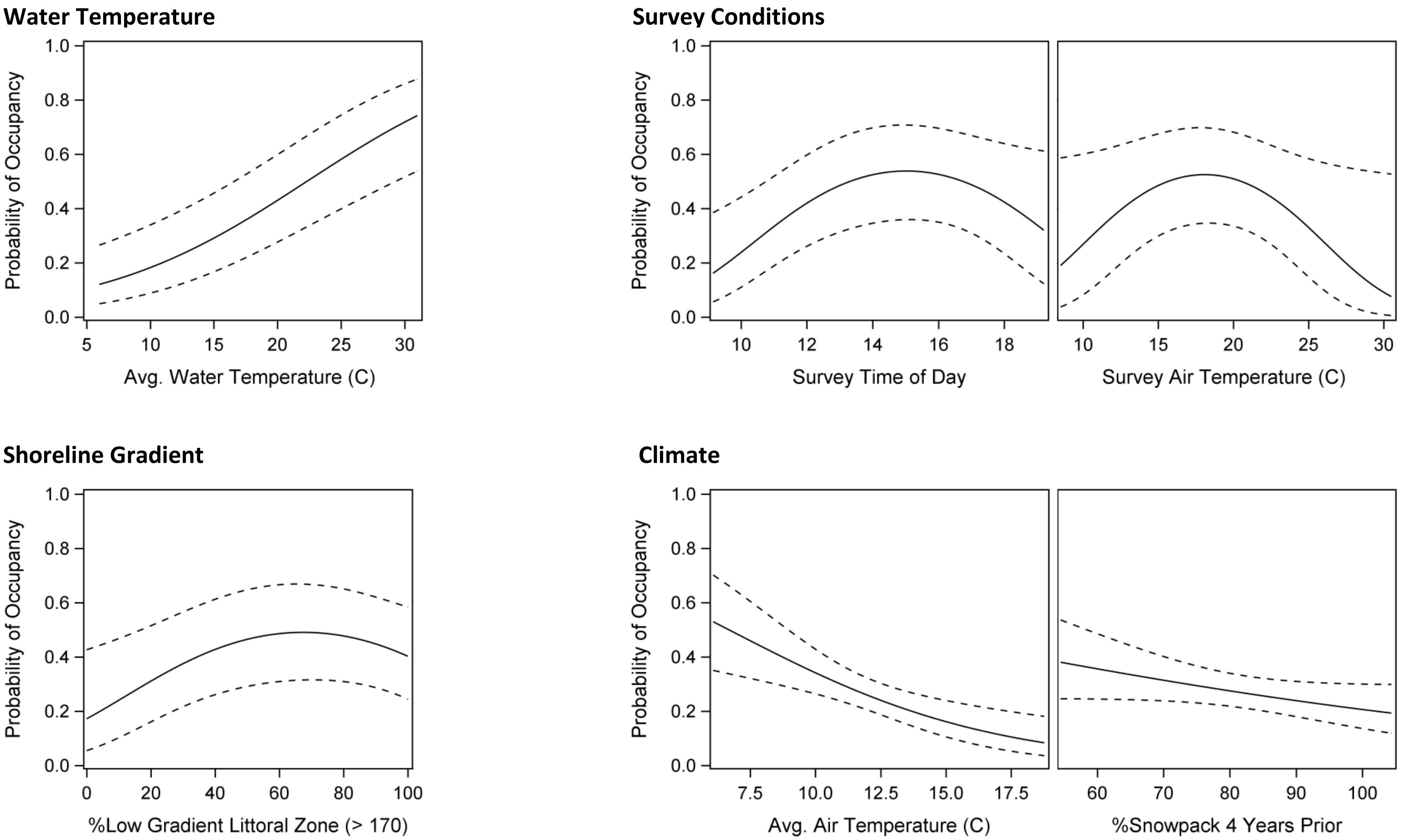


Figure 1.3. Continued.


Water Temperature

Survey Conditions

Shoreline Gradient

Climate

Figure 1.3. Continued.

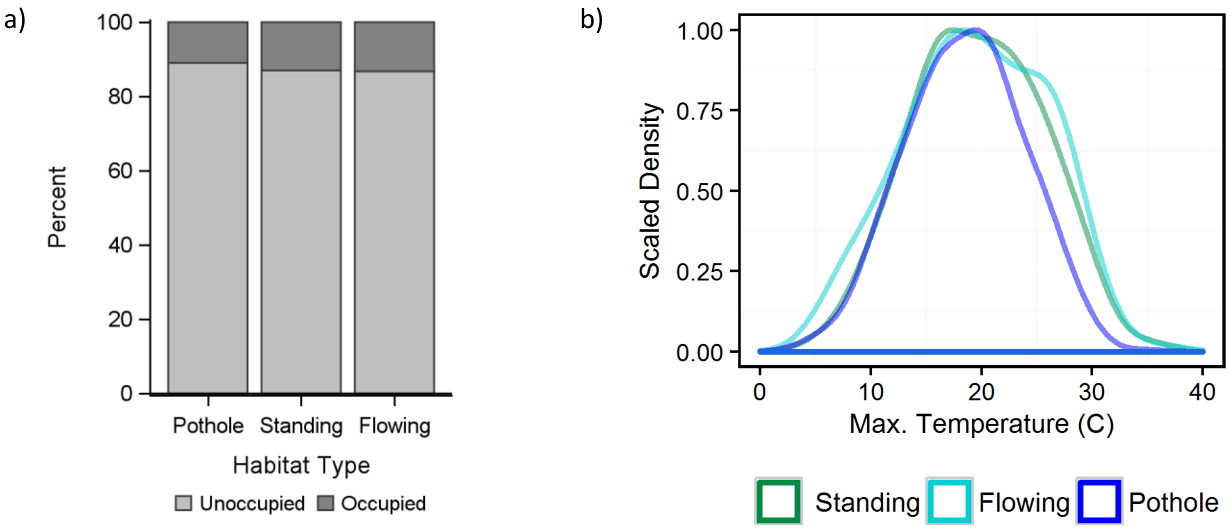
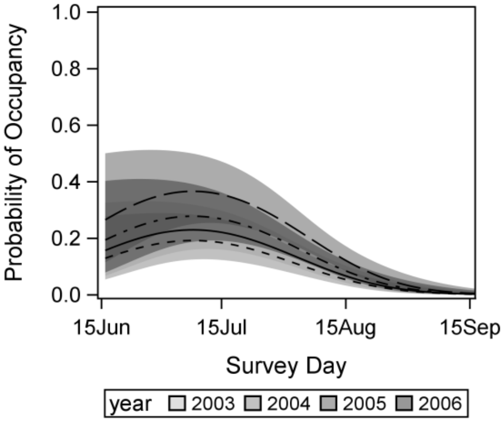
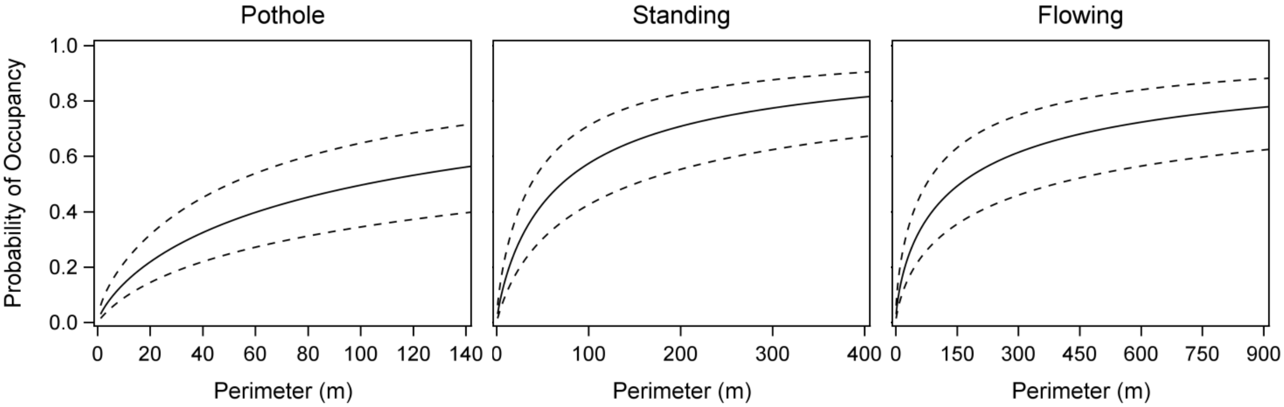


Figure 1.4. Percent of pothole, flooded standing, and flooded flowing habitats used by Yosemite toads at the breeding area scale (a) and density plots comparing maximum water temperatures among the habitat types (b) in wet meadows. Surveys were conducted from 2003-2006 on National Forest lands across the Yosemite toad's range in the Sierra Nevada, CA.

Survey Day From Baseline



Water



Cover

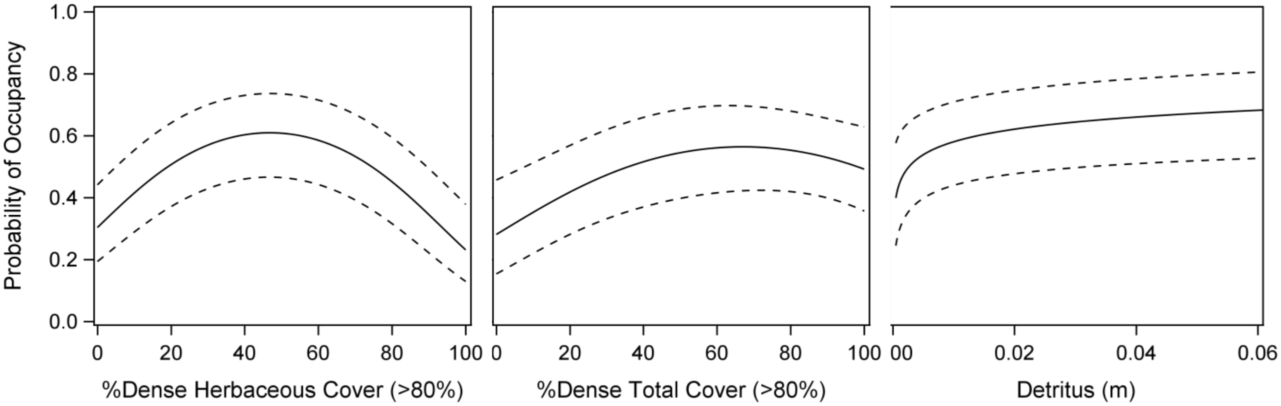
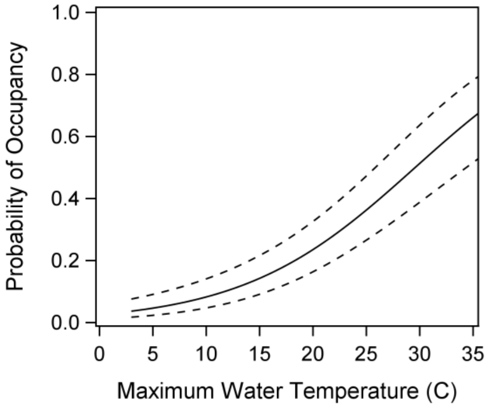
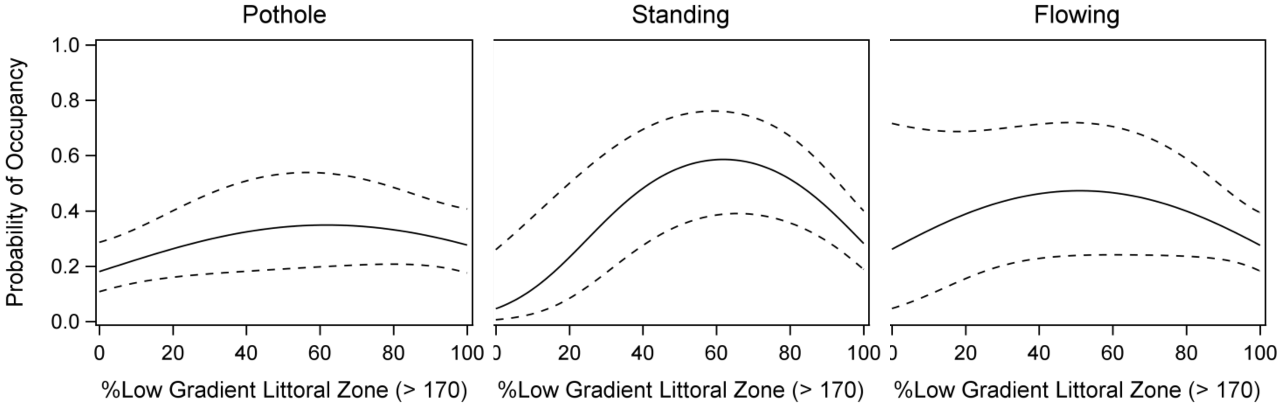


Figure 1.5. Estimated logistic regression relationships between breeding occupancy and habitat variables at the breeding area scale by ecological function group for pothole, flooded standing, and flooded flowing habitat types in wet meadows. Meadows were surveyed from 2003-2006 on National Forest lands across the Yosemite toad’s range in the Sierra Nevada, CA. Differences among years are shown for survey day.

Water Temperature



Shoreline Gradient



Survey Conditions

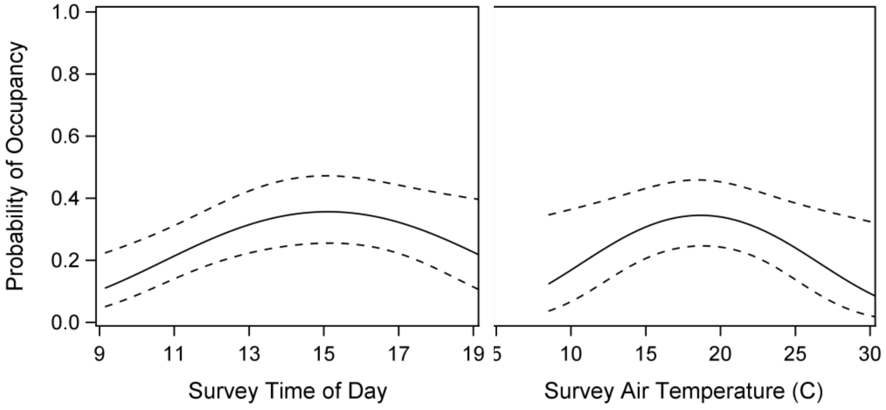


Figure 1.5. Continued.

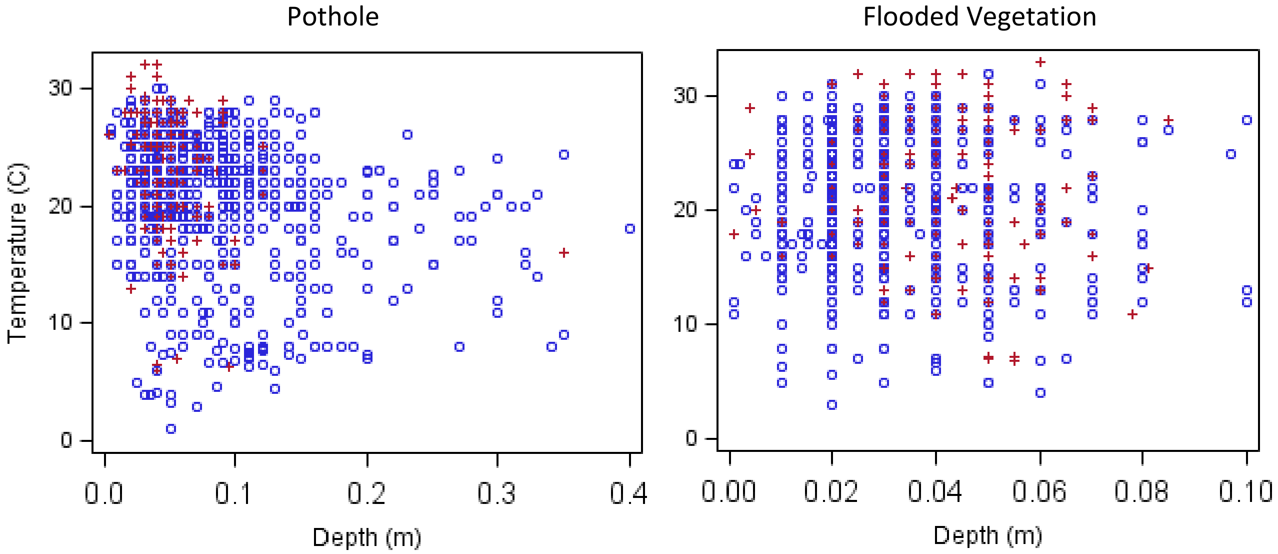
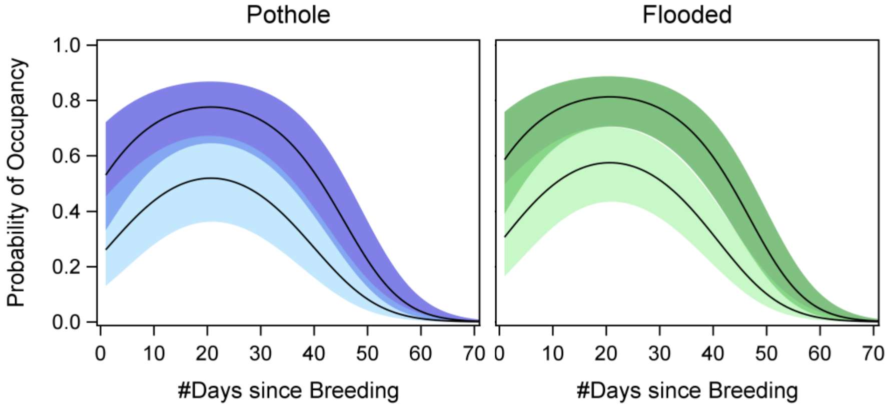


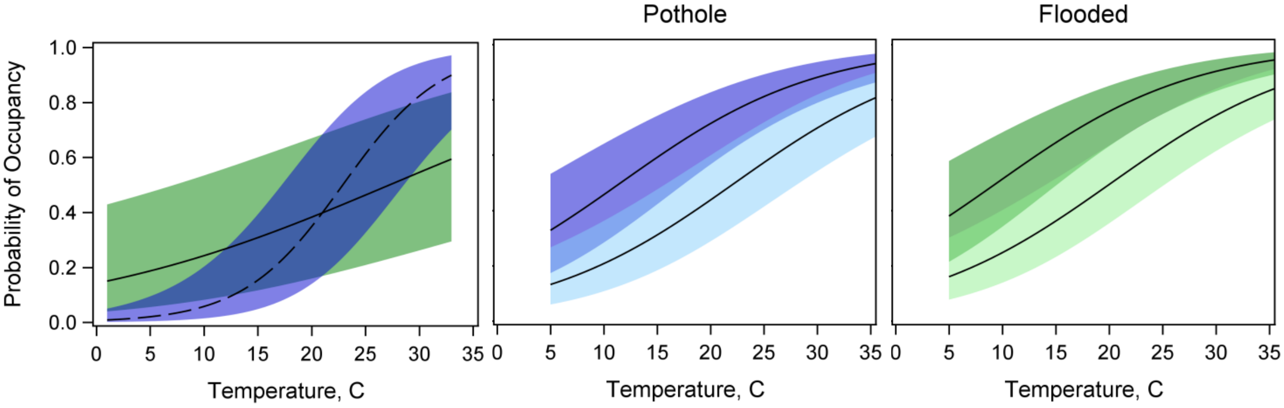
Figure 1.6. Interaction of water depth and temperature for occupied and unoccupied Yosemite toad egg mass plots for pothole and flooded vegetation breeding areas in seven wet meadows surveyed 2006-2016. Red plus signs are occupied and blue circles are unoccupied plots. Depth and temperature axes are scaled to reflect the true available habitat; depths are shallower in flooded vegetation than potholes.

