CONSERVATION SCIENCE PARTNERS

FINAL REPORT

(29 February 2016)

Effects of pool characteristics on California tiger salamander larval density at Dutchman Creek Conservation Bank, Merced County

(Objective 1)

For the project entitled:

Effects of changing hydroperiod on reproductive occupancy of threated Central California tiger salamander in vernal pool and seasonal wetlands in the CA Central Valley under different climate scenarios

Submitted to:

The California Landscape Conservation Cooperative

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Introduction

The California tiger salamander (*Ambystoma californiense*; hereafter, CTS) is classified as a federally threatened species (U.S. Fish and Wildlife Service 2004). Consequently, much research has been done to provide information for its management and conservation. However, previous research has primarily focused on the use of upland, terrestrial environments by CTS (Trenham and Shaffer 2005), the demography of populations (Trenham et al. 2001), and the effects of hybridization between CTS and the introduced, barred tiger salamander (*Ambystoma tigrinum mavortium*; Johnson et al. 2013). Information on the characteristics of wetlands (e.g., hydrologic regime) that support reproduction and metamorphosis by CTS is limited. Understanding the relationship between the hydrologic regime of pools and use of the pool by CTS for reproduction is critical, because scenarios of climate change predict warmer temperatures and less precipitation across the species' range. If these scenarios are realized, the hydrologic regimes of pools are expected to be affected. The timing of inundation and the period over which wetlands contain standing water could change, which could have negative consequences for CTS.

Westervelt Ecological Services has contracted data collection on CTS at the Dutchman Creek Conservation Bank (DCCB). We originally proposed to use occupancy models (MacKenzie et al. 2006) to analyze those data, to guide the design of our sampling at the refuge complex and regional scales and develop preliminary inferences regarding the wetland characteristics that were associated with use of wetlands by CTS. While the data were not appropriate for use with occupancy models, we have applied an alternative approach to generate insights towards effects of pond characteristics on CTS larval density at DCCB, and to guide our future sampling efforts to the extent possible.

Our goal with this report was to analyze existing data collected at the DCCB to: 1) evaluate relationships between CTS larval density and pool characteristics assessed at this site (pool depth, area, type, and water temperature); and 2) develop insights and sampling considerations useful for data collection efforts underway over larger spatial extents.

Study Area

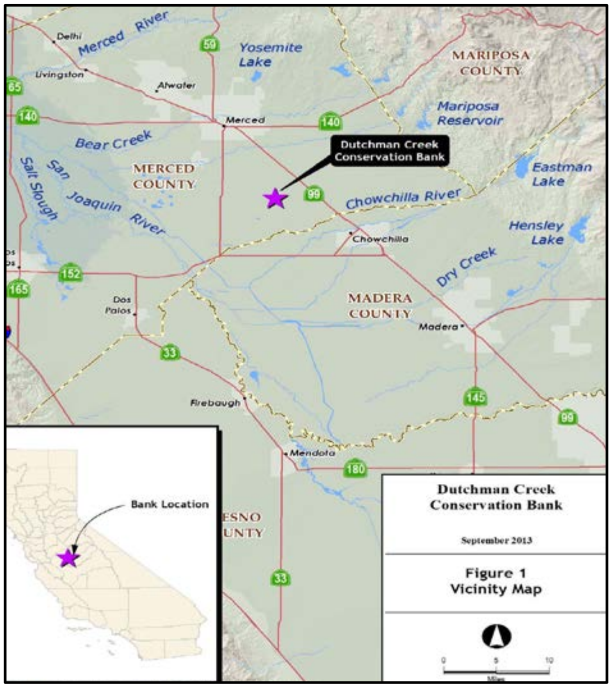

Figure 1. Location of Dutchman Creek Conservation Bank

Data were collected at the DCCB located in south-central Merced County (Figure 1). The site covers approximately 504 acres and consists of 36.9 acres designated as jurisdictional wetlands, including 2.2 acres freshwater marsh, 4.8 acres playa pool, 17.3 acres vernal pool, 10.9 acres vernal pool-vernal swale complex, and 1.6 acres vernal swale (Helm, 2010). Vernal pools and playa pools generally occurred in shallow to deep depressions, while vernal pool-vernal swales occurred as a complex with evidence of connecting drainages (Butterworth 2009). Wetland hydrology of the three pool types was supported primarily by incident precipitation (Butterworth 2009). The northern edge of the DCCB is bounded by Sandy Mush Road, whereas the other three edges are directly adjacent to irrigated cropland. The DCCB is used for dryland livestock grazing, but there is no evidence that the site as ever been plowed or cultivated (Butterworth 2009).

The site is characterized by mound-intermound microtopography ranging from approximately 2-4 feet, in which a mosaic of vernal and playa pools and vernal swales occur within a matrix of non-native grasslands (Butterworth 2009). Ground squirrel burrows occur throughout the uplands of DCCB. Dutchman Creek, which flows through the northern portion of the site, is incised 8-15 feet and has ephemeral flows (Butterworth 2009). The site is located in a Mediterranean climate in which most precipitation falls between November and March.

Methods

Field data collection

Surveys of CTS larvae were conducted by Helm Biological Consulting at the DCCB during 2013, 2014, and 2015 (Helm Biological Consulting 2013, 2015). A primary goal of the sampling effort was to document CTS larval presence and metamorphosis for the purpose of establishing mitigation credits. As such, sampling was purposefully conducted in pools where larvae were expected to occur, based in part on pool ponding characteristics as well as on knowledge of which pools had previously provided CTS breeding habitat (Helm Biological Consulting 2015). Twenty-six ponds were sampled between one to six times during 3 field seasons, 2013-2015 (Table 1). All required procedures for CTS sampling were conducted by Helm Biological Consulting under permit TE-795930-8/9 of Section 10(a)(1)(A) of the federal Endangered Species Act, 16 U.S.C. 1531 and its implementing regulations (Helm Biological Consulting 2015).

Table 1. Wet-season sampling conducted on 6 occasions over 3 years (2013-2015) at 26 pools at Dutchman Creek Conservation Bank. A '1' indicates that a pool was sampled on a particular date, whereas a '0' indicates that no sampling occurred at that pool on that date. The 'Total' Column at the right indicates the number of times each pool was sampled.

Pool	**Pool area**	**Sampling Dates**						
Name	**(ha)[1]**	**3/22/2013**	**4/16/2013**	**4/9/2014**	**1/27/2015**	**2/26/2015**	**3/22/2015**	**Total**
PP1	**0.06**	0	0	0	1	1	0	**2**
PP2	**0.19**	1	1	1	1	1	1	**6**
PP5	**0.05**	1	0	0	1	1	0	**3**
PP6	**0.04**	0	0	0	0	1	0	**1**
PP7	**0.15**	1	0	0	1	1	0	**3**
PP9	**0.03**	0	0	0	1	1	0	**2**
PP10	**0.08**	1	0	0	1	1	0	**3**
PP11	**0.05**	1	0	0	1	1	1	**4**
PP12	**0.22**	1	0	1	1	1	0	**4**
PP13	**0.24**	1	0	0	1	1	0	**3**
PP14	**0.51**	1	0	0	1	1	0	**3**
PP15	**0.08**	0	0	1	1	1	0	**3**
PP16	**0.02**	0	0	0	0	1	0	**1**
PP17	**0.09**	1	0	0	0	1	0	**2**
VP75	**0.06**	0	0	0	1	1	0	**2**
VP144	**0.02**	0	0	1	1	1	0	**3**
VP216	**0.01**	0	0	0	1	0	0	**1**
VP339	**0.07**	0	0	0	1	1	0	**2**
VP489	**0.04**	0	0	0	0	1	0	**1**
VP559	**0.28**	0	0	0	1	1	0	**2**
VP570	**0.04**	1	0	0	1	1	0	**3**
VP571	**0.11**	1	0	0	1	1	0	**3**
VP678	**0.02**	0	0	0	0	1	0	**1**
VPVS14	**0.17**	1	0	0	1	1	0	**3**
VPVS20	**0.13**	0	0	0	1	0	0	**1**
VPVS76	**0.1**	0	0	0	1	1	0	**2**
Total pools sampled		**12**	**1**	**4**	**21**	**24**	**2**	**64**

[1] *Pool areas from Butterworth (2009) survey of delineated wetlands.*

Larval surveys

One of two possible survey methods was used at each visit to a pool: dipnets (6- to 12-inch wide openings) or seines (8 to 12 X 4 feet with 1/8 inch mesh). Dipnets were typically used in pools less than 3 inches deep or less than 500 square feet area (Helm Biological Consulting 2015). The area of sampled pools designated as wetland varied from 109.3 to 5086.9 square meters (Table 1; Butterworth 2009). To accommodate this wide variation in pool size, the surface area sampled (seine or dipnet total pull length X width) per pool and date ranged from 0.69-345.6 square meters. The proportion of pools sampled ranged from 0.03-14.66% of delineated pool areas.