Eggs **Tadpoles**

Time of Survey (tadpoles only)



Temperature



Depth

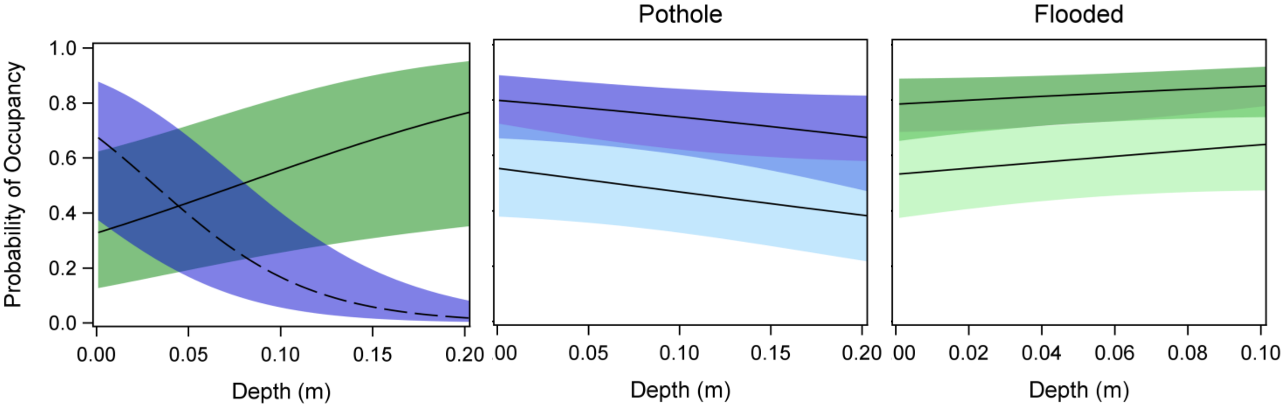


Figure 1.7. Estimated logistic regression relationships between Yosemite toad egg mass and tadpole microhabitat occupancy with habitat variables for pothole and flooded vegetation breeding areas in wet meadows. Microhabitat data was collected in seven meadows 2006–2016 for egg masses and in seven meadows in 2006 and 8 meadows in 2016 for tadpoles in the Sierra Nevada, CA. Blue colors are pothole and green are flooded vegetation habitats. For tadpoles, dark bands are 2006 and light are 2016.

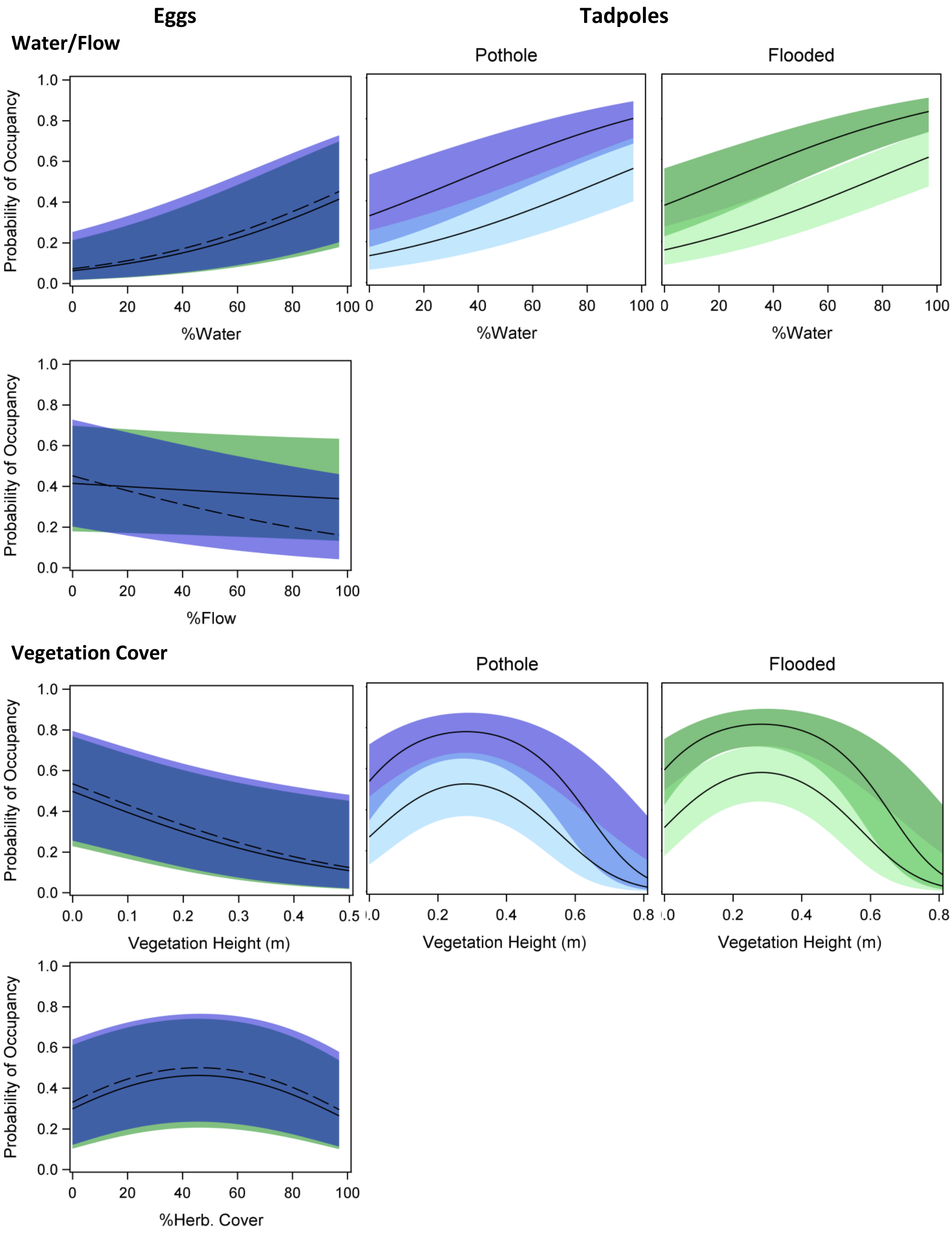


Figure 1.7. Continued.

CHAPTER 2

Untangling Multi-Scale Habitat Relationships of an Endangered Frog in Streams to Inform Reintroduction Programs

***In publication as:**

Brown, C., A. J. Nowakowski, N. C. Keung, S. P. Lawler, and B. D. Todd. 2021. Untangling multi-scale habitat relationships of an endangered frog in streams to inform reintroduction programs. Ecosphere 12(10):e03799. 10.1002/ecs2.3799

ABSTRACT

Successful reintroductions of endangered species rely on comprehensive knowledge of habitat requirements across spatial and temporal scales. To inform designs of reintroduction programs, we studied habitat selection of the federally endangered Sierra Nevada yellow-legged frog (*Rana sierrae*) in streams in its northern range. We quantified multi-scale habitat use across diverse streams, habitat types within streams, and seasonal flows. In one intermittent stream, we compared habitat selection between wild and captive-reared frogs that were released as a population augmentation. We analyzed habitat selection of seasonal habitat unit types (e.g., pool, riffle, cascade) and dimensions, and microhabitat hydrology and cover. *Rana sierrae* appeared able to meet its ecological requirements in a variety of habitats. Study streams ranged from third-order perennial streams to first-order intermittent headwater creeks. Perennial streams retained a variety of habitats across flows whereas intermittent streams dried to just a few pools. Frogs used all seasonal habitat types but selected deeper pools most often relative to available habitats in intermittent streams and selected riffles or showed no preference in perennial streams. Frogs avoided fast deep-water microhabitats, but otherwise, preferred flowing water or deeper water without flow. Our results suggest that diverse streams can be considered candidates for reintroductions. Within intermittent streams, deeper perennial pools may provide more stable release sites, whereas non-pool habitats may be safer in perennial streams with fish. Importantly, captive-reared frogs selected similar habitats as wild frogs, suggesting that captivity does not alter habitat selection behaviors post-release and that reintroduction designs for this species can be based on wild frog preferences. By improving our understanding of how habitat use varies among different types of streams and differs seasonally in response to changing habitat conditions, our results inform the design of effective frog reintroduction programs in streams.

INTRODUCTION

Successful reintroductions of endangered species depend on a thorough knowledge of their habitat use to ensure reintroduction sites meet critical biological needs (Dodd and Seigel 1991, Armstrong and Seddon 2008, Stadtmann and Seddon 2018). Reintroducing species to low quality habitats or habitats where causes of declines still remain can lead to failed reintroductions (Fellers et al. 2007, Bennett et al. 2013, Berger-Tal et al. 2020). Understanding habitat needs can identify habitat-related causes of declines (Jachowski et al. 2011, Bennet et al. 2013), elements necessary for population persistence (Cheyne 2006, Zhang et al. 2017), and effective reintroduction strategies (Rantanen et al. 2010, Bodinof et al. 2012).

Failure of reintroduced animals to recognize suitable habitat is a common reason for unsuccessful reintroductions (Stamps and Swaisgood 2007, Berger-Tal et al. 2020). Released animals often leave seemingly high-quality habitats leading to higher mortality risks both when traveling (Tuberville et al. 2005, Germano and Bishop 2009) and if they ultimately settle in poorer quality habitats (Stamps and Swaisgood 2007, Rantanen et al. 2010, Berger-Tal et al. 2020). This may be particularly problematic for captive-reared animals whose rearing environments differ from those in the wild (Jule et al. 2008, Tetzlaff et al. 2019). Understanding whether habitat selection behaviors are innate or learned from cues based on early experiences can inform reintroduction designs, including whether conditioning tactics like soft-releases, environmental enrichments, or behavioral trainings are warranted (Tuberville et al. 2005, Stamps and Swaisgood 2007, Tetzlaff et al. 2018). It has been suggested that captive-reared amphibians and reptiles tend to retain ecologically appropriate behaviors (Bloxam and Tonge 1995, Germano and Bishop 2009, Escobar et al. 2010). Recent studies, however, suggest behaviors in these taxa may be more complex than previously recognized (Roe et al. 2010, Burghardt 2013, Hammond et al. 2021). Comparing habitat use of captive-reared and wild individuals can provide valuable insights into whether captive-rearing affects post-release habitat selection which can affect survival of reintroduced animals and success of reintroduction designs (Seddon et al. 2007, Roe et al. 2010, Tetzlaff et al. 2018).

Habitat use includes the diversity of macro- and micro-habitats used by a species as well as how use varies over space and time (Roe and Georges 2007, Matthews and Preisler 2010, Stadtmann and Seddon 2018). Determining these relationships 33can be particularly important in streams because of the high degree of temporal and spatial variability that often characterizes these systems (Kupferberg 1996, Dong et al. 2016, Cecala et al. 2018). Temporally, hydrographs and hydroperiods vary across years and seasons depending on climate, weather, and sometimes human activities (Bonada et al. 2006). Spatially, streams are a nested hierarchy of physical habitats, each of which depends on larger-scale processes (Frissell et al. 1986, Cecala et al. 2018). Flow and sediment transport processes create and maintain typical stream habitats like pools, riffles, and cascades (Hawkins et al. 1993, Thomson et al. 2001). As flows change temporally, the characteristics of the habitats also change. For example, a geomorphic cascade may behave as a cascade at high water and a series of pools at low water. Habitats present at a particular time may be viewed as seasonal units. Given all of these sources of variation, understanding habitat selection at multiple temporal and spatial scales is critical for designing successful reintroductions of endangered species to stream habitats.

Reintroductions are being considered to recover the federally endangered (USFWS 2014) Sierra Nevada yellow-legged frog (*Rana sierrae*, MYLF ITT 2018). *Rana sierrae* was once one of the most abundant and widespread amphibians in the Sierra Nevada mountains of California, USA (Grinnell and Storer 1924). It has disappeared from >90% of historical localities

(Vredenburg et al. 2007) and an estimated 50% of watersheds where reproduction was documented before 1990 (Brown et al. 2014). *Rana sierrae* has been well-studied in high elevation lakes (Knapp et al. 2003, Matthews and Preisler 2010), but the applicability of these results to streams is poorly known. *Rana sierrae* is highly aquatic at all life stages, has most commonly been found in lakes, and its multi-year tadpole stage requires permanent water that does not freeze in winter. These characteristics suggest stream-dwelling *R. sierrae* would prefer pools and be restricted to perennial streams with flows or depths sufficient to prevent freezing. The few studies on *R. sierrae* in streams, however, suggest more complex habitat relationships. Larger populations were found in intermittent creeks than in perennial streams in one study (Brown et al. 2020). Further, frogs used varying microhabitats in diverse streams, preferred pools in three of four streams (Brown et al. 2019), and preferred shallow, slow water which could be found in various geomorphic unit types (e.g., pools, riffles, cascades, Yarnell et al. 2019). Here we advance our understanding of *R. sierrae* habitat use in streams by explicitly modeling variation in habitat selection across temporal and spatial scales, and by comparing habitat selection of wild and captive-reared frogs. Although reintroductions of wild and captive-reared *R. sierrae* have occurred (Fellers et al. 2007, Joseph and Knapp 2018, MYLF ITT 2018), there has been no study of the behavior and habitat selection of released animals, and no comparison of habitat use between wild and captive-reared frogs. To design effective reintroduction programs for *R. sierrae* in streams, managers need a more complete understanding of its habitat selection across the temporal and spatial diversity of available habitats, as well as of habitat selection of captive-reared frogs.

We studied habitat selection of wild and captive-reared *R. sierrae* at multiple scales in four diverse streams in the Sierra Nevada, CA. Our objectives were to (1) characterize the variety of streams *R. sierrae* uses to inform selection of potential reintroduction sites; (2) quantify habitat selection at the scales of seasonal units (pool, riffle, etc. present at the time of survey) and microhabitats (specific location of frogs) to identify selection of release locations within streams; and (3) quantify temporal and spatial variation in habitat selection as available habitat changes. We then (4) examined whether captive-reared frogs used the same habitats as wild frogs across the spatial and temporal variation of available habitat. We added additional data (years and streams) to build on prior studies by analyzing habitat use versus availability at the three spatial scales, accounting for the diverse streams, spatial diversity, and temporal variation (high to low flows). Finally, we compared habitat selection of wild versus captive-reared frogs as part of a population augmentation. Results from this study will inform the design of effective reintroduction programs for frogs in streams.

METHODS

Study streams

We studied six occupied reaches of four streams in *R. sierrae*'s northern range that varied by size, hydrology, geomorphology, and frog abundance (Figure 2.1, Yarnell et al. 2019). Perennial Lone Rock Creek (2.7 km) and Independence Creek (0.66 m) are low to moderate gradient, second and third-order reaches (Figure 2.2). Lone Rock flows through a deep valley in open forest into a recreational reservoir, is downcut with large sections of eroded banks, and beaver dams create long, deep pools and runs. Independence flows through a meadow with multiple braided side channels, floodplains, and off-channel habitats that are inundated at snowmelt, generally dry by mid-summer, and reappear in fall from water releases from an upstream reservoir. Native and nonnative fishes were present in Independence and abundant in Lone Rock (Brown et al. 2020).

Intermittent South Fork (SF) Rock Creek and Mossy Pond Creek are small, high gradient headwater creeks with large sections that dry, usually by mid-summer (Figure 2.2). SF Rock includes three study reaches, the headwaters of the main channel (1.0 km length), SF Tributary 1 (1.2 km length), and SF Tributary 2 (0.4 km length). These reaches are connected in the spring, but disconnect as water dries. Fishes occasionally occur in the main channel and lower sections of SF Tributary 1, but the intermittent water limits their presence. Mossy Pond (0.9 km length) is surrounded by granitic bedrock and flows from a shallow lake into a reservoir. A steep barrier prevents fish from entering the creek.

Populations were small on the perennial streams (<25 adults) and larger on the intermittent streams (≤60 adults in the SF Rock reaches, ~100-200 in Mossy Pond, Brown et al. 2020).

Field methods

We collected aquatic habitat use versus available habitat data four times per active season, approximately monthly from June-October, 2016-2018. We quantified physical, hydrologic, and cover components at three scales. We characterized habitat at each study reach, and quantified habitat selection at the seasonal habitat unit (pool, riffle, cascade, etc. at the time of survey) and microhabitat (closest aquatic habitat to frog's location) scales. We only collected data in wadable water, but streams were usually wadable. In spring 2017, high flows impeded early season surveys at the perennial streams.

At the reach scale, we measured water stage using Solinst pressure transducers, programmed to collect data every 15 min, installed near the downstream end of each reach. In spring 2016, we mapped and classified the geomorphic units of each reach as cascade, bedrock chute, high gradient riffle, low gradient riffle, run, pool, step pool, or wood-formed pool. This classification of the individual geomorphic units did not change appreciably during the study.

For available habitat, we established permanent transects on each reach perpendicular to the channel. We chose permanent transects rather than a case-control design (collection of data at random points near frog locations, Manly et al. 2002) to more systematically quantify changes in available habitat across the active season. We used a systematic random design meaning we used a random start point and distributed transects evenly along the reach proportional among geomorphic unit types. We aimed to sample 5–7 points on average per transect each survey, and only collected data at transects with water. Using a systematic random design, we measured habitat at points along each transect spaced 0.25-1 m based on wetted width of the transect at the time of survey. In the intermittent streams many transects dried over the active seasons; thus, we added transects in 2018 to increase the sample size of available habitat. We dropped transects in the perennial streams to balance the workload. At each point along the transects, we recorded water depth and, in water too shallow to measure velocity (depths < 0.06 m), presence/absence of visible flow. In depths ≥0.06 m, we measured water velocity using a Marsh-McBirney flow meter. We used the combination of velocity measurements from deeper water and presence of visible flow in shallow water to create a variable reflecting presence/absence of visible flow for the entire dataset. We estimated the percent herbaceous and total cover in a 1-m^2 plot centered on the point and < 1 m above the stream. Total cover included anything large enough to hide a 2 cm animal (e.g., substrate, vegetation, woody debris). At each point, we visually estimated the percent of overhead canopy cover (0, 0-25, 25-50, 50-75, 75-100) which we treated as an ordinal variable, and recorded substrate type (silt/organics=<0.06mm, sand=0.06-2mm, fine gravel=2-32mm, coarse gravel=32-64mm, cobble=64-256mm, boulder= >256mm, bedrock). We categorized substrate as silt, sand/fine gravel/coarse gravel, cobble/boulder, and bedrock. Finally, at each transect, we recorded the type (using geomorphic unit definitions, Hawkins et al. 1993) and dimensions (maximum width, maximum depth) of the seasonal habitat unit (hereafter, ‘seasonal unit’).

For habitat used, we surveyed approximately two passes of each stream over a ~4-d period each visit. We searched banks and all wadable water for frogs, including channels, mouths of tributaries, side channels, backwaters, and floodplains. We began surveys after 0930 and started in different locations on each pass. For each frog, we recorded life stage and collected habitat data at its location when found in the water or at the closest water it faced when found out of water. We recorded the frog’s location as main channel, side channel, floodplains, or other, whether it was in or out of water, and the distance it was from the water’s edge. We recorded hydrologic metrics (water depth, presence/absence of visible flow, water velocity for depths $\geq$0.06 m), cover metrics (herbaceous, total, canopy, substrate), and the type and dimensions (maximum width, maximum depth) of the seasonal unit using the same methods as available habitat.

Our sample included 11,237 available habitat data points, and 1,011 wild adult, 311 wild subadult, and 178 captive-reared use data points.

Captive rearing and frog release

We collected tadpoles from SF Rock in 2016 that were reared into adults at the San Francisco Zoo and Gardens (Brown et al. 2020). We released 22 frogs on 18 July 2017 divided evenly among two pools on each of the two SF Rock tributaries. We released 60 frogs on 5 July 2018 divided evenly among three pools on the main channel and SF Tributary 1, and two pools on SF Tributary 2. We chose the release pools based on water presence into mid-October and wild frog presence in 2016.

Analysis

Hydrologic stream flow index definition.—Stream habitats were highly dynamic, varying among years and within summers due to stream flow fluctuations. Annual precipitation varied among years with 1 April snowpacks 94% of normal in 2016, 148% of normal in 2017, and 46% of normal in 2018 (http://cdec.water.ca.gov /snowapp/sweq.action). Flows decreased over the summer and large sections of the intermittent streams dried. To account for the different annual and seasonal flow conditions, we created a hydrologic index that reflected high, medium, or low flows. We assigned each survey to one of the three categories, and for this particular metric, used different methods for perennial and intermittent streams. For perennial streams, we used stream stage based on hydrographs from the loggers, where high was flood stage, low was stage at base flow, and medium was intermediate between the two. Stage was not a good indicator for the intermittent streams; loggers were located in pools that were, to some extent, buffered from the decreasing flows over the summer. Thus, for the intermittent streams, we defined categories reflecting water retention using the percent of transects that had water (high flows= >60%, medium flows= 30-60%, low flows= <30%). We examined habitat use across these different hydrologic index categories.

Reach scale characterization.—We plotted stage height over time for each stream and year (2017, 2018) where available. For each reach, we summed the lengths of geomorphic unit types. For further analysis of the seasonal units, we collapsed the types into three categories, cascades (cascades, chutes), riffles (high and low gradient), pools (all other categories). We included runs with pools because in the streams where these were prevalent, they were more similar to pools than riffles. We described graphically the dimensions of each reach by seasonal unit type and hydrologic index using maximum widths and depths of seasonal units, and the maximum velocity (maximum of transect points) at each transect. We calculated the percent of each substrate type by seasonal unit type for each reach. To quantify the desiccation of the intermittent reaches, we calculated the percent of transects with water for each survey each year. Finally, we compared the percent of seasonal unit type by geomorphic unit type across hydrologic index categories for each reach. Based on these reach scale characteristics, we collapsed the three SF Rock reaches for the habitat selection analyses.

Habitat selection analyses.—We first used generalized linear models to examine whether frogs preferred specific geomorphic and seasonal habitat types across the different hydrologic index categories while accounting for the diverse streams. We modeled the probability of selected versus available habitat as the response and habitat type (geomorphic or seasonal unit), hydrologic index (high, medium, or low), and stream as the predictors (SAS 9.4/Glimmix, SAS Institute Inc., Cary, NC). Sample sizes were too small to model the three-way interaction among habitat type, hydrologic index, and streams so we only included the three two-way interactions. Model results were similar between the geomorphic and seasonal habitats so we used seasonal unit types for further analyses; this is the habitat experienced by the frog.

We then compared selected versus available habitat variables at the seasonal unit and microhabitat scales using a Bayesian implementation of a generalized linear mixed model with binomial probability distribution. Specifically, we used a hierarchical random slope model to examine selection of seasonal unit dimensions (maximum wetted width, maximum depth), hydrologic factors (water depth, presence of visible flow, velocity), and cover (total, herbaceous, canopy, and substrate type). We included random slope terms for each of these predictor variables to allow selection coefficients to vary across the different seasonal units, hydrologic index categories, and streams. As an exploratory analysis, we used descriptive statistics and generalized additive models (GAM) to examine correlation among variables and to select the best functional parametric form for each predictor variable. Because the maximum Pearsons correlation coefficient was <0.7 for all variables, no variables were excluded, and GAMs indicated that no polynomial terms were warranted. Finally, we standardized and rescaled all continuous predictor variables to have a mean of zero and a standard deviation of one.

As our study is of an endangered amphibian, our sample was small and unbalanced, with more available sample points relative to frog use points in the perennial streams and fewer available sample points relative to frog use points in the intermittent streams. This limited our ability to model the full suite of interactions among the seasonal unit types and hydrologic index categories that might affect microhabitat selection. Exploratory analyses indicated that, because our streams are diverse, the averaging effect of combining categories (e.g., streams, seasonal units, hydrologic index) obscured important variation. To solve this, we created a random factor that had 36 potential values combining the three seasonal units, three hydrologic index categories, and four streams. We then used this random factor in a hierarchical model with a random slope that allowed the relationships between the probability of use and the habitat predictors to vary among the 36 categories. This in turn allowed us to explore how habitat selection varied among seasonal units across the hydrologic index categories while accounting for the diverse streams. Further, this approach not only reduced the number of parameters estimated but allowed us to ‘borrow’ from categories with more data to make inferences for categories with sparse data. In our interpretation of results, we are transparent about sample sizes, focus on broad patterns and defensible differences, and emphasize categories with larger sample sizes. The general model was structured as follows:

$$Y[i] \sim Binomial(p_i, N_i)$$

$$logit(p_i) = \alpha 0 + \alpha 1[year[i]] + \alpha 2[compositeRE[i]] + \beta[compositeRE[i]]) * X[i]$$

where Y indicates whether the ith observation is from used versus available habitat measurements. We modeled a mean population intercept, $\alpha 0$, a varying intercept of year, $\alpha 1$, and varying intercepts and slopes, $\alpha 2$ and β respectively, for the composite random factor (accounting for seasonal units, hydrologic index, and stream). X is a model matrix wherein columns are additive habitat variables; maximum width and maximum depth are seasonal unit scale variables; the remainder reflect microhabitats:

- maximum depth of seasonal unit + maximum width of seasonal unit + water depth at microhabitat + presence of flow (yes/no) + %total cover + %herbaceous cover + %canopy cover + substrate

Models were fit using diffuse, normally distributed priors for varying intercepts and slopes:

$$\alpha 1 \sim Norm(0, \sigma^2_{\alpha 1})$$

$$\alpha 2 \sim Norm(0, \sigma^2_{\alpha 1})$$

$$\beta \sim Norm(\mu_\beta, \sigma^2_\beta)$$

Models were fit with 20,000 total iterations and 1,000 iterations discarded as burnin. We assessed convergence by examining Gelman-Rubin statistics and traceplots. Gelman-Rubin statistics were ~1 for all models. We assessed model fit using Bayesian R^2 and posterior predictive checks based on sums of squares (Kery and Royle 2016, Gelman et al. 2019). Bayesian R^2 ranged from 0.37–0.63 and Bayesian p-values for goodness of fit ranged from 0.10–0.59, both indicating adequate model fit. Models were run with JAGS using the jagsUI package to interface with R (R Core Team 2018).

Because we did not use a case-control design where use and available points were matched, we developed separate models to compare subgroups rather than, for example, comparing wild versus captive-reared frogs within a single model. Thus, we developed separate models for wild adults and subadults on main channels at all streams. We developed models (separately for adults and subadults) for side channels at Independence since these were only present at this stream and their habitats were quite different than the main channel. Finally, we developed a model for captive-reared frogs at SF Rock. Because our primary interest was in assessing the relative importance of the different habitat variables for each subgroup, we compared selection coefficients within a single model, as is the common approach with Bayesian hierarchical models (Kery and Royle 2016) rather than a comparison of models representing a priori hypotheses (e.g., Burnham and Anderson 2002). We present posterior population mean estimates for coefficients of predictor variables. However, because our primary study objectives were to explore spatial and temporal variability in habitat use, we also focus on variability among the random factor categories. In our presentation of model results, we further this objective by synthesizing output from the subgroup models organized by the function of the predictor variables (seasonal unit dimensions, hydrologic, cover) rather than by models (i.e., all variables from a single model).

Up to a third of our non-edge data points (available=30%, use=24%) were in depths shallower than the Marsh-McBirney flow meter could measure. Our exploratory analysis suggested there was an interaction between water depth and both visible flow presence and velocity. Thus, our primary models were additive and included visible flow presence as a predictor. We then developed models further exploring the interaction of water depth with visible flow presence and the absolute value of velocity. Models with velocity only included data where depths were ≥0.06 m.

RESULTS

Reach scale characterization

Hydrographs of our study reaches were typical of Sierra Nevada streams with most precipitation from winter snowpacks (Appendix 1: Figure S1). Flows were high during spring and declined through summer. High snowpacks in 2017 led to later spring run-off and higher flows later than the other years. These high flows flooded much of the off-channel habitat at Independence well into the summer.

Geomorphology varied among the study streams (Appendix 1: Figure S2). The perennial Lone Rock had mostly pools and runs with a few steep cascades and low gradient riffles, whereas Independence was mostly riffles and pools. The intermittent Mossy Pond was dominated by cascades and chutes, whereas cascades, pools, and riffles occurred in the South Fork reaches.

The streams were relatively small, with wetted widths averaging 2.4–6.3 m (Appendix 1: Table S1, Figure S3). In general, the intermittent streams were slightly narrower, shallower, and had slower flow than the perennial streams. SF Tributary 1 had a few deep pools, and its average maximum depth was almost twice that of the other intermittent reaches; its median, however, was similar. Substrate varied by reach more than by habitat type (Appendix 1: Figure S4). Lone Rock was the most diverse reach; its cascades had more boulders, riffles more gravel, and pools more sand. Independence was dominated by cobble and boulders, Mossy Pond by bedrock and boulders, and the SF Rock reaches by boulders and bedrock.

As flows decreased, wetted widths, depths, and velocities decreased in all streams (Appendix 1: Figure S3). Intermittent streams dried to a low of 3%–30% of transects (Appendix 1: Figure S5). As flows decreased, the habitat types available to frogs remained relatively consistent in the perennial streams, but in the intermittent streams, both the amount and type of habitat changed (Appendix 1: Figure S6). Riffles and cascades tended to dry out or change to pools. Thus, only a few pools comprised the available habitat in the intermittent reaches for much of the active season.

Habitat selection

Sixty percent of frog sightings were in water, and the remaining were on shore or on emergent objects (e.g., boulders, logs). All but three frog sightings were within 1 m of water, with a maximum of 3 m. Only Independence and Lone Rock had available aquatic habitats off the main channels. At Independence in the high-water year 2017, all frog sightings except one were in off-channel pools, floodplains, or side channels (Appendix 2: Figure S1a). In 2016 and 2018, all frog sightings were in the main channel and in side channels with tadpoles; the other side channels and off-channel habitat were dry for most of the summer. In the drier years, 59% of adults were found in the main channel whereas 74% of subadults were found in the breeding side channels. At Lone Rock, a few frogs (11 subadults and small adults) were found in the few off-channel pools that formed on floodplains early in the season. In the intermittent streams, frogs tended to cluster around remaining pools as the streams dried (Appendix 2: Figure S1b). Other than these patterns, frogs did not appear to select particular areas of the streams.

Wild adults.—1. Seasonal units.—Wild adults were found in all habitat types on all streams, but they preferentially selected some specific habitats. At Lone Rock at low water, for example, similar numbers of adults were found in pools as riffles, but pools comprised 86% of the available habitat; at SF Rock at high water, similar numbers of adults were found in cascades as pools, but cascades comprised 59% of the habitat. Accounting for available habitat, wild adults did not prefer pools in the perennial streams but did prefer pools in the intermittent streams (Figure 2.3). On Lone Rock during low flows, the estimated probability of use was highest in riffles, then cascades, and smallest in pools (Figure 2.3). Conversely, there was no difference in the estimated probability of use among habitat types in the main channel at Independence. In the side channels of Independence, the estimated probability of use by wild adults was slightly higher in riffles. In the intermittent streams, the estimated probability of use was highest in pools.

Overall, the estimated probability of use for wild adults was negatively associated with maximum wetted width of seasonal units (Table 2.1), though this association was most significant in pools and cascades on intermittent streams (Figure 2.4, Appendix 2: S2). Although the estimated population mean coefficient for maximum water depth was not significant (Table 2.1), this resulted from opposing trends in intermittent and perennial streams. The estimated probability of use for wild adults was associated with shallower pools in the perennial streams and deeper pools in the intermittent streams (Figure 2.4, Appendix 2: S2). There were no major associations between estimated probability of use and seasonal unit dimensions for wild adults in Independence side channels (Table 2.1, Figure 2.4).

2. Microhabitat—Wild adults appeared to avoid fast, deep water, particularly in the perennial streams, but otherwise, they preferred the presence of visible flow and, in the intermittent streams, deeper water. Using an additive model with the full dataset, the estimated probability of use was positively associated with the presence of visible flow in most streams and habitats (Table 2.1, Figure 2.5). The estimated population mean coefficient for maximum water depth was not significant (Table 2.1), but there was variability between intermittent and perennial streams. The estimated probability of use of wild adults was either negatively or not associated with water depth in the perennial streams, and positively associated with water depth in the intermittent streams. However, the interaction models with water depth x presence of visible flow (full dataset) and water depth x velocity (depths ≥0.06 m) showed more complex relationships. Using the water depth x presence of visible flow model, the estimated probability of use for wild adults was positively associated with water depth where visible flow was not present (Figure 2.6a, Appendix S2: Figures S3, S4). In the presence of visible flow, the estimated probability of use was negatively associated with water depth in perennial streams (Figure 2.6b, Appendix S2: Figure S3) and varied by habitat type and reach in intermittent streams (Figure 2.6b, Appendix S2: Figure S4). Similarly, using the depth x velocity model, the estimated probability of use was negatively associated with water velocity in the perennial streams, and varied by habitat type and stream in intermittent streams (Figure 2.7, Appendix S2: Figure S5).

Wild adults had weak associations with the cover variables. The estimated population mean coefficient was positive for total cover, negative for herbaceous cover, positive but non-significant for canopy cover, and non-significant for substrates (Table 2.1, Figure 2.8, Appendix S2: Figure S6).

Captive-reared adults.—1. Seasonal Units.—Most SF Rock captive-reared adults were found in pools and the estimated probability of use was highest in pools (Figure 2.3). We released frogs into pools, and they tended to stay. Similar to wild adults, the estimated probability of use of captive-reared adults was negatively associated with maximum wetted width and positively associated with maximum water depth of seasonal units (Table 2.1, Figure 2.4, Appendix 2: Figure S2).

2. Microhabitat.—Captive-reared adults showed the same general flow and water depth preferences as wild adults in SF Rock (Table 2.1, Figure 2.5, Appendix S2: Figures S4, S5). The estimated population mean coefficients were not significant for the cover variables (Table 2.1). However, when comparing the variability among seasonal units and the hydrologic index, the relationships generally were similar to wild SF Rock adults (Figure 2.8, Appendix S2: S-6).

Wild subadults.—1. Seasonal units.—Overall, wild subadults selected similar seasonal unit types as wild adults, though the preference for riffles in Lone Rock was more evident (Appendix S3: Figure S1). Subadults tended to be found in more narrow and shallower seasonal units in the perennial streams, except that they preferred deeper riffles at low water at Lone Rock (Table 2.1, Appendix S3: Figures S2, S3). In Mossy Pond, subadults shifted from using narrow and deeper pools at high and medium flows to wider and shallower pools at low water. The same general pattern occurred at SF Rock but to a lesser degree.

2. Microhabitat—Wild subadults showed similar flow and water depth preferences as wild adults (Table 2.1, Appendix S3: Figures S2, S4, S5, S6), with the exception that in Independence side channels, they tended to be in deeper water and no visible flow presence (Appendix S3: Figure S2). There were few strong associations with subadult use and cover, though the estimated population mean coefficient for canopy cover was significantly negative (Table 2.1, Appendix S3: Figure 2.7).

DISCUSSION

Understanding the breadth of habitat selection at multiple spatial and temporal scales for both wild and captive-reared animals can lead to more successful reintroduction strategies (Cochran-Biederman et al. 2015, Zhang et al. 2017, Stadtmann and Seddon 2018). This may be particularly true for stream-dwellers whose available habitats change across space and time (Yarnell 2013, Dong et al. 2016, Cecala et al. 2018). Our study suggests that *R. sierrae* is an aquatic generalist (Cabral Eterovick 2003, Sredl and Jennings 2005) at multiple spatial scales. Frogs appeared able to meet their ecological requirements in a variety of habitats, with some site-specific constraints affecting habitat selection. Our results suggest that understanding how annual and seasonal flow patterns affect available habitats is key to selecting reintroduction streams. Finally, captive-rearing did not appear to alter habitat selection, supporting the use of wild frog habitat preferences to develop reintroduction designs and suggesting simple releases may be sufficient.

Rana sierrae's use of diverse stream habitats is most apparent at our largest scale—the stream. *R. sierrae* was found in streams that ranged from first to third-order, high to low gradient, occurred in open forest or meadows, included both perennial and intermittent waters with fast-flowing and still-water, and differed geomorphologically. Intermittent streams in the study were similar to those described by early studies of *R. sierrae* (Zweifel 1955, Mullally and Cunningham 1956) and to those used by the closely related *R. muscosa* in southern California (Mullally 1959, Santana 2012).

The use of small intermittent streams by such a highly aquatic frog like *R. sierrae* may seem counterintuitive. Nonetheless, others have reported higher abundances in intermittent creeks than in perennial streams (Mullally and Cunningham 1956, Brown et al. 2020), emphasizing the importance of these headwater habitats (Olson and Weaver 2007, Richardson and Danehy 2007). Across its broader range, *R. sierrae* commonly uses lentic waters (Knapp et al. 2003), habitats akin to pools of intermittent streams (Cabral Eterovick 2003). Frogs appeared to survive in intermittent streams by moving to the few persistent pools as water dried, including moving long distances (Mullally and Cunningham 1956, Mullally 1959, Keung et al. 2021). Populations in these streams may be vulnerable during droughts, and it is unknown whether *R. sierrae* can withstand total desiccation of their habitats like some Ranid frogs in arid environments (Hinderer et al. 2021). The absence of fish, a known cause of declines for *R. sierrae* (Knapp and Matthews 2000), may contribute to the higher abundances in the intermittent streams. Nonnative trout now occur in almost all perennial streams in the species' range. The current populations in intermittent streams may be remnants of formerly larger populations in downstream perennial reaches before the introduction of fishes (Brown et al. 2020). Regardless of the species' historical use of intermittent streams, given current conditions, these creeks may provide refuge from fishes and merit appropriate management.