Surveyors summed the number of larvae collected across all dipnets or seine pulls conducted for each pool and date sampled (see Helm Biological Consulting 2015 for more detailed methods). To gain an assessment of larvae size while minimizing stress to larvae, surveyors also measured the total length (mm) of the first 20 larvae collected.

Maximum larval length was small (< 15mm) during the January, 2015 survey and increased from February through April, 2013-2015 surveys to sizes greater than 75mm total length sufficient for metamorphosis to occur (Alvarez 2013, cited in Helm Biological Consulting 2015; Table 2). There was substantial inter-annual variability in the numbers of larvae for the same date across years (Table 2) likely driven in large part by changes in precipitation. Given the range in potential influences we decided to evaluate the numbers of larvae in 2 ways. First, we considered all larvae detected across the 6 surveys. Second, we considered only data collected during 3 surveys in February and March to represent the "peak" larval period, to try to account for potential early- and/or late-season variability in timing of rainfall that may influence larval growth and/or timing of metamorphosis.

Table 2. Number of pools sampled, number of larvae sampled, and average of maximum length of larvae by sampling date.

	Sampling Dates						
	1/27/2015	**2/26/2015**	**3/22/2013**	**3/22/2015**	**4/9/2014**	**4/16/2013**	**Total**
Pools sampled	**21**	**24**	**12**	**2**	**4**	**1**	**64**
CTS larvae sampled	**68**	**725**	**204**	**9**	**0**	**7**	**1013**
Avg of max length (mm)	**15.0**	**50.8**	**71.2**	**78.0**	**-**	**85.0**	

Pond covariates

In addition to larval counts, surveyors also recorded several pool characteristics. First, each pool was categorized as vernal pool, playa pool, or vernal pool-vernal swale complex. The delineated wetland area (ha) for each surveyed pool (from Butterworth 2009) was converted to square meters for analysis. Pool depth was measured using net handles marked with 1 inch increments then converted to cm. Maximum depth (deepest measured depth per pool) and average depth (weighted average of depth for proportion of pool sampled) were strongly correlated (correlation coefficient = 0.7023). However, maximum depth was more vulnerable to outliers (e.g., uncharacteristically deep areas in pools caused by tire ruts) and so we used average pond depth, which we considered a surrogate for hydroperiod. Surveyors measured the pool water temperature for 50 of 64 ponds surveyed. Finally, all pools were categorized as "milky" or "turbid", though these terms were used interchangeably, so there was no basis to assess variation in turbidity.

Data analysis

While the original intent of our proposal was to conduct an occupancy analysis with these data, we determined that there were insufficient replications within a season to meet the closure assumption necessary to model occupancy. As such, we used available data to conduct regression analyses to assess pond characteristics relative to the number of larvae captured per pool and date.

Due to the wide variation in survey effort among pools, it was necessary to adjust for survey effort. We felt that the total surface area (total pull length X width) provided a more precise measure of sampling effort than volume (total pull length X width X average depth sampled), since larvae occurred almost entirely on the pool-bottoms rather than in the water column, and the pools were relatively flat (Brent Helm, *personal communication*). As such, we used larval density (larvae per surface area sampled), to reflect potential benthic habitat capacity within pools. We developed the outcome variable for regression analysis as the number of larvae divided by the sampled surface area multiplied by 100, to obtain a CTS larval density per 100 m^2, the approximate size of the smallest sampled pools.

As a first step in modeling, we assessed distributions of variables using graphs and diagnostic tests, and assessed potential relationships between predictor variables. We found low correlations between water temperature with pond area (correlation coefficient = -0.1126; n=50) and with pool depth (correlation coefficient = -0.3176; n=50); however, the relationship between larval density and water temp was not linear, so we considered water temperature as a predictor variable by itself using non-parametric, locally weighted regression (lowess smoothing) of water temperature on larval density.

We found that pond type was related with pond area, with greater pond area for playa pools compared with vernal pools, so we considered pool type as a predictor itself. We also considered pool depth by itself, and due to a slight relationship between pond area and depth, considered pool area after accounting for pool depth.

The outcome variables (larval density during January-April, and for "peak" periods during February-march) were slightly skewed, so we used generalized least squares (GLS) random-effects regression, a semi-parametric approach, and used the Huber-White sandwich estimator to obtain robust standard error estimates. We used pool as a random effect to account for repeated measures by pool over time. We conducted this analysis for CTS density as a function of pool type, water temperature, and pool depth each considered in separate models, as well as for pond area after accounting for pool depth. We conducted this analysis for all data (January – April) and for peak data (February – March).