At the seasonal unit scale, we predicted that wild frogs would prefer deep pools based on assumptions from *R. sierrae* in lakes. Frogs did prefer deeper pools in the intermittent streams which, by retaining more water during dry periods, were the primary habitat available most of the summer. This was similar to *R. muscosa* in southern California streams (Santana 2012). Surprisingly, *R. sierrae* also used cascades and riffles in the intermittent streams when available. In the perennial streams, frogs did not prefer pools relative to availability. Pools may have been the least safe habitats in these streams because of the presence of fishes. Lone Rock, in particular, had large numbers of both native and nonnative fishes and the sandy substrate of the long deep pool-runs provided little cover. Fish presence also may explain the frogs' preference for shallower seasonal units in the perennial streams (Hunter and Smith 2013). The fast flow of riffles in the perennial streams or deep water of pools in the intermittent streams may facilitate quick escape from predators (Gillespie and Hollis 1996). We observed frogs escaping into fast-flowing water as well as taking cover at the bottom of deep pools. Also, higher periphyton growth in riffles and cascades can lead to more emerging macroinvertebrate prey (McKie et al. 2018), possibly increasing use of these habitats. This behavioral flexibility may allow *R. sierrae* to adjust at this scale to site-specific opportunities and constraints like prey availability, fish predation, and seasonal desiccation of habitats (Cabral Eterovick 2003).

At the microhabitat scale, we predicted that frogs would prefer deep water with low or no flow, again based on assumptions from *R. sierrae* in lakes. Frogs generally did avoid high velocity deep water, which may be difficult to navigate. This may explain the shift to off-channel habitats at Independence and use of floodplain pools by subadults at Lone Rock during high flows. This generally agrees with Yarnell et al. (2019) who found that frogs preferred shallow and low velocity water, which they find in a variety of geomorphic unit types. Our analyses add nuance to this result, however, by using a random slope model that explicitly accounted for habitats available at the time of survey (seasonal units), flows (hydrologic index), and streams, and by explicitly modeling the interaction between depths and flow (presence and water velocity). Overall, our model found frogs more often in water with visible flow and deeper water where flow was not visible. Supporting these results, we commonly found frogs basking next to cascades or riffles, in pools at the bottom of cascades, on rocks in shallow water trickles or sheetflow, and occasionally in the spray zone behind small waterfalls. These areas may provide moist foraging habitat or help with thermoregulation (Gillespie and Hollis 1996). In addition, frogs used flowing water to escape, and we observed no signs that frogs avoided or were not able to navigate flowing water.

Rana sierrae showed some preference for areas with higher total cover, but not specifically for cover associated with herbaceous vegetation or specific substrates like silt, cobble, and boulders. This differs from many amphibian species like giant salamanders (Bodinof et al. 2012, Zhang et al. 2017), tailed frogs (*Ascaphus truei*, Adams and Bury 2002), and *Leiopelma hochstetteri* (Nájera-Hillman et al. 2009) which are all strongly associated with cobble and boulders that provide protective cover. Stream-dwelling *Rana luteiventris* (Arkle and Pilliod 2015) and *Lithobates chiricahuensis* (Hinderer et al. 2021), were associated with emergent vegetation. In contrast with these species that have clear affinities with specific cover types, *R. sierrae* showed behavioral flexibility in their use of cover.

Finally, whether captive-reared animals develop behaviors that are deleterious in the wild can affect the success of a reintroduction program (Seddon et al. 2007, Germano and Bishop 2009, Santos et al. 2009). Captive-reared frogs released into an intermittent stream exhibited the same behavioral flexibility as wild frogs from the same stream. Not only did they select similar habitats as wild frogs as shown here, they also have been shown to exhibit similar movement distances (Keung et al. 2021). Behavioral flexibility in both habitat selection and movements may help reintroduced animals stay in release habitats as well as acclimate to changing habitats (Cabral Eterovick 2003, Santos et al. 2009, Keung et al. 2021). Our results add to a small but growing number of studies that indicate captive-reared amphibians tend to retain natural behaviors (Germano and Bishop 2009, Berger-Tal et al. 2020, Roznik and Reichling 2021).

The results here suggest that soft-releases or behavioral acclimation may not be necessary for reintroductions of *R. sierrae* or similar species in streams. However, our still-water release pools likely were similar hydrologically to the aquarium environments where the captive frogs were reared (Burghardt 2013). Whether frogs released into fast-water riffles or cascades would show similar behaviors or benefit from conditioning remains unknown. We also purposefully released frogs in small groups into pools that had been occupied by wild frogs, assuming this reflected high quality habitat. Whether this may have influenced the behavior of the captive-reared frogs merits further study. In other taxa, releasing animals with conspecifics may help them stay in release habitats (Lloyd et al. 2019). Anecdotally, we found that frogs left one release pool earlier than frogs released into other pools. Understanding whether such behaviors were related to habitat quality, competition with resident wild frogs, or behaviors such as exploration and kin aggregation would inform effective reintroduction designs (Blaustein et al. 1984, Bell et al. 2004, Hammond et al. 2021). Ultimately, factors that contribute to reintroduction success are complex, and long-term monitoring may help elucidate whether our results are consistent long-term and their applicability to other systems (Roe et al. 2010, Roe et al. 2015).

Candidate Streams for Reintroductions

Rana sierrae appears able to meet its ecological requirements in a variety of habitats. Thus, while we have extensively quantified habitats at multiple scales (also Brown et al. 2019), we suggest these relationships be used as guidance and not prescriptive, and caution against using simple habitat suitability cutoffs to make specific management and conservation decisions (Cecala et al. 2018). While our results provide such guidance for identifying candidate reintroduction streams, we do not show data for breeding or overwintering habitats, two components critical for population persistence that warrant further study (Brown et al. 2019). Importantly, our study suggests that release designs can be based on wild frog habitat preferences.

Ideal candidate reintroduction streams would have suitable habitat present across appropriate spatial and temporal scales (Stadtmann and Seddon 2018). For both perennial and intermittent streams, understanding how available habitat changes both annually and seasonally is critical, including across both wet and dry years.

Our results suggest candidate streams may include a broad array of first to third-order stream types (Mullally and Cunningham 1956, Brown et al. 2019). At a minimum, streams need permanent water, though this may be continuously flowing in perennial streams or patches on intermittent streams. Ideal candidate streams would be fish-free, although small populations of frogs coexist with fish on several study streams. Although reintroducing frogs to intermittent streams may seem counterintuitive for this highly aquatic species, they may provide important fish-free refuge.

Although reaches with numerous deep pools that retain water through summer seem an intuitive choice, this presumed lentic species also used non-pool habitats when available. The presence of fish also may influence habitat selection at the within-stream scale. Thus, streams with a mix of seasonal unit types (pools, riffles, cascades) that include permanent water refuges may be optimal (Yarnell 2013, McCoy et al. 2014). *R. sierrae* displacement distances (likely while streams had water) can provide guidance on spatial configurations of permanent water patches that the species may be able to tolerate. On intermittent streams, average maximum displacement distances of wild frogs ranged from 36–84 m with a maximum of 670 m (Keung et al. 2021).

Refuge from high spring runoff flows may be important, particularly in perennial streams. In streams from the current study, this was provided by off-channel habitats in one stream and presumably on-channel habitats at the others.

At microhabitat scales, sufficient cover and basking perches are important, and for *R. sierrae*, these can take various forms. We observed frogs taking cover in silt, cobbles, boulders, undercut banks, woody debris, and fast-flowing water. Examples of basking perches included substrate of all types, wood, and shorelines.

Ideally, release sites, an important component of reintroduction designs, would provide safe, stable habitats where reintroduced frogs would remain until acclimated (Slater and Altman 2011, Bodinof et al. 2012). For *R. sierrae*, these may be shallower flowing water in perennial streams with fish, and deep pools in the absence of fish and where water is limited.

In conclusion, our results provide insights for designing effective reintroduction programs for an endangered species in stream environments, taking into account spatial and temporal changes in available habitat as well as the full diversity of a species' habitat use (Dodd and Siegel 1991, McCoy et al. 2014, Cecala et al. 2018). Reintroduction designs that take into account physical and biological constraints when selecting streams and release sites may be most effective. For stream-dwelling *R. sierrae*, physical constraints include changes in available habitats from early season high flows to late season desiccation. Biological constraints include the distribution of fish predators in different seasonal units, an area that needs further research. Finally, our results suggest that captive-reared *R sierrae* will likely behave similarly to wild frogs. Our results demonstrate the importance of basing reintroduction designs on the full spectrum of habitats used by a species, moving beyond general knowledge from a single or most commonly used habitat at a single point in time.

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Table 2.1. Population mean coefficients from random slope generalized linear models for wild and captive-reared *R. sierrae* adults and wild subadults in four study streams. Bold italicized coefficients indicate 95% credible intervals do not overlap 0.

	Main Channel				Side Channels	
	Wild		Captive-reared		Independence	
Variable	Mean	95% CI	Mean	95% CI	Mean	95% CI
Adults						
Intercept (no visible flow, silt)	-4.66	-7.39 – -1.98	-9.71	-18.25 – -1.66	-5.49	-11.29 – 0.04
Seasonal unit dimensions						
Maximum wetted width	***-0.69***	***-1.08 – -0.31***	***-1.15***	***-1.73 – -0.52***	-0.14	-0.86 – 0.62
Maximum water depth	0.14	-0.34 – 0.59	0.91	-0.06 – 1.80	0.33	-0.58 – 1.29
Hydrologic characteristics						
Water depth	0.42	-0.01 – 0.84	***0.91***	***0.27 – 1.66***	0.00	-0.89 – 0.77
Visible flow presence	***1.28***	***0.57 – 2.00***	***1.35***	***0.10 – 3.48***	***2.05***	***0.63 – 3.69***
Cover						
Total	***0.38***	***0.18 – 0.57***	0.44	-1.59 – 2.14	0.49	-0.19 – 1.10
Herbaceous	***-0.27***	***-0.56 – -0.00***	-0.49	-1.63 – 0.13	***-0.93***	***-1.85 – -0.18***
Canopy	0.16	-0.08 – 0.38	0.38	-0.18 – 0.84	0.11	-0.35 – 0.54
Sand/gravel substrate	0.05	-0.94 – 0.88	1.70	-0.57 – 3.94	***-1.58***	***-3.62 – -0.15***
Cobble/boulder substrate	0.12	-0.57 – 0.80	0.24	-1.21 – 1.50	-0.40	-1.95 – 1.03
Bedrock substrate	0.35	-0.66 – 1.48	0.46	-1.66 – 2.09	24.04	-0.34 – 65.46
Subadults						
Intercept (no visible flow, silt)	-6.98	-10.74 – -3.47			-9.25	-17.72 – -2.34
Seasonal unit dimensions						
Maximum wetted width	***-1.12***	***-1.95 – -0.39***			-0.58	-3.08 – 1.67
Maximum water depth	-0.02	-0.69 – 0.57			0.66	-1.87 – 3.77
Hydrologic characteristics						
Water depth	0.54	-0.15 – 1.23			1.15	-0.68 – 2.47
Visible flow presence	0.80	-0.61 – 2.18			-0.41	-3.68 – 3.21
Cover						
Total	0.24	-0.22 – 0.64			0.18	-2.03 – 2.57
Herbaceous	-0.22	-0.78 – 0.21			0.12	-2.03 – 2.38
Canopy	***-0.34***	***-0.61 – -0.08***			-0.31	-2.00 – 1.21
Sand/gravel substrate	-1.30	-3.59 – 0.39			-2.03	-9.56 – 4.07
Cobble/boulder substrate	-0.55	-1.99 – 0.85			-2.70	-9.64 – 0.35
Bedrock substrate	0.00	-1.98 – 2.10			-2.68	-66.43 – 59.31

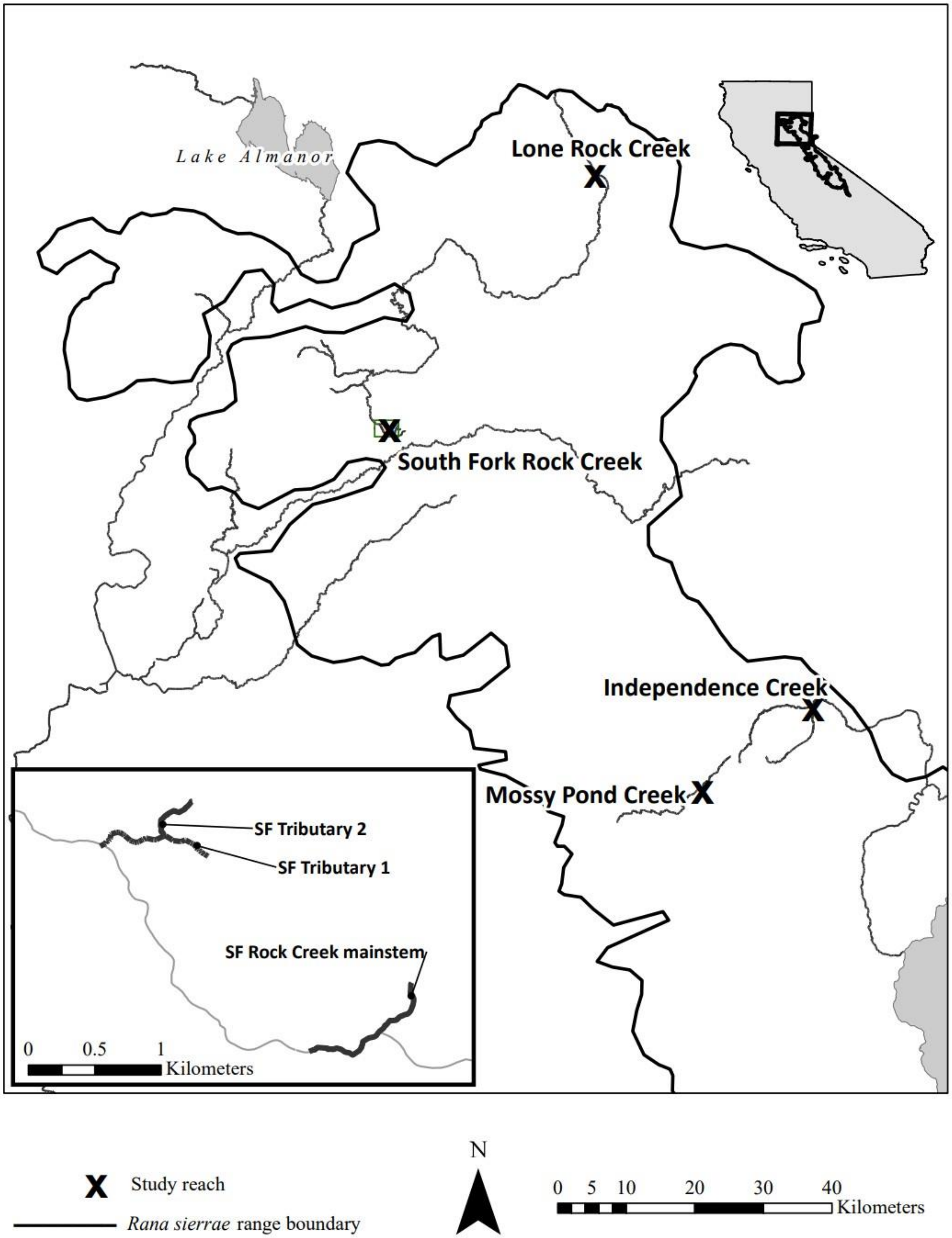

Figure 2.1. Locations of *Rana sierrae* study streams in the northern Sierra Nevada, CA.

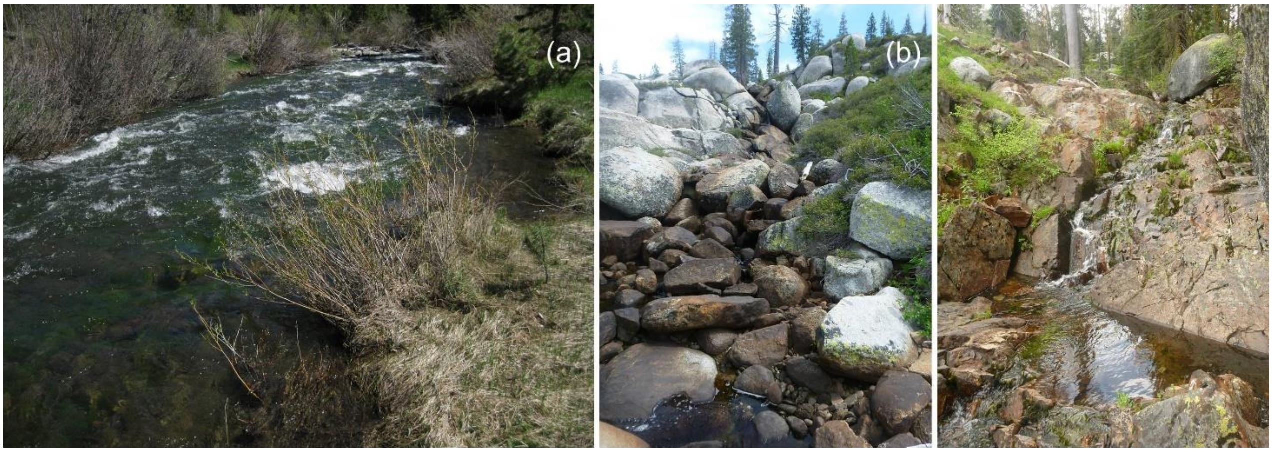

Figure 2.2. *Rana sierrae* study streams were diverse first to third-order streams in their northern range. Perennial streams (a) retained a diversity of habitats while intermittent streams (b) dried to a few pools over the summer.

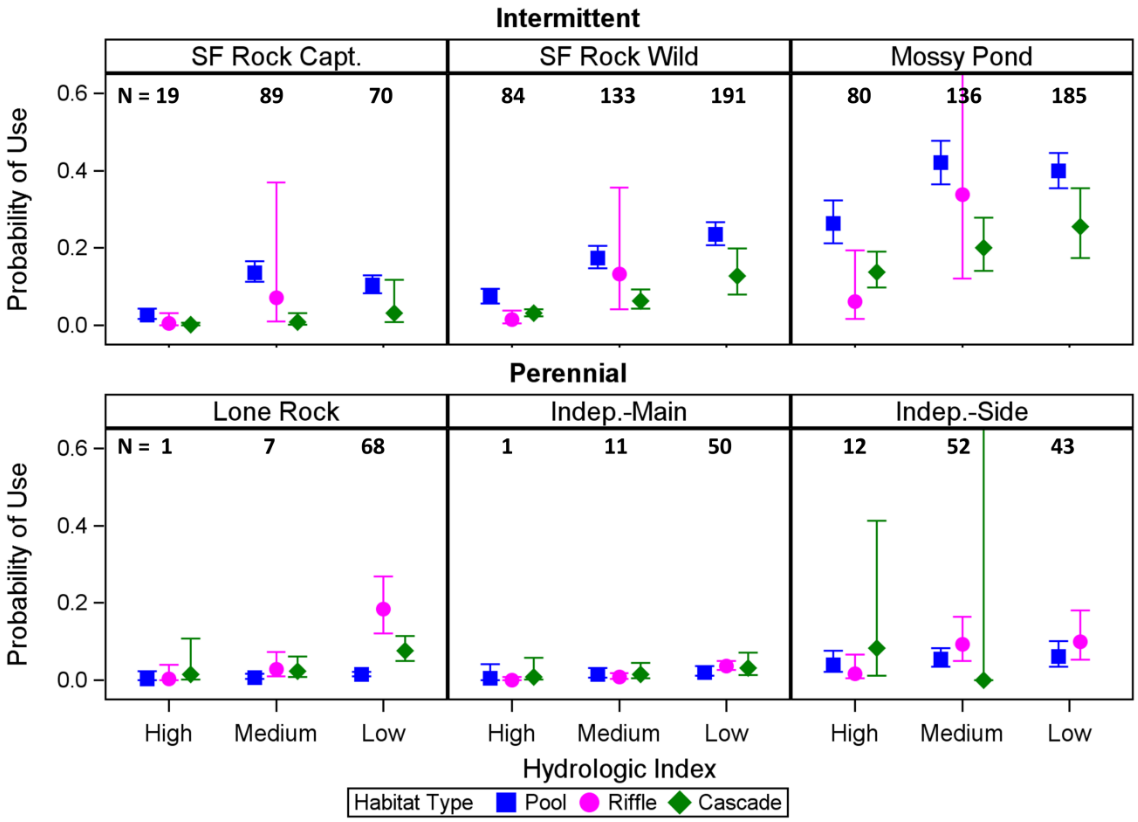

Figure 2.3. Comparison of probability of use among seasonal unit types for wild and captive-reared *Rana sierrae* adults across high, medium, and low hydrologic index categories in four study streams. Indep.=Independence. N=number of frogs found per hydrologic index category per stream. Separate models were run for captive-reared vs. wild frogs and main vs. side channels; see text for explanation. Results for subadult models are shown in Appendix 3: Figure S1. Adults were found in all seasonal unit types, but selected pools in the intermittent streams and riffles at low flows at Lone Rock.

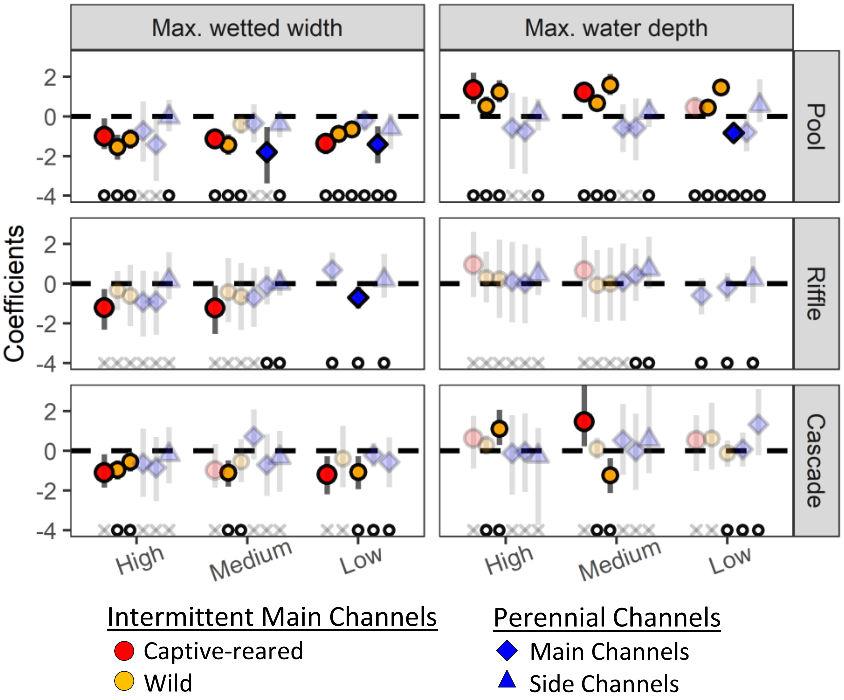

Figure 2.4. Comparison of coefficients from random slope generalized linear models showing relationships between seasonal habitat dimensions and use by wild and captive-reared *Rana sierrae* adults across seasonal units and hydrologic index categories. Points are random effect coefficients for streams. Vertical bars show 95% credible intervals. Separate models were run for captive-reared vs. wild frogs and main vs. side channels; see text for explanation. Results for subadult models are shown in Appendix 3: Figure S2. Highlighted points are significant at the 95% level. Highlighted circles at the bottom of each panel show random effect categories with at least 5 frog use points.

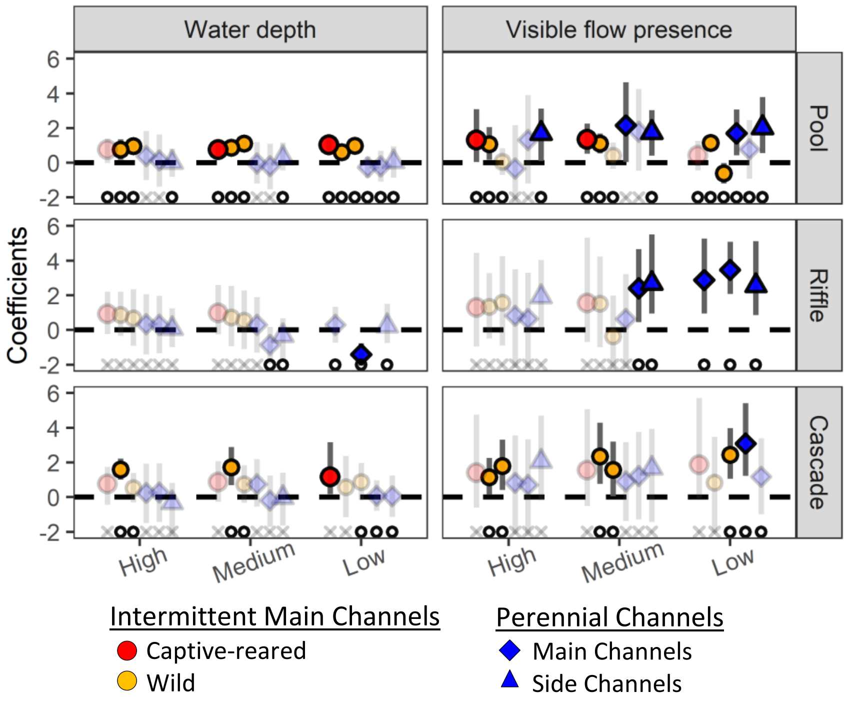

Figure 2.5. Comparison of coefficients from random slope generalized linear models showing additive relationships between hydrological characteristics and use by wild and captive-reared *Rana sierrae* adults across seasonal units and hydrologic index categories. Points are random effect coefficients for streams. Vertical bars show 95% credible intervals. Separate models were run for captive-reared vs. wild frogs and main vs. side channels; see text for explanation. Results for subadult models are shown in Appendix 3: Figure S2. Highlighted points are significant at the 95% level. Highlighted circles at the bottom of each panel show random effect categories with at least 5 frog use points.

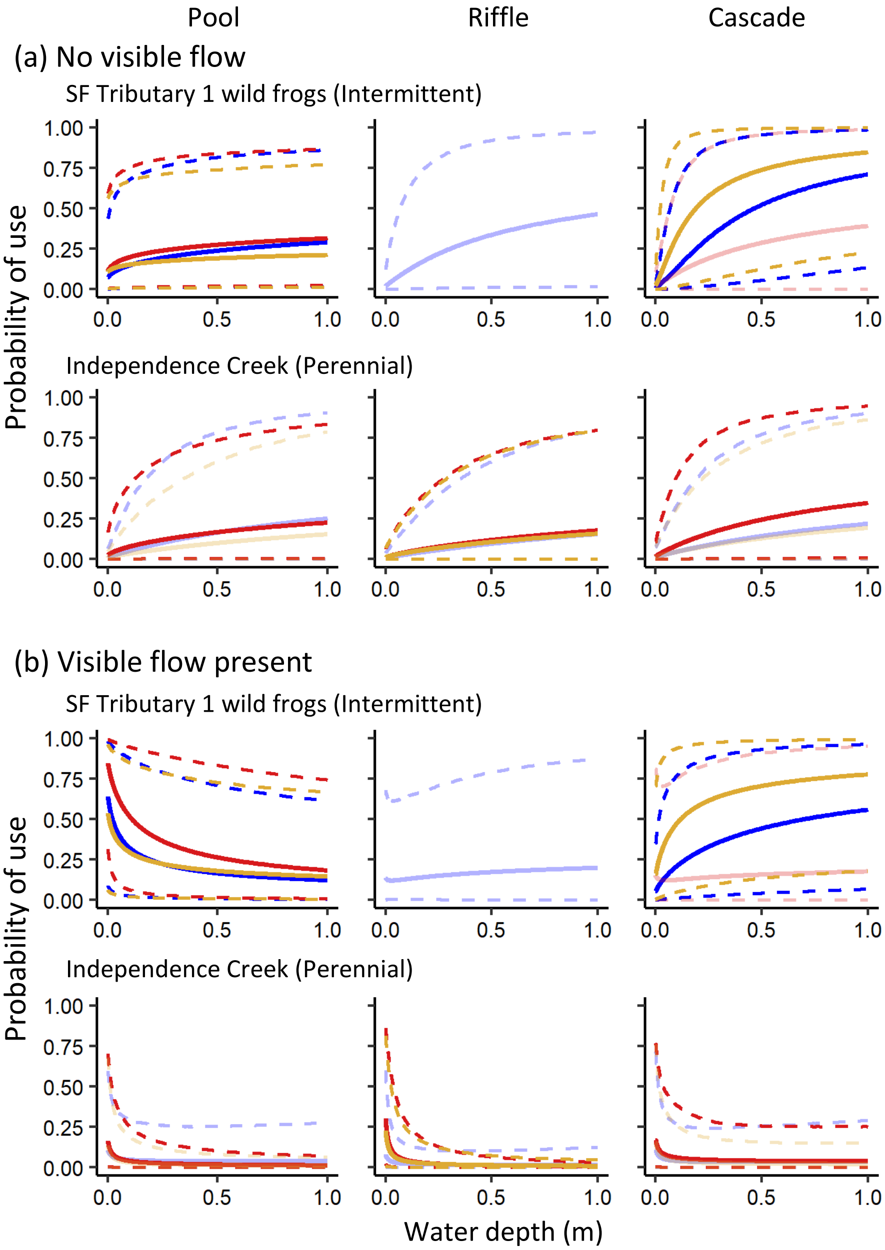

Figure 2.6. Examples of estimated relationships between habitat use and water depth (a) without and (b) with the presence of visible flow in pools, riffles, and cascades in perennial (Independence) and intermittent (SF Tributary 1) streams for wild adult *Rana sierrae*. Estimated relationships are based on models with interaction terms for water depth and the presence of flow using the entire dataset. Blue lines are high water, yellow medium water, and red low water. Dotted lines are 95% credible intervals. Faded lines are categories with low sample sizes (N<5). Water depth x flow model results differed among the SF Rock reaches, shown for all streams for adults in Appendix S2: Figures S3–S4 and for subadults in Appendix S3: Figures S4–S5.

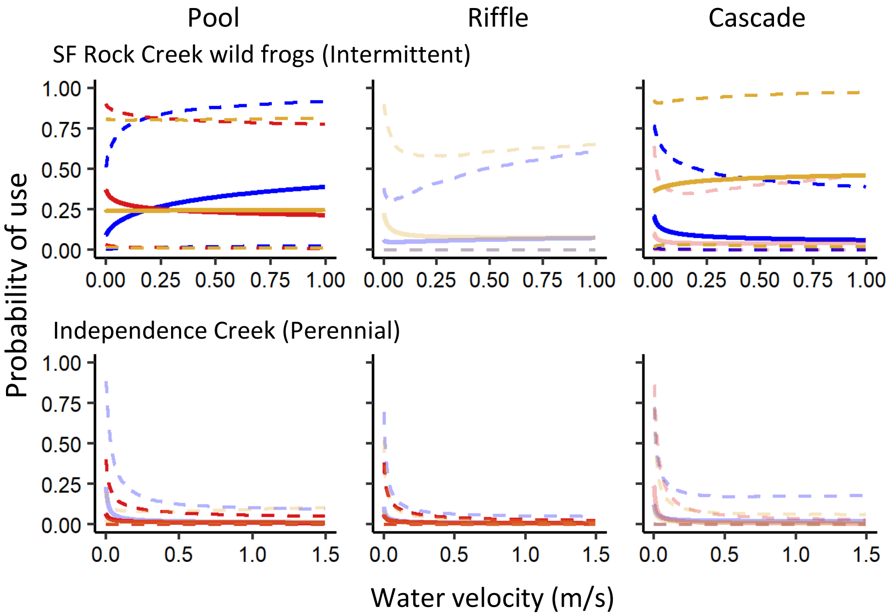

Figure 2.7. Examples of estimated relationships between habitat use and water velocity in pools, riffles, and cascades in perennial (Independence) and intermittent (SF Rock reaches combined) streams for wild adult *Rana sierrae*. Estimated relationships are based on models with interaction terms for water depth and velocity using data where water depths are ≥0.06 m. Blue lines are high water, yellow medium water, and red low water. Dotted lines are 95% credible intervals. Faded lines are categories with low sample sizes (N<5). Results for all streams for adults are shown in Appendix S2: Figure S5 and for subadults in Appendix S3: Figure S6.

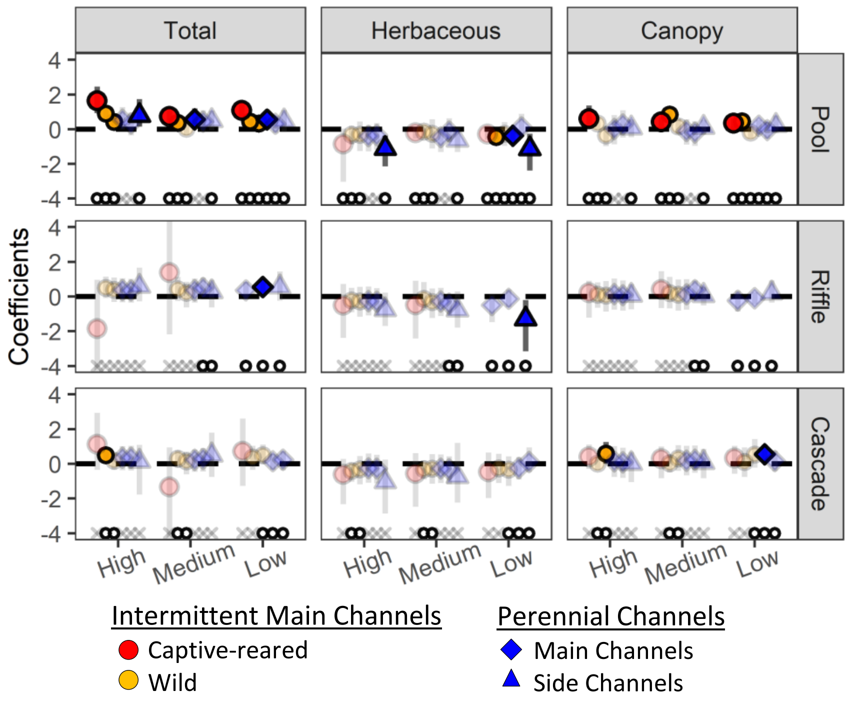

Figure 2.8. Comparison of coefficients from random slope generalized linear models showing relationships between cover variables and use by wild and captive-reared *Rana sierrae* adults across seasonal units and hydrologic index categories. Points are random effect coefficients for streams. Vertical bars show 95% credible intervals. Separate models were run for captive-reared vs. wild frogs and main vs. side channels; see text for explanation. Results for subadult models are shown in Appendix 3: Figure S2. Highlighted points are significant at the 95% level. Highlighted circles at the bottom of each panel show random effect categories with at least 5 frog use points.

CHAPTER 3

Using Demography to Evaluate Reintroductions for Conservation of the Endangered Frog, *Rana sierrae,* in Streams

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ABSTRACT

Reintroductions are an important recovery tool for endangered species but have had varying success. We used demographic data to evaluate the use of reintroductions for the recovery of the federally endangered Sierra Nevada yellow-legged frog (*Rana sierrae*) in stream populations in its northern range where the species is particularly at risk. We conducted a capture–mark–recapture study from 2016–2018 in four diverse streams. One of the streams contained a high abundance of frogs that allowed us to investigate demography in a relatively healthy population. In another of the streams, we tested a reintroduction using captive-reared frogs. We used robust design Huggins and Pradel models to estimate abundance, apparent overwinter survival, recruitment, longevity, and sex ratios. Annual abundance estimates were small, with ≤ 52 wild adult frogs at three streams and few tadpoles or subadults seen in any of the streams. The oldest frog in our streams was at least 13 years. Estimates of apparent survival rates of wild adults ranged from 0.55 ± 0.05 SE to 0.90 ± 0.05 SE and estimates of annual recruitment ranged from 0.02 ± 0.11 SE to 0.26 ± 0.03 SE per site. High survival rates leading to long-lived adults may facilitate persistence of these stream populations, whereas low recruitment may limit population growth and recovery. The two largest populations were in intermittent streams, which may reflect their roles as refuge from fishes. At least 52% of captive-reared frogs survived their first summer after release and at least 36% survived their first winter. Apparent overwinter survival of captive-reared frogs was lower than for wild frogs, ranging from 0.29 ± 0.13 SE to 0.56 ± 0.14 SE. Because of the apparent low survival of wild eggs, tadpoles, and subadults, releasing adults, as was done in this study, rather than younger life stages may be more successful. Our results offer promise for the use of reintroductions to augment depleted populations and suggest that further research on factors affecting recruitment and survival of younger life stages is needed.

INTRODUCTION

Reintroductions are increasingly used as a recovery tool for endangered species (Bar-David et al. 2005; Hunter et al. 2010; Forstner and Crump 2011; Miskelly and Powlesland 2013; Tuberville et al. 2019). We define reintroductions broadly to include the release of translocated or captive-reared individuals to augment existing populations (Hunter et al. 1999; Bertolero et al. 2007; Hunter 2007), to re-establish populations in habitats where the species is locally extinct (Denton et al. 1997; Tavecchia et al. 2009), and to establish new populations in areas where the species either did not occur or its prior status is unknown (Denton et al. 1997; Bell et al. 2004). The success of reintroductions has varied resulting from factors that range from existing environmental conditions at release sites to the design of release programs (Dodd and Seigel 1991; Bloxam and Tonge 1995; Fischer and Lindenmayer 2000; Germano and Bishop 2009). Existing conditions include habitat quality and the presence of threats (Germano and Bishop 2009; Joseph and Knapp 2018). Designs of release programs include criteria such as life stages, number of animals, and number of batches released (Reynolds et al. 2013; Rummel et al. 2016). As a result, there is a growing awareness of the need to first evaluate whether a reintroduction may succeed and is the best choice for a given conservation challenge, and second, to evaluate the best design for an effective release program (Dodd and Seigel 1991; Denton et al. 1997; Fischer and Lindenmayer 2000; Armstrong and Seddon 2008; Germano and Bishop 2009). Small-scale or pilot reintroductions can be pivotal to avoiding costly mistakes before full implementation in conservation programs.