Results and discussion

CTS Larval Surveys

An average of 15.8 larvae ± 35.9 SD (range from 0-233 larvae) were captured across the 64 pool/date surveys. The maximum length of larvae averaged 42.6 mm ± 25.9mm SD (range from 15-96mm) across all dates, and 59.5 mm ± 16.8 mm SD (range from 26-96mm) for the peak larval period.

Water temperature and other water quality parameters

Water temperature in sampled pools ranged from 49.7 to 84°F (9.8 to 28.9°C) over all dates sampled. For both time periods there was not a strong linear relationship between larval density and water temperature. However, when assessed using locally weighted regression, there was an apparent optimal range of temperature (approximately 56-68°F) that maintained highest larval densities for both periods (Figure 2).

Additional water quality variables such as pH or dissolved oxygen were not available in this dataset. However these and other parameters are expected to be important (Ryan et al. 2013) and may likewise show optimal ranges to maximize larval presence or abundance.

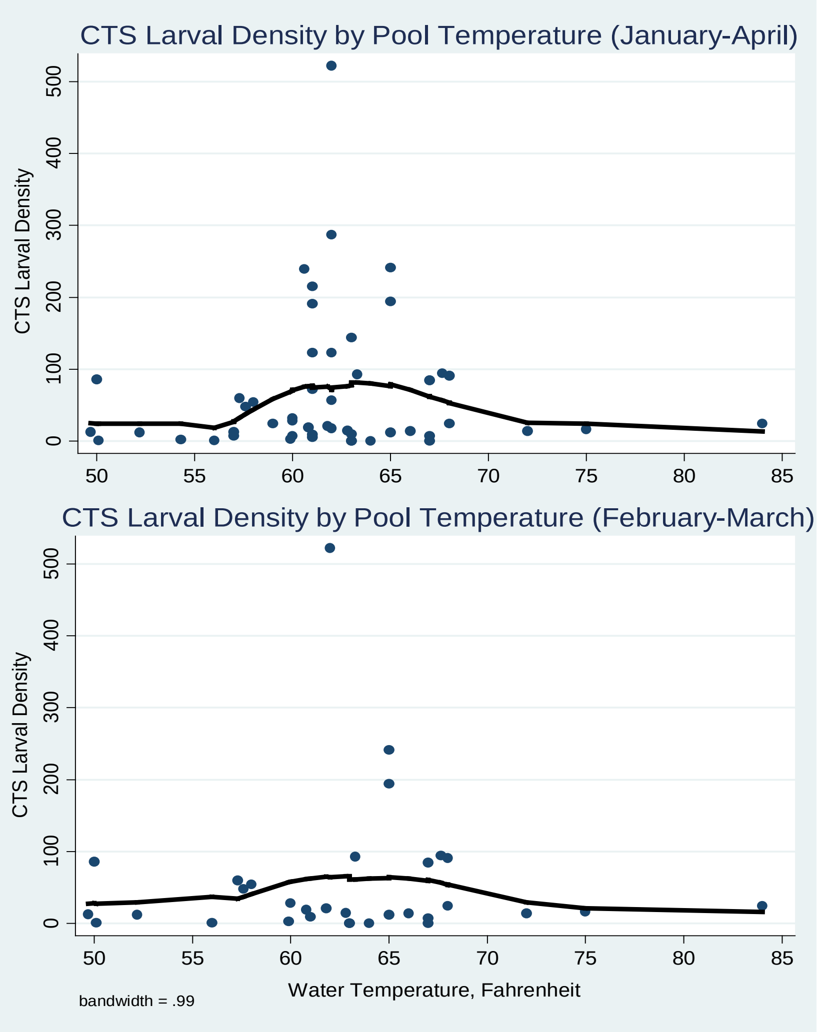

Figure 2. CTS Larval Density as a function of pool water temperature for all data and peak periods

Turbidity is also expected to play an important role in water temperature, evaporation rates, hydroperiod, and in minimizing predation risk by herons and egrets (Croel and Kneitel 2011, Helm Biological Consulting 2015). In this study, all pools were classified as turbid, suggesting that high turbidity is likely to be an important habitat characteristic for larval density at DCCB. Based on the inability to discern variation using qualitative characterization of turbidity in this study, however, future data collection efforts could consider applying quantitative measures of turbidity (e.g., using a turbidometer) to characterize a range of variation that may show additional, meaningful effects on larval density.