Demographic information about a species is paramount for developing an effective reintroduction program (Bell et al. 2004; Bar-David et al. 2005; Cayuela et al. 2019). Knowledge of a species' demography guides reasonable expectations for the outcome of a reintroduction

(Seddon et al. 2007), and aids in designing a successful program (Tocher et al. 2006; Collazo et al. 2013; Reynolds et al. 2013). Developing reasonable expectations requires understanding the biological constraints of a species such as typical abundances, survival rates, and potential for population growth (Dodd and Siegel 1991; Cayuela et al. 2019; Honeycutt et al. 2019). Designs of reintroduction programs ideally incorporate species-specific data on age structure, survival of different life stages, sex ratios, and reproductive potential (Dodd and Siegel 1991; Biek et al. 2002; Hunter 2007; Muths et al. 2014). Quantitative information on these demographic parameters provides insights into which life stages to release (Muths et al. 2014; Cayuela et al. 2019), whether sex ratios affect population establishment (Dodd and Siegel 1991), and the best configurations of numbers of individuals, frequency, and timing of releases (Tocher et al. 2006; Germano and Bishop 2009; Collazo et al. 2013; Reynolds et al. 2013). Finally, demographic data can inform decisions that increase chances of successful reintroductions while minimizing impacts to source populations caused by the collection of animals (Tocher et al. 2006).

Reintroductions, including the use of captive-reared animals, are being considered as a potential conservation action for Sierra Nevada yellow-legged frogs (*Rana sierrae*, MYLF ITT 2018). Once considered one of the most abundant and widespread amphibians in the Sierra Nevada mountains of California (Grinnell and Storer 1924; Wright and Wright 1949), the endemic *R. sierrae* is now a federally listed endangered species (USFWS 2014). Estimates of rangewide declines of *R. sierrae* and the closely related *Rana muscosa* indicate disappearances from more than 90% of historical localities (Vredenburg et al. 2007) and about 50% of watersheds where reproduction had been documented prior to 1990 (Brown et al. 2014a). Primary causes of rangewide declines include the introduction of nonnative fishes to formerly fishless habitats (Knapp and Matthews 2000; Vredenburg 2004; Knapp 2005) and the amphibian chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*), which is responsible for amphibian declines worldwide (Rachowicz et al. 2006; Scheele et al. 2019). Given the small size and isolation of many remaining populations, recovery of *R. sierrae* will likely require reintroductions (Joseph and Knapp 2018; MYLF ITT 2018).

The demography of *R. sierrae* has primarily been studied in the context of understanding threats and recovery actions (Vredenburg 2004; Briggs et al. 2010; Joseph and Knapp 2018), though a few studies provide baseline vital rates (Fellers et al. 2013; Brown et al. 2019). These studies suggest reintroductions may be a viable recovery tool, at least in alpine lakes where the species has been most studied. In lakes, *R. sierrae* and the closely related *R. muscosa* have the potential for rapid recovery if the causes of declines are removed. In the absence of other threats, for example, removal of introduced fish predators from lakes has led to rapid increases in frog abundances (Vredenburg 2004; Knapp et al. 2007). However, if the causes of decline remain, reintroductions may not be successful (Dodd and Seigel 1991; Fischer and Lindenmayer 2000). Experimental reintroductions of these frogs in high elevation mountain lakes containing *Bd*, for example, have had mixed success (Fellers et al. 2007; Joseph and Knapp 2018).

Rana sierrae is particularly at risk in its northern range where the few remaining populations have low abundances (Brown et al. 2014b; MYLF ITT 2018; Brown et al. 2019). Populations in the northern range also commonly occur in streams, a habitat type where the species is much less studied. The lack of knowledge of the ecology of these northern stream populations has limited the ability to evaluate restoration options, including reintroductions. There are little historical or demographic data, and population monitoring in four streams has found low abundances of < 15 adult frogs (Brown et al. 2019). Further, very little is known about the breeding ecology and recruitment of *R. sierrae* in northern streams. Although tadpoles and subadults are occasionally found in these streams, there are only a few known breeding areas that are located in shallow in- and off-channel habitats (Brown et al. 2019). These habitats are very different from the 4–5 m depths assumed to be required based on lake studies (Bradford 1983; Knapp et al. 2003; Knapp 2005). Thus, further information on the demography and breeding ecology of stream populations in the northern Sierra is needed to evaluate the efficacy of reintroductions as a recovery tool (MYLF ITT 2018).

We quantified the demography of *R. sierrae* in four diverse northern Sierra Nevada streams and pilot-tested population augmentation in one of the streams. Our objectives were to: (1) quantify changes in abundance and survival and estimate other vital rates in three at-risk stream populations first monitored four years earlier; (2) quantify abundance and vital rates in a larger, presumably healthy, stream population and in two additional headwater creeks; (3) increase our knowledge of *R. sierrae* breeding ecology and recruitment in streams; and (4) pilot-test a population augmentation form of reintroduction. Results from this study will provide valuable baseline information to evaluate whether reintroductions are a feasible recovery tool for *R. sierrae* in northern streams, and if so, how best to design a reintroduction program.

METHODS

Study Streams

We selected four streams across the northern range of *R. sierrae* known to be occupied by the species and that varied by size, geomorphology, hydrology, and population abundance (Figure 3.1). Three of the streams had been monitored from 2009–2011 (Brown et al. 2019) and were included in this study to assess demographic changes over the intervening period. We added Mossy Pond Creek to provide demographic data for a presumed healthy population.

The two perennial stream reaches, Independence Creek (1966 m elevation, 0.66 km length) and Lone Rock Creek (1547 m elevation, 2.7 km length), are low to moderate gradient, second and third-order reaches associated with reservoirs. Independence Creek is fairly uniform and dominated by riffles and pools. Flows at Independence Creek are regulated by an upstream reservoir that is located on a preserve. This reach flows through a meadow with multiple braided side channels, floodplains, and off-channel habitats. The total length of the side channels is greater than the length of the main channel. This mosaic of wet habitats outside the main channel is inundated at snowmelt, generally dry by mid-summer, and may reappear in the fall from water releases from the upstream dam. *Rana sierrae* breeds in two intermittent side channels of Independence Creek that have sections retaining water all summer. Lone Rock Creek, in contrast, flows through open forest, has a deeper channel valley, is relatively downcut with large sections of eroded banks, and flows into a large reservoir popular with recreationists and anglers. This more diverse stream is dominated by long deep pools and runs, interspersed with higher gradient cascades and low gradient riffles. Prior surveys found *R. sierrae* populations to be small in both Independence Creek and Lone Rock Creek (< 15 adults; Brown et al. 2019). Native and nonnative fishes were present in Independence Creek and abundant in Lone Rock Creek.

The two intermittent streams, South Fork (SF) Rock Creek and Mossy Pond Creek, are small, high gradient headwater creeks with large sections that dry, typically by mid-summer. Frogs concentrate around the remaining pools. SF Rock Creek consists of three reaches: the headwaters of the main channel (1651 m elevation, 1.0 km length) and two tributaries, SF Tributary 1 (1425 m elevation, 1.2 km length) and SF Tributary 2 (1487 m elevation, 0.4 km length; Figure 3.1). The three reaches are connected by water in the spring, but become disconnected as water dries. These reaches are dominated by medium-gradient cascades and pools. The intermittent water limits fishes from persisting in the SF Rock Creek reaches, though they are occasionally found in the main channel and lower sections of SF Tributary 1. Prior surveys in the SF Rock Creek main channel found very small numbers of frogs (< 5 adults; Brown et al. 2019), only a few frogs had been found in SF Tributary 1 during limited surveys, and the SF Tributary 2 population was a new discovery during this study. Mossy Pond Creek (2034 m elevation, 0.9 km length) flows from a shallow lake into a reservoir and is surrounded by granitic bedrock. Chute, cascade, and pool geomorphology dominates this stream. A steep barrier at the confluence with the reservoir prevents fishes from entering Mossy Pond Creek including the headwater lake. Preliminary surveys of Mossy Pond Creek found a relatively large number of frogs (California Department of Fish and Wildlife unpublished data).

Mark–Recapture Surveys

We conducted capture–mark–recapture surveys from 2016–2018 using a robust design (Pollock 1982). The robust design has two tiers that include primary sampling periods over longer intervals that allow population gains (recruitment/immigration) and losses (mortality/emigration), and secondary sampling occasions within each primary period where the population is assumed to be closed. In each year (primary period), we surveyed each of the four streams four times (secondary occasions) approximately monthly, from early summer (June) into fall (September to mid-October). Annual precipitation varied among years; snowpacks on 1 April were 94% of normal for 2016, 148% of normal in 2017, and 46% of normal in 2018 (available at http://cdec.water.ca.gov/snowapp/sweq.action). High spring flows in 2017 limited early season surveys of the Independence Creek main channel and several side channels, and sections of Lone Rock Creek. During the 2017 high flows at Independence Creek, we found frogs in the mosaic of off-channel aquatic habitats. For each secondary occasion, we walked the entire reach length at least twice, usually over multiple days, conducting surveys after 0930 h to maximize detection and starting in different locations each pass to minimize detection bias related to time of day.

We surveyed all wadable water, including stream channels, mouths of tributaries, side channels, backwaters, and nearby floodplains. We caught frogs by hand or with a handnet. We recorded sex, weight, length (snout–urostyle length [SUL]), and unique passive integrated transponder (PIT) tag number for each captured frog. We PIT-tagged all newly captured adults ≥ 45 mm SUL using Avid MUSICC PIT tags or, in some cases, ≥ 40 mm SUL using BioMark Microchips^{TM} (Biomark, Inc.). We considered frogs ≥ 40 mm to be adults (Matthews and Miaud 2007; Fellers et al. 2013). We counted frogs too small to mark and tadpoles individually when possible, or rounded to the nearest 10 for numbers up to 100, and to the nearest 100 for numbers greater than 100. We visually estimated the number of fishes using the same approach.

Captive-Rearing and Frog Release

Prior to this study, the only known breeding sites in these streams were on Independence Creek. On 26 May 2016, Plumas National Forest crews found a small (~25 m^2) breeding pool with several hundred tadpoles just off the main channel at SF Tributary 2 (Figure 3.1). On 27 May 2016, we collected 105 tadpoles from this pool plus a few other locations on the SF Rock Creek tributaries, and transported them to the San Francisco Zoo and Gardens following protocols specified in the Interagency Mountain Yellow-legged Frog Conservation Strategy (MYLF ITT 2018). Of these 105 tadpoles, 90 died within a few weeks due to an error in the installation of a new water filtration system allowing entry of chlorine over several days before it was discovered and repaired. The tadpoles that were only a few weeks old were especially susceptible to the low levels of chlorine in their environment. In October 2016, we collected an additional 80 older tadpoles from the same pool and transported them to the San Francisco Zoo. The life-support system was fixed and 92 of the remaining 95 tadpoles survived to adulthood.

We reared the tadpoles through metamorphosis at the San Francisco Zoo following strict quarantine holding (Poole and Grow 2012). We kept frogs and tadpoles in large water volumes in enclosures constructed of glass for easy disinfection and with screened lids to prevent frogs from escaping. Water depth was 25–30 cm for swimming larvae and 15–30 cm for metamorphosing larvae and small frogs. Enclosures included hiding spots, basking logs and aquatic perches. Ambient temperatures were 10–18°C with a specific hot spot of 29–32°C to provide a thermal gradient. Water temperature was between 10–18°C with seasonal changes to mimic natural temperatures. Compact fluorescent lights on a timer simulated natural sunlight at levels approximating wild habitat. Mercury vapor UVB lamps provided ultraviolet B lighting.

We used filtration in conjunction with UV sterilizers to reduce the density of aquatic bacteria and parasites and maintain stable water quality. To reduce buildup of organic waste, we changed 25–50% of the water in the tanks twice weekly with reconstituted reverse osmosis water. We tested water quality weekly for frogs and daily for tadpoles. We maintained water quality at near-neutral pH and very low conductivity based on other successful collection locations that were typically granitic basins in the central and southern Sierra Nevada.

Tadpoles were offered food twice daily using Spirulina-based fish foods including an algae gel (Repashy Superfoods Soilent Green meal replacement gel; Repashy Ventures Inc.) and a reconstituted powdered feed plated for grazing (sera Micron Nature; sera Ltd.). Frogs were fed daily for the first year and then three times per week with a variety of invertebrates, including domestic crickets, mealworm larvae, mealworm adult beetles, flightless houseflies, flightless fruit flies, and wax worm larvae. Feeder insects were gut loaded and dusted with calcium with D_3

and multivitamin. Food was offered alive to elicit and reinforce natural foraging behaviors. To maintain innate anti-predation behaviors, staff handled frogs only during necessary health examinations. In total, 92 tadpoles were reared to the adult stage. We know wild tadpoles in the SF Rock Creek reaches overwinter at least once, and subadults probably take at least two years to mature. In the controlled conditions in the zoo, all tadpoles developed into adults within two years.

All frogs released to the wild in 2017 and two-thirds of the frogs released in 2018 were exposed to *Bd* and subsequently cleared with the antifungal drug itraconazole prior to release in an effort to stimulate their immune system to recognize *Bd* (Garner et al. 2008; Jones et al. 2012). One third of the 2018 release were left untreated as a control. Prior to release, we marked all frogs with a PIT tag and clipped the tip of the outer toe on the front foot as a year batch mark.

On 18 July 2017, we released 22 frogs into their natal watershed, SF Rock Creek. We released the frogs into two pools on each of the two SF Rock Creek tributaries, divided evenly among the pools. We chose the four pools based on water presence into mid-October and frog presence in 2016. On 5 July 2018, we released 60 frogs into the two SF Rock Creek tributaries plus the main channel. We selected pools using the same criteria as 2017 and included the 2017 release pools. We released 20 frogs into each reach divided equally among three pools on the main channel and SF Tributary 1, and two pools on SF Tributary 2. We assigned frogs to each pool using a balanced design incorporating *Bd* exposure and sex. In both years, we monitored the released frogs during the two days immediately following release and subsequently during population surveys over the remaining season. We documented a few mortality events using radio-telemetry data.

In 2018, to investigate the effect of the *Bd* exposure and the role *Bd* may play in the survival of the captive-reared frogs, we collected skin swabs from all captured frogs (wild and captive-reared) in the SF Rock Creek reaches using standard protocols (Hyatt et al. 2007; Vredenburg et al. 2010; Joseph and Knapp 2018). We analyzed skin swabs using standard *Bd* DNA extraction and qPCR methods (Boyle et al. 2004; Kriger et al. 2006; Vredenburg et al. 2010; Joseph and Knapp 2018).

Data Analysis

For the four study streams, we used the Huggins and Pradel forms of Pollock's robust design (Pollock 1982) to estimate abundance, apparent overwinter survival, and recruitment of adults for each stream, year, and sex, and to compare the apparent survival of captive-reared frogs released in 2017 to 2018 with that of wild adults. For both forms of the model, we assumed the probability of capture (p) was constant across secondary occasions (model M_o; White et al. 1982). The robust design assumes population closure across secondary occasions (summer in our case), meaning there is no recruitment into or loss of individuals (through movement or birth/death) from the study population. Violations of this assumption would lead to underestimates in p, which would lead to overestimates in population size. Abundance estimates would be unbiased if such inputs or outputs to the study population were random (Kendall 1999). There were no indications of nonrandom inputs or outputs to our stream populations and, as a double-check, we ran a simple Cormack–Jolly–Seber model to estimate monthly summer survival. This resulted in a survival estimate of 0.85 ± 0.01 for the summer, indicating the populations were largely closed over the study period.

We used Huggins robust design to estimate abundance and apparent survival. We compared all combinations of additive models where apparent survival (Φ) and the probability of capture (*p*) were either held constant or allowed to vary among streams, years, sex, and captive-reared versus wild frogs. Although temporary emigration may have occurred at our streams, models were not able to estimate this parameter because of the low numbers of frogs and short duration of our study (3 yr). Thus, we fixed temporary emigration to 0. We compared models using Akaike Information Criterion adjusted for small sample sizes (AIC_c; Burnham and Anderson 2002). All models converged, and final estimates were calculated by model averaging. We report the models comprising the top 95% cumulative weight in the model set for each run.

We used Pradel robust design models (Pradel 1996; Schueller and Peterson 2010; Pederson et al. 2012) to estimate recruitment for wild frogs. We chose the Pradel formulation that estimates recruitment and survival. We used the probability of capture (*p*) structure from the parsimonious models (ΔAICc < 2) of the Huggins robust design (stream and year) with the omission of sex and captive-reared versus wild frogs. We omitted sex to simplify the models given our small sample sizes and because model averages of probability of capture from the Huggins models were essentially the same between the sexes (differences ≤ 0.05 with similar confidence intervals, see Appendix). We excluded captive-reared frogs because their recruitment was a given. We compared all combinations of additive models where recruitment rate (*f*) and survival (Φ) were held constant or allowed to vary among streams and years. Similar to the Huggins analyses, we compared models using AIC_c and calculated final estimates by model averaging (Burnham and Anderson 2002). Model fitting and averaging were conducted using the R (v3.5.2–3.6.1; R Core Team 2018) package RMARK (Laake 2013). Other data processing and analysis were conducted using SAS v9.4 (SAS Institute Inc., Cary, NC).

For unmarked subadult and tadpole stages, we reported the maximum number of individuals found on a given day. Since so few frogs were infected with *Bd,* we descriptively summarized frequencies of infection. We calculated sex ratios for each stream using the capture–mark–recapture abundance estimates and tested for biased sex ratios using the normal approximation to the binomial test (Kachigan 1986). We reported known mortality events of radio-tracked frogs.

We included data from prior monitoring (Brown et al. 2019) to examine longevity of adults. We estimated longevity using adults first captured 2009–2011 from the three streams where surveys spanned 10 years (Lone Rock Creek, Independence Creek, SF Rock Creek main channel) plus adults first captured in 2013 Independence Creek surveys. We reported the number of years from first to last capture because we do not know the length of the tadpole and subadult stages. For the last capture, we used all available data including surveys from 2009–2011, 2013 in Independence Creek, 2015 in Lone Rock Creek and SF Rock Creek main channel, and 2016–2018. We know tadpoles in our streams overwinter at least once (Brown et al. 2019), and subadults probably take at least two years to mature. Thus, we assumed a conservative 14–16-month tadpole and 2-yr subadult stage, making our age estimates conservative minimums that are at least three years less than the true age.

RESULTS

Demography of Adult Wild Frogs

Overall, abundances of wild frogs were low, with larger numbers of frogs in the intermittent creeks than the perennial streams (Table 3.1). The number of unique individuals found per stream was 19 in both Lone Rock and Independence Creeks, 9 in the SF Rock Creek main channel, 51 in the two SF Rock Creek tributaries combined, and 227 in Mossy Pond Creek. Model abundance estimates were similar to the counts.

Four of the Huggins robust design models had equal support with ΔAIC < 2 and relatively equal weights summing to 0.92 (Table 3.2). The remaining models differed in rank by more than 4 AIC each with weights < 0.03. Probability of capture varied by stream, year, sex, and captive-reared versus wild frogs. In the parsimonious models, apparent survival varied by stream and captive-reared versus wild frogs, and there was some support for differences among sexes and years. Two of the Pradel robust design models had equal support with ΔAIC < 2 summing to 0.68 of the weight (Table 3.2). In the parsimonious Pradel models, apparent survival varied by stream with some support for differences among years. Recruitment varied by stream. Probability of capture was essentially the same for both Huggins and Pradel models and ranged from 0.21 ± 0.05 SE to 0.66 ± 0.02 SE (see Appendix).

Annual abundance estimates of wild frogs from the Huggins models were < 30 adults at all reaches except Mossy Pond Creek. The smallest annual abundance estimate was in the main channel of SF Rock Creek with a maximum of 3.4 ± 0.7 SE females and 4.8 ± 1.0 SE males in 2017. The two SF Rock Creek tributaries combined had larger numbers of frogs compared with Independence Creek, Lone Rock Creek, and the SF Rock Creek main channel. The two tributaries were in close proximity, were connected hydrologically, and frogs occasionally moved between them; a long stretch of seasonally dry streambed separated the SF Rock Creek main channel reach from the mouth of SF Tributary 1 and no frogs were documented moving between these reaches. Mossy Pond Creek had the most frogs, as expected. Average densities in Mossy Pond Creek were 47.0 to 144.6 frogs/100 m wetted stream length greater than the perennial streams. Average densities in the SF Rock Creek tributaries were 4.1 to 18.9 frogs/100 m wetted stream length greater than the perennial streams.

Apparent survival of adults from the Huggins models was consistent among years and varied among the streams with no pattern between perennial and intermittent streams (Table 3.1). Apparent survival was relatively low at Lone Rock Creek (e.g., females = 0.56 ± 0.13 SE) and Mossy Pond Creek (e.g., females = 0.55 ± 0.05 SE), and greater at Independence Creek (e.g., females = 0.83 ± 0.09 SE) and the three SF Rock Creek reaches (e.g., females at SF Tributary 1 = 0.89 ± 0.05 SE). One frog was eaten by a Sierra gartersnake (*Thamnophis couchii)* on SF Tributary 2 and one was eaten by a gartersnake of unknown species (*Thamnophis* spp.) on Mossy Pond Creek.

Annual recruitment estimates from the Pradel models over the three-year period were low, with a maximum of 0.26 ± 0.03 SE at Mossy Pond Creek (Table 3.1). However, over a longer period, new frogs were added to the populations. In the three streams with prior monitoring data, the majority of adults found during this study (2016–2018) had not been found during the previous surveys (Lone Rock Creek = 91%, Independence Creek = 63%, SF Rock Creek main channel = 83%).

With a few exceptions, sex ratios were close to 1:1 (*P* from binomial test range = 0.18–0.98; Table 3.3). At Independence Creek, more females were found in 2016 (*P* = 0.09) and more males in 2018 (*P* = 0.04). The numbers of estimated individuals were too low for the binomial test for Lone Rock Creek in 2017, SF Rock Creek main channel in all years, and SF Tributary 2 in 2018. At Lone Rock Creek in 2017, an estimated 4.2 females and no males were found.

A total of 38 individual adult frogs were first captured 2009–2013 in the three streams with surveys spanning 10 yr (excluding Mossy Pond Creek and the two SF Rock Creek tributaries). One male on the SF Rock Creek main channel was caught 10 yr apart (first caught 2009, last caught 2018) making it a minimum of 13 yr old (Figure 3.2). Four frogs were caught 9 yr apart and 5 frogs, 8 yr apart. Of the 14 frogs found at least 5 yr apart, 8 were from Independence Creek. Ages appeared equal between the sexes.

Reproduction

We distinguished breeding areas as locations where there were larger numbers of tadpoles clustered in one location relative to locations with a few scattered tadpoles, which we sometimes found. We found tadpoles in previously known and new breeding areas. We found tadpoles each year in the two side channels at Independence Creek where breeding was previously known to occur. In spring 2016, we found the two breeding pools at the upper end of SF Tributary 2. These SF Tributary 2 pools were hydrologically connected to the main channel during snowmelt, but disconnected most of the summer. One of the pools dried each year, stranding tadpoles, and the other pool was spring-fed, retaining water all summer. In 2018, breeding areas were found in three new locations: the upper part of the SF Rock Creek main channel and in two pools on SF Tributary 1. Each year we found a few tadpoles scattered in additional locations on the SF Rock Creek tributaries, Mossy Pond Creek, and Lone Rock Creek.

Overall, we found few of the younger life stages in any of the stream reaches (Table 3.4). In the known breeding areas, we reliably found tadpoles each year, but numbers were small with maximum counts on a given day ranging from 18–158 at Independence Creek and 18–525 at SF Tributary 2. Egg masses generally have 100–300 eggs, so 525 tadpoles may come from only a few masses. The maximum counts of subadults per day were also very small. The most subadults found was at Mossy Pond Creek, with numbers that ranged from only 11–26 individuals.

There were indications at several streams that 2018 was a relatively good year for breeding compared with prior years (Table 3.4). We found larger numbers of tadpoles in the known breeding areas as well as the discovery of the three new areas. For the first time in six years of surveys, we found evidence that at least one egg mass had been laid in the SF Rock Creek main channel. In addition, for the first time in this reach, three older tadpoles with legs were found in the spring, indicating that breeding had occurred in prior years. Recently metamorphosed individuals were found about a month later, and then small subadults, possibly the same individuals, were occasionally found during the summer. One of the new areas with tadpoles on SF Tributary 1 was a pool where captive-reared frogs had been released in 2017. Finally, a maximum count of 23 subadults was found on Lone Rock Creek, compared with only 4 during the prior two years.

Population Augmentation of Captive-Reared Frogs

Of the 82 captive-reared frogs released into the SF Rock Creek reaches in 2017 and 2018, 52% were known to be alive at the last survey of 2018, 13% were last found mid-summer of their release year, 7% were known or presumed mortalities, and 27% were never seen after their release period (Figure 3.3). At least 8 of the 22 frogs released in 2017 survived to 2018. For two of these frogs, the 2018 sighting was the first time they were seen after their release period. From the Huggins models, the estimated apparent survival of captive-reared frogs from 2017 to 2018 was lower than for wild frogs and ranged from 0.29 ± 0.13 SE to 0.56 ± 0.14 SE with no difference between the sexes (Table 3.5). Three confirmed mortalities of captive-reared frogs occurred in 2017, two to *T. couchii* predation and one from unknown causes. We were not able to model within-summer survival with our design, so we examined observed numbers. Based on counts, survival may have been higher in 2017 with at least 63% of 22 released frogs surviving to the end of the season compared with 48% of 60 frogs in 2018. More than twice as many frogs were found alive at the end of 2018 in the SF Rock Creek main channel and SF Tributary 1 than in SF Tributary 2 (Figure 3.3).

Bd **Infection and Fish**

Data from the *Bd* exposure in the zoo suggested different susceptibility to infection than data from skin swabs collected from frogs in the field. In the zoo, our captive-reared frogs seemed particularly susceptible to *Bd* infection compared with captive-reared frogs collected from other locations in the Sierra Nevada. Our frogs became infected quickly after exposure to *Bd*, and in 2018, eight (17%) died during immunization even though we reduced the exposure period by a third of the time. In contrast, we found very little infection in the field. After the release in 2018, we collected 257 *Bd* swabs from wild and captive-reared frogs in the SF Rock reaches, including 93 adults and 13 subadults. Only eight adult frogs tested positive for *Bd* and the levels of infection in all eight individuals were low (median = 260, range = 18–8722 ITS1 copies per swab). These levels of infection are much lower than the 600,000 ITS1 copies per swab (= 10,000 zoospore equivalents) that are associated with severe disease (Vredenburg et al. 2010; see Joseph and Knapp 2018 for details on the conversion between zoospore equivalents and ITS1 copies). Of the eight infected frogs, five were wild and three were captive-reared. All five wild frogs were from the two SF Rock Creek tributaries, and one infected zoo frog was found on each of the three reaches. Our sample size was very low, but based on these raw data, there were no patterns of infection among release locations, sexes, or *Bd* exposure. All three infected captive-reared frogs were exposed to *Bd* in the zoo prior to their release and none of the control frogs tested positive. All but one of the eight infected frogs were captured more than once, and all but one infection was found in late-August to mid-October. The two frogs with the lowest *Bd* loads (< 20 ITS1 copies per swab) were found again two weeks later with no infection.

Maximum counts of fish per day at Lone Rock Creek were > 450 trout in all three years, 82 Sacramento suckers (*Catostomus occidentalis*) in 2017, and 372 Sacramento suckers in 2018.

At Independence Creek, maximum counts per day were 501 trout in 2016, 50 in 2017, and 66 in 2018. We also found a few Paiute sculpin (*Cottus beldingi*) in this stream. A maximum of 3 trout per day were counted in the SF Rock Creek main channel in 2016, and maximum counts per day in the bottom of SF Tributary 1 were 2 trout in 2016, 1 in 2017, and 4 in 2018. No fish were found in Mossy Pond Creek or SF Tributary 2.

DISCUSSION

The objectives of this study were to explore the potential of reintroductions as a recovery tool for stream-dwelling *R. sierrae* in their northern range and, if promising, to use demographic data to inform reintroduction designs. In our pilot population augmentation, more than half of the captive-reared frogs survived their first summer, suggesting that further experimentation with reintroductions has merit. In addition, our demographic results provide insights into possible reasons for both the persistence of the small populations in our northern streams and for their low numbers. Our populations had high survival rates of long-lived adults, whereas recruitment and survival of tadpoles and subadults appeared low. Interestingly, populations were larger in intermittent than perennial streams, possibly because fish are less common where water is not flowing year-round. Our results help identify considerations for designing reintroductions as well as areas of further research to increase chances of reintroduction success.

Populations in our study streams were small relative to the hundreds of frogs that can be found in some high elevation lakes (Vredenburg et al. 2010; Brown et al. 2014a; Knapp et al. 2016). The three study reaches surveyed four years earlier continued to have very low abundances of wild frogs (Brown et al. 2019), and these populations likely still remain vulnerable due to their small size (Shaffer 1981). An intermittent stream, Mossy Pond Creek, had the highest abundance, as expected from preliminary surveys. Interestingly, the second highest abundance of wild frogs was found in the intermittent tributaries of SF Rock Creek, which were new to this study. The number of wild frogs in these reaches was similar to those reported by Fellers et al. (2013) in a central Sierra Nevada stream. In that meadow-stream complex, numbers of adults varied annually from 45–115 with no particular trend. Also, the sex ratio in that stream was equal, similar to most of our study streams.

Violations of the population closure assumption of the mark–recapture models would result in overestimates of abundance. Although assumptions like this are ideals that are never fully met, it is important to evaluate the degree they are violated. We did document mortality and it is likely new adults entered our populations during the summer. However, our estimates of low recruitment and high survival suggest that inputs and outputs to the population were relatively small, and our abundance estimates were generally similar to counts. Further, Cormack–Jolly–Seber summer survival estimates were relatively high. These minor violations of the closed population assumption would not change our overall conclusions that populations were small, with larger populations in the intermittent streams.

Rana sierrae generally has been considered a highly aquatic, perennial water species that requires deep lakes for breeding (Bradford 1983; Knapp et al. 2003). Thus, we did not expect to find the larger populations in intermittent streams. How the frogs persist in these intermittent streams is an important question, and studies on habitat use and movements are underway to address this topic. Examining causes of population sizes was beyond the scope of our study, but a likely explanation for the higher frog abundances in the intermittent streams is the lack of fishes which are known to be predators of *R. sierrae* and a primary cause of population declines in mountain lakes (Knapp and Matthews 2000; Vredenburg 2004). A steep barrier protects Mossy Pond Creek frogs from the fishes in the downstream reservoir, and large sections of dry streambed protect the SF Rock Creek frogs from fishes for most of the summer. In contrast, fishes inhabit both perennial streams. Independence Creek is downstream from a Lahontan cutthroat trout (*Oncorhynchus clarkii henshawi*) conservation area, and we found fishes in the main channel as well as numerous juveniles in the breeding side channels. Lone Rock Creek flows into a large popular recreational fishing reservoir stocked with nonnative trout. We have observed hundreds of adult and juvenile trout and Sacramento suckers in this reach. Nonnative trout are present in almost all perennial streams within *R. sierrae*'s range, presenting a major conservation challenge for this species. Historical patterns of frog occurrence in intermittent and perennial streams are not known. It is possible that the current populations are isolated remnants of larger populations that once inhabited downstream perennial reaches prior to the introduction of fish. Still, although intermittent streams may not be the most intuitive choice for reintroductions of a highly aquatic species, they may provide important refuges given current conditions and be a realistic alternative to more permanent water given the widespread presence of nonnative trout (Corlett 2016).

Bd is the other known cause of small *R. sierrae* populations in alpine lakes (Rachowicz et al. 2006; Vredenburg et al. 2010). The low *Bd* loads in the SF Rock Creek reaches in 2018 were similar to those reported for streams in 2009–2011 (Brown et al. 2019). These loads were well below the 600,000 ITS1 copies/swab level associated with morbidity and mortality (Vredenburg et al. 2010; Joseph and Knapp 2018). In addition, we did not find any field mortalities that appeared related to *Bd*. In contrast, the captive-reared frogs appeared to be highly susceptible to the disease based on their reaction to the *Bd* exposure. Future monitoring is needed to determine whether the released captive-reared frogs will become infected with *Bd* or whether the increased population size due to augmentation may change the disease dynamics in the streams (Briggs et al. 2010). The central Sierra Nevada stream population monitored by Fellers et al. (2013) has persisted with low levels of *Bd*, though the effect of the disease on abundances was unknown.

There is no information on historical frog numbers in our study streams to guide expectations for population recovery. It is likely that current numbers reflect the rangewide declines of *R. sierrae* (Vredenburg et al. 2007; Brown et al. 2014a), though it is also possible that these streams may never have supported the large populations once common in lakes. In streams that dry to just a few small pools in the summer, the carrying capacity may be low relative to alpine lakes. Studies on productivity, food supply, and other factors that may affect carrying capacity in these streams would help guide expectations for abundances of recovered populations.

Both survival and recruitment affect population growth rates, persistence, and the potential for recovery (Schmidt et al. 2005; Muths et al. 2011). In species with long lifespans, high survival of adults can sustain populations through periods of low recruitment (Taylor et al. 2006) and population growth is often sensitive to changes in adult survival (Biek et al. 2002; Schmidt et al. 2005; Bertolero et al. 2018). But, without sufficient recruitment, populations can slowly decline (Muths and Scherer 2011). Amphibians like *R. sierrae* that lay many eggs have high reproductive potential, and thus, in the absence of other factors that may depress recruitment, a capacity for fast recovery or population growth from introduced animals (Pechmann et al. 1991; Alford and Richards 1999; Vredenburg 2004; Knapp et al. 2007). However, there may be a lag between reintroductions and population increases due to long tadpole and/or subadult development (Hunter et al. 2010; Joseph and Knapp 2018).

We found that *R. sierrae* adults can be relatively long-lived, as found in other studies (Matthews and Miaud 2007; Fellers et al. 2013). Although the specific methods for determining ages differed, Matthews and Miaud (2007) and Fellers et al. (2013) estimated maximum ages ranging from 14–16 yr old; the oldest frog in our study was at least 13 yr. Apparent survival of adults was relatively high at two of our study streams, but low at the other two streams. Low apparent survival at Lone Rock Creek may be attributed to the abundance of nonnative trout in the stream. However, frogs at Mossy Pond Creek also had low apparent survival despite the absence of fish. Frogs in this stream may be susceptible to other causes of mortality, but the low apparent survival also may result from emigration to other nearby aquatic habitats. Apparent survival estimates cannot distinguish between mortality and emigration. The demography and movements of frogs in this watershed are currently under study (I. Chellman, personal communication). Apparent overwinter survival rates during this study were similar at Independence Creek, lower at Lone Rock Creek, and higher at SF Rock Creek compared with estimates from 2009–2011 (Brown et al. 2019). Overwinter survival estimates by Fellers et al. (2013) ranged from 0.45–0.95. Thus, apparent overwinter survival can vary among streams and among years.

Recruitment was low in our streams based on estimated recruitment rate, low counts of tadpoles and subadults, and few known breeding areas. However, there was at least some recruitment over a longer time period at the three streams that had been monitored previously. High mortality of eggs, tadpoles, and subadults is common in many amphibians (Alford and Richards 1999). For *R. sierrae*, these younger life stages may be particularly vulnerable because of their prolonged development. The apparent low survival of tadpoles and subadults argues for releasing older individuals.

Low recruitment into the populations may result from a variety of factors. First, few egg masses may be laid due to Allee effects in small populations such as an unbalanced sex ratio, trouble finding mates, or lower reproduction in older individuals (Shaffer 1981). Further, in temperate zones with short growing seasons, females of some amphibians may not breed every year (Muths et al. 2010). In the small populations in our study streams, there were only a few unequal sex ratios in some years with the bias in opposite directions, though it is possible that the pool of available females may be limited in some years. Our study streams were all ≤ 2.7 km and frogs are capable of moving relatively long distances with movements documented up to 1.3 km (Brown et al. 2019; Keung et al. 2021). Thus, it seems unlikely that frogs would not be able to find mates if present. Population augmentation may help alleviate some of these small population risks. Second, poor placement of egg masses may lead to desiccation or loss from high flows. Each year at least one egg mass was laid in a small pool that dried, stranding the tadpoles. On the other hand, the three breeding locations with tadpoles each year were relatively protected from high spring flows, and the one egg mass we found was on the underside of a small boulder in a small protected tributary. Note that we did not search extensively for egg masses in most of our study streams so further research on this is warranted. Third, predation on tadpoles and subadults by fishes (Knapp and Matthews 2000) likely affects their survival in Lone Rock Creek and Independence Creek. Predation by nonnative crayfish (*Pacifastacus leniusculus*; Wiseman et al. 2005) may also occur in Lone Rock Creek. Fourth, newly metamorphosed frogs are particularly susceptible to *Bd* (Rachowicz et al. 2006) though, as discussed, *Bd* loads were low in our streams. Finally, high overwintering mortality of younger life stages may be common in species like *R. sierrae*; little is known about how any of the *R. sierrae* life stages overwinter in streams. Further research on factors affecting survival of eggs, tadpoles, and subadults is needed.

Evaluating reintroductions such as this population augmentation pilot requires some definition of what constitutes success (Seddon 1999). Success can range from the ultimate goal of building a self-sustaining population (Dodd and Seigel 1991) to a series of short-term objectives that provide opportunities for learning (Denton et al. 1997; Hunter et al. 2010; Miller et al. 2014). Our population augmentation in SF Rock Creek served as a pilot test to determine whether reintroductions should even be considered as a restoration tool for the recovery of this endangered species in streams. In the short-term, the release of captive-reared animals was relatively successful. At least half the animals survived to the end of their release summer, and at least 36% of individuals released in 2017 survived to 2018. Further, a companion radio-tracking study found frogs remained near their release pools with no frogs dispersing from the stream (Keung et al. 2021).

The lower survival of the captive-reared frogs compared with wild frogs is common in reintroductions (Tavecchia et al. 2009; Bertolero et al. 2018; Cayuela et al. 2019). However, this ‘cost of release’ may diminish in subsequent generations (Bar-David et al. 2005; Bertolero et al. 2018; Cayuela et al. 2019). Reasons for lower survival are not well known, but suggestions have included dispersal from the stream, higher risk of predation, and stress from a new environment (Armstrong and Seddon 2008). The landscape surrounding our release streams is dry, so it is unlikely frogs would have had incentive to leave the stream channel. Moreover, radio-tracked captive-reared frogs did not leave the streams (Keung et al. 2021). We did see a few instances of snake predation in our study, but there did not appear to be a higher rate for captive-reared frogs than wild frogs. Captive-reared frogs were, on average, smaller than wild adult frogs and thus may have been more susceptible to predation. We cannot evaluate stress, though one frog did die of unknown causes shortly after release. Finally, it is possible that more frogs survived but were cryptic and hard to find; two of the 2017 frogs were not found after their release until 2018.

It is possible that 2017 captive-reared frogs contributed to the increased reproduction in 2018 in the two SF Rock Creek tributaries. For instance, one captive-reared female was found at the breeding pool at SF Tributary 2 in spring of 2018 and a second captive-reared frog was observed depositing eggs in spring of 2019. Also, captive-reared frogs do seem to be reproducing; nine egg masses were found in the SF Rock Creek main channel in 2019, after our study concluded, which exceeds the numbers of wild frogs known in this reach. Further years of monitoring are needed to clarify the long-term success of the population augmentation.

Reintroductions of at-risk species can be challenging and have had variable success (Dodd and Siegel 1991; Fischer and Lindenmayer 2000; Germano and Bishop 2009). Although our pilot captive-rearing and augmentation seems successful in the short-term, it was not without risk. Our first batch of tadpoles brought into captivity died from a life-support system failure, leading us to collect older, more resilient tadpoles for the second batch. Once the cause of the mortality was identified and resolved, we had 97% success rearing tadpoles to adults. Also, overwinter survival of captive-reared adults was lower than in wild counterparts. On the other hand, there are also inherent risks in doing nothing for critically endangered species (Hunter et al. 1999). Further, there may be opportunities to lessen the risk. For example, we have the opportunity to salvage tadpoles that would otherwise die in a desiccating pool. We are experimenting with releasing some of these tadpoles in other locations in our streams to increase their survival and would use salvaged tadpoles should further augmentation be warranted. Finally, although risks cannot be eliminated and unforeseen events can occur, they also provide opportunity for learning and improving techniques. Monitoring is crucial to maximizing learning from both successes and failures of reintroduction programs, with the ultimate goal of increasing their effectiveness (Armstrong and Seddon 2008; Miller et al. 2014; Muths et al. 2014).

Our demographic data indicated several release design considerations. First, releasing adults seems prudent given their high survival rates and the apparently low survival of the younger life stages. Second, releasing equal numbers of males and females may best reflect ratios in healthy populations. Finally, although there is not sufficient information to set abundance goals for a recovered population, our two largest populations may provide guidelines for what is possible under current conditions. Ultimately, however, stream-specific characteristics may contribute to higher and lower abundances in streams, even under ideal conditions.

Our study also identified information gaps. First, further research is needed on factors that affect recruitment and the survival of eggs, tadpoles, and subadults in streams. Second, information on productivity and other factors affecting the carrying capacities of small intermittent streams could help establish realistic abundance goals for recovered populations in these systems and guide decisions on numbers of frogs to release. Third, studies on interactions between frogs, fish, and crayfish in streams would help develop strategies to promote recovery in streams where these taxa overlap. Finally, longer term monitoring will assess the ultimate success of our population augmentation as well as provide a longer time series for evaluating demographic parameters. A longer time series could also detect the occurrence, frequency, and implications of population pulses such as those that may have occurred with reproduction in 2018. Studying conditions before and during these pulses may help us understand factors affecting reproductive success.

We set out to evaluate the potential of reintroductions as a recovery tool and to inform the design of a reintroduction program using demographic information and a pilot population augmentation. In the northern range of *R. sierrae*, we found that populations remained small in three of our study reaches four years after surveys were first conducted, suggesting that conservation actions such as reintroductions may be warranted. However, several challenges remain. The persistence of small populations may be due to the longevity and high survival rates of adults, but low recruitment may prevent these populations from reaching greater abundances or complete recovery. Although apparent survival of captive-reared frogs was lower than wild frogs, sufficient numbers survived their first summer and winter to merit further experimentation with reintroductions. Decisions about whether to use reintroductions depend on many factors, but our results suggest that this tool may be a promising option for the endangered *R. sierrae* in streams.

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Table 3.1. Demographic parameter estimates for wild *Rana sierrae* in four northern Sierra Nevada streams surveyed 2016–2018. The top half shows counts of unique individuals. The bottom half shows model estimates. Abundance and survival estimates are model averages from Huggins robust design models. Recruitment estimates are model averages from Pradel robust design models. F = Females, M = Males.

	Perennial streams						Intermittent streams											
										South Fork Rock Creek reaches								
	Lone Rock Creek			Independence Creek			Mossy Pond Creek			Main Channel			Tributary 1			Tributary 2		
Count of unique individuals from 2016–2018																		
F	11			9			118			5			14			9		
M	8			10			109			4			17			11		
	Est.	SE	CI	Est.	SE	CI	Est.	SE	CI	Est.	SE	CI	Est.	SE	CI	Est.	SE	CI
Abundance																		
Female																		
2016	9.1	2.5	6.8–18.3	11.6	2.6	9.0–20.8	70.5	3.0	66.7–79.5	3.6	0.8	3.1–7.8	15.3	2.2	13.0–22.9	6.3	1.3	5.2–11.9
2017	4.2	1.4	3.2–10.4	9.5	2.0	7.6–17.1	63.4	2.4	60.6–70.9	3.4	0.7	3.0–7.2	10.9	1.6	9.5–17.0	6.0	1.1	5.2–11.0
2018	6.7	1.0	6.1–11.4	3.3	0.6	3.0–6.6	59.8	0.9	59.1–63.9	4.1	0.4	4.0–6.4	11.6	0.8	11.1–15.4	5.2	0.5	5.0–8.1
Male																		
2016	10.0	3.0	7.1–20.6	4.7	1.7	3.3–11.9	73.2	3.7	68.2–83.7	2.5	0.8	2.1–6.7	12.2	2.3	9.9–20.1	12.1	2.2	9.9–20.0
2017	0.0			11.7	2.6	9.1–20.9	63.1	2.9	59.5–71.7	4.8	1.0	4.1–9.5	14.1	2.1	11.9–21.5	10.1	1.8	8.5–16.8
2018	4.7	1.0	4.1–9.3	11.4	1.4	10.3–17.2	56.2	1.1	55.2–60.8	3.1	0.4	3.0–5.5	12.9	1.0	12.2–17.4	4.3	0.6	4.0–7.4
Survival[1]																		
Female																		
17–18	0.56	0.13	0.31–0.77	0.83	0.09	0.59–0.95	0.55	0.05	0.46–0.64	0.86	0.11	0.51–0.97	0.89	0.05	0.75–0.96	0.74	0.09	0.53–0.88
Male																		
17–18	0.58	0.13	0.33–0.79	0.85	0.08	0.61–0.95	0.57	0.05	0.48–0.66	0.87	0.10	0.53–0.97	0.90	0.05	0.76–0.96	0.76	0.09	0.55–0.89
Recruitment	0.19	0.09	0.07–0.41	0.02	0.11	0.0–1.0	0.26	0.03	0.20–0.32	0.15	0.12	0.03–0.53	0.09	0.07	0.02–0.33	0.11	0.07	0.03–0.33

[1] Survival was similar for both years so only data for 2017–2018 are shown.

Table 3.2. Huggins and Pradel robust design model selection for demographic parameter estimates for *Rana sierrae* in four northern Sierra Nevada streams surveyed 2016–2018. K is the number of parameters in the model. AICc is the Akaike Information Criterion adjusted for small sample sizes. ΔAICc is the difference in AIC value from the parsimonious model. w_i is the Akaike weight for comparing models. Φ is apparent survival. *f* is recruitment. *p* is probability of capture. Only top models with cumulative weight of 95% are shown.

Model	K	Deviance	AICc	ΔAICc	w_i
Huggins robust design					
Φ(stream + wild/zoo), *p*(stream + year + sex + wild/zoo)	19	4505.8	3888.9	0.00	0.35
Φ(stream + year + wild/zoo), *p*(stream + year + sex + wild/zoo)	20	4504.6	3889.8	0.90	0.22
Φ(stream + sex + wild/zoo), *p*(stream + year + sex + wild/zoo)	20	4504.7	3889.9	0.99	0.21
Φ(stream + year + sex + wild/zoo), *p*(stream + year + sex + wild/zoo)	21	4503.5	3890.7	1.87	0.14
Φ(stream + wild/zoo), *p*(stream + year + wild/zoo)	17	4515.1	3894.0	5.17	0.03
Pradel robust design					
Φ(stream), *f* (stream), *p*(stream + year)	20	1595.3	4006.5	0.00	0.44
Φ(stream + year), *f*(stream), *p*(stream + year)	21	1594.5	4007.8	1.27	0.24
Φ(stream), *f*(stream + year), *p*(stream + year)	21	1595.3	4008.6	2.08	0.16
Φ(stream + year), *f*(stream + year), *p*(stream + year)	22	1594.5	4009.9	3.35	0.08
Φ(stream), *f*(.), *p*(stream + year)	15	1610.5	4011.3	4.84	0.04

Table 3.3. Sex ratios (Female:Male) for wild *Rana sierrae* in four northern Sierra Nevada streams surveyed 2016–2018. Sex ratios are based on abundance estimates, *P*-values test for biased sex ratio, and confidence intervals (CI) show proportion of females.

	Perennial streams						Intermittent streams											
										South Fork Rock Creek reaches								
	Lone Rock Creek			Independence Creek			Mossy Pond Creek			Main Channel			Tributary 1			Tributary 2		
Year	Ratio	*P*	CI	Ratio	*P*	CI	Ratio	*P*	CI	Ratio	*P*	CI	Ratio	*P*	CI	Ratio	*P*	CI
2016	0.9:1	0.84	0.36–0.59	2.5:1	0.09	0.60–0.82	1.0:1	0.82	0.45–0.53	1.4:1		0.39–0.79	1.3:1	0.55	0.46–0.65	0.5:1	0.18	0.23–0.45
2017	4.2:1			0.8:1	0.63	0.34–0.56	1.0:1	0.98	0.46–0.55	0.7:1		0.24–0.59	0.8:1	0.52	0.34–0.54	0.6:1	0.31	0.25–0.49
2018	1.4:1	0.55	0.44–0.73	0.3:1	0.03	0.12–0.33	1.1:1	0.74	0.47–0.56	1.3:1		0.38–0.75	0.9:1	0.79	0.37–0.57	1.2:1		0.39–0.71

Table 3.4. Maximum number of subadults and tadpoles counted on a single day for wild *Rana sierrae* in four northern Sierra Nevada streams surveyed 2016–2018.

	Perennial streams		Intermittent streams			
				South Fork Rock Creek reaches		
	Lone Rock Creek	Independence Creek	Mossy Pond Creek	Main Channel	Tributary 1	Tributary 2
Subadults						
2016	4	6	26	1	1	1
2017	4	2	18	0	3	2
2018	23	6	11	3	4	7
Tadpoles						
2016	1	18	9	1	1	107
2017	8	59	4	0	3	18
2018	1	158	4	87	126	525

Table 3.5. Estimates of 2018 abundance and overwinter survival rates for captive-reared *Rana sierrae* released in 2017 in the South Fork Rock Creek reaches. Demographic parameter estimates are model averages from Huggins robust design models. F = Females, M = Males. Number of frogs released in 2017 were 3 F, 8 M in SF Tributary 1; and 6 F, 5 M in SF Tributary 2. Number of frogs released in 2018 were 11 F, 9 M in SF Main Channel; 12 F, 8 M in SF Tributary 1; and 12 F, 8 M in SF Tributary 2.

	South Fork Rock Creek reaches					
	Tributary 1			Tributary 2		
	Est.	SE	CI	Est.	SE	CI
Estimated number of frogs released in 2017 and alive in 2018						
F	1.0 (of 3)	0.1	1.0–1.9	2.0 (of 6)	0.2	2.0–3.2
M	5.1 (of 8)	0.4	5.0–7.4	0.0 (of 5)		
Survival rate overwinter from 2017 to 2018						
F	0.54	0.14	0.27–0.79	0.29	0.13	0.10–0.59
M	0.56	0.14	0.29–0.80	0.31	0.13	0.11–0.60

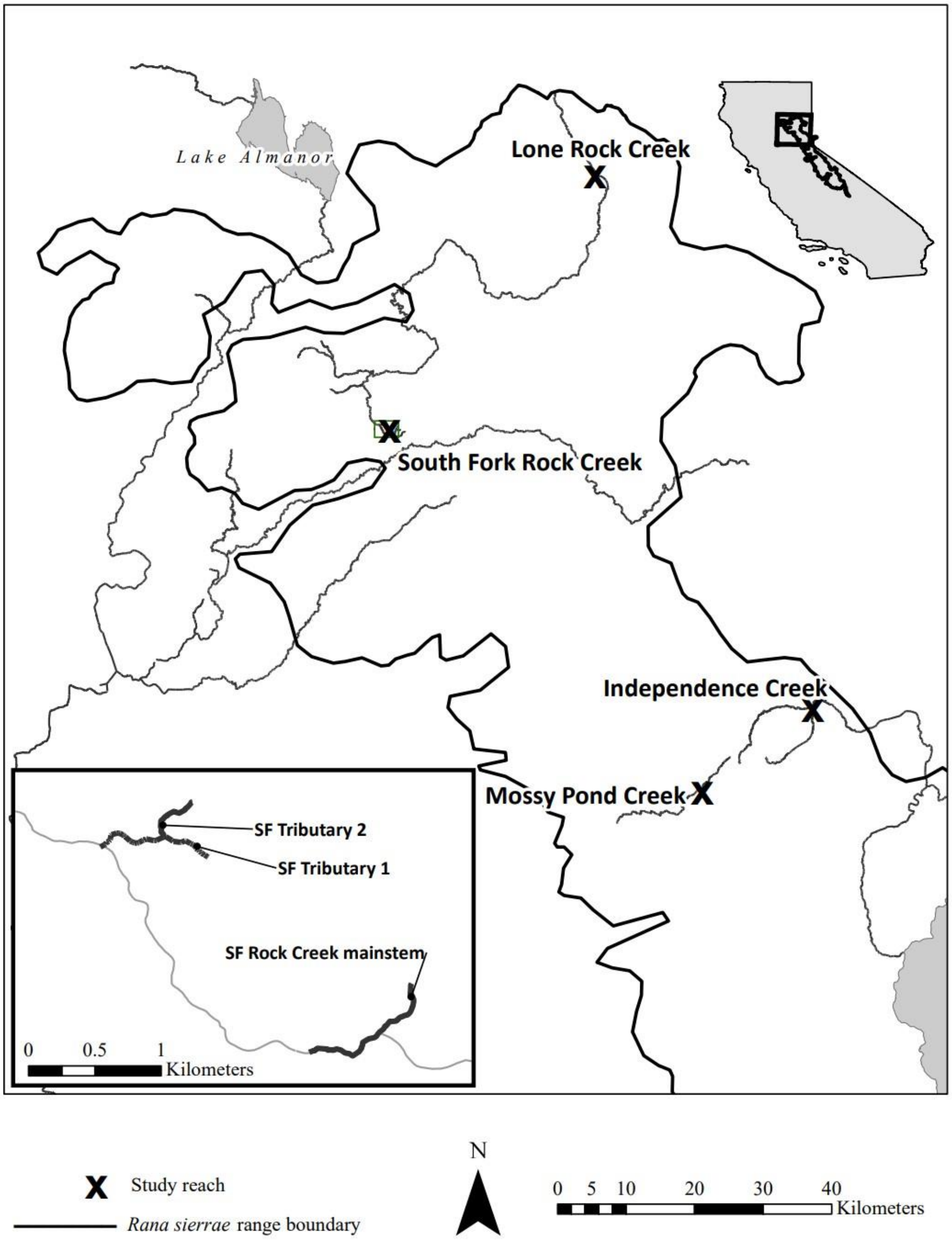

Figure 3.1. Locations of *Rana sierrae* streams surveyed 2016–2018. Insets show breeding areas.

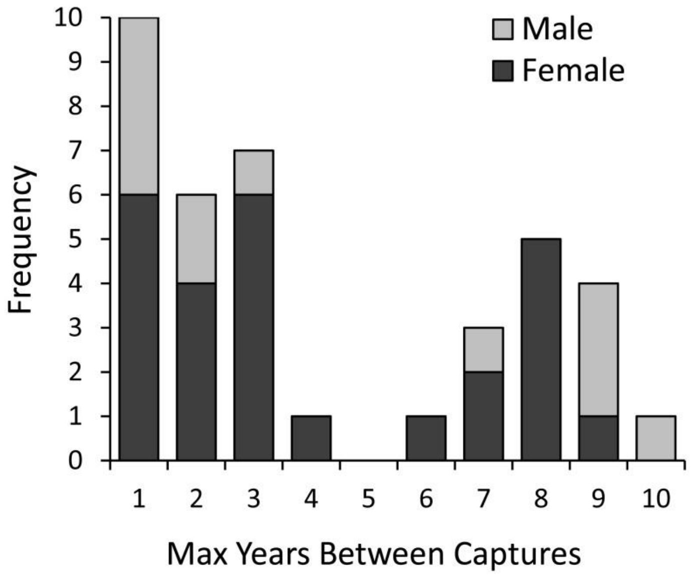

Figure 3.2. Maximum number of years between captures of marked *Rana sierrae* first captured 2009–2013 in three northern Sierra Nevada streams. First captures occurred 2009–2011 in Lone Rock Creek and SF Rock Creek main channel, or 2009–2011 or 2013 in Independence Creek. Last captures occurred 2009–2011 in all three streams, 2013 in Independence Creek, 2015 in Lone Rock Creek and SF Rock Creek main channel, or 2016–2018 in all three streams.

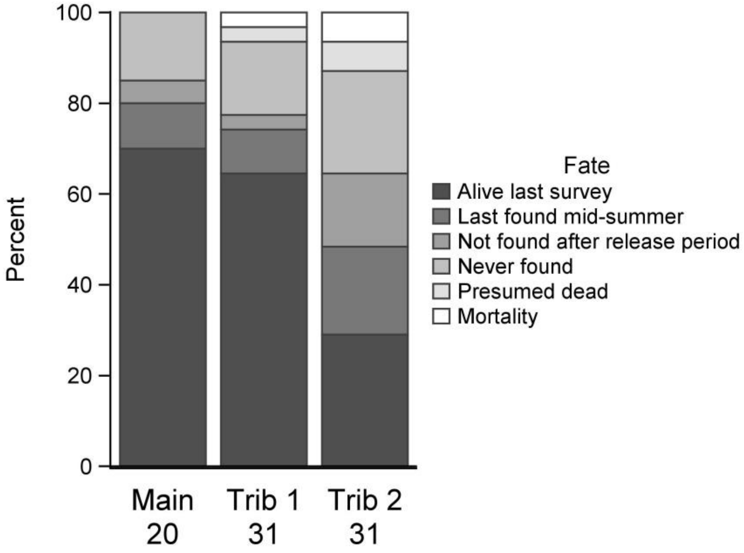

Figure 3.3. Fate of captive-reared frogs released into the South Fork Rock Creek reaches in 2017 and 2018. The number of frogs released into each stream is shown below each bar.