Pool type

We assessed larval density in relation to pool type. There was an indication of lower larval density in water features classified as vernal pools compared with playa pools or vernal pool-vernal swale complex, though 95% confidence intervals among pool types overlapped for both full and peak season data (Figure 3).

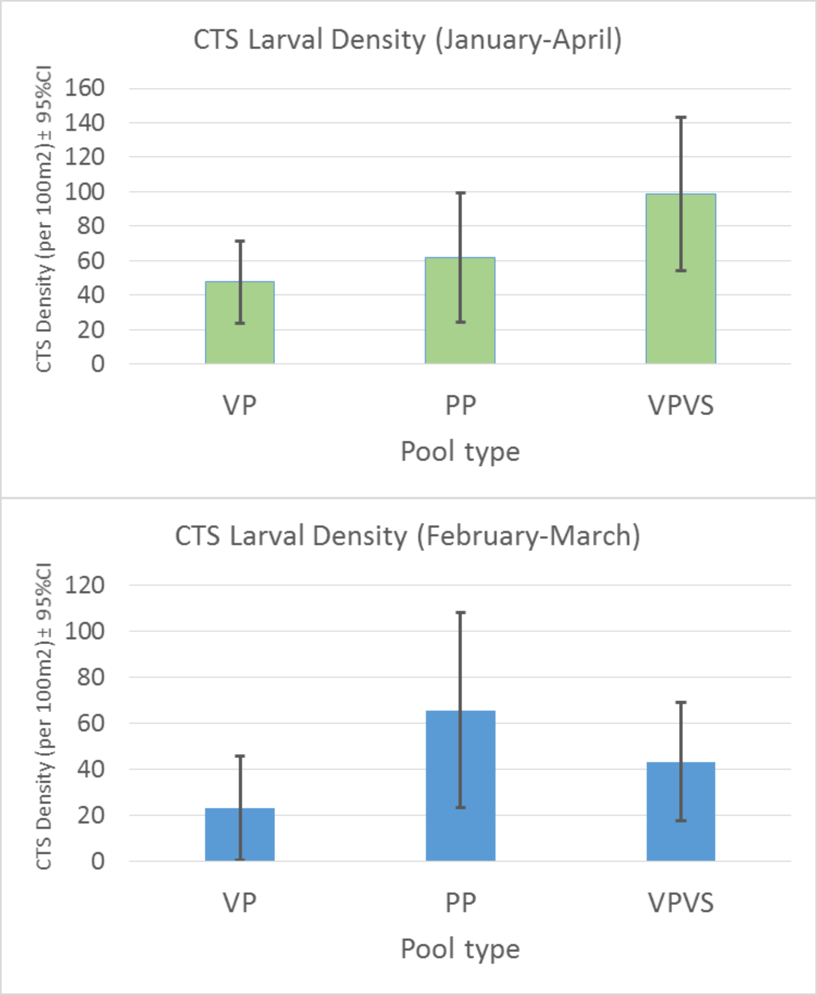

Figure 3. CTS larval density by pool type (VP=Vernal Pool, PP = Playa Pool, and VPVS = Vernal Pool-Vernal Swale Complex) for all data and peak periods

While pool area was not itself predictive of CTS larval density, pool type was related with pool area. Playa pools were larger on average than vernal pools by 706.4 square meters (SE = 310.5, p=0.026); there was no difference in pond area of vernal pool-vernal swale compared with vernal pools. Additionally, there was no relationship between pool type and pool depth. In addition to pool area, there may be one or more collinear factors driving potential differences in larval density as a function of pool type. Future work could include additional covariates to try to tease out the relevant pond or upland factors that may influence larval or metamorphic occupancy or density.

Pool depth

Average pool depth varied 10-fold and ranged from 2.1 to 21.1 cm across surveyed pools. We found that average pond depth was an important predictor of CTS density. When considering all 6 sampling dates that spanned January-April, we found that CTS larval density increased by an average of 5 larvae per 100 square meters for every 1 cm increase in pool depth (slope coefficient = 5.1, p=0.020, n=64; Figure 4). When considering 3 sampling dates that spanned the "peak" larval period from February and March, we found that CTS larval density increased by almost 7 larvae per 100 square meters for every 1cm increase in pool depth (slope coefficient = 6.7, p=0.017, n=38; Figure 4).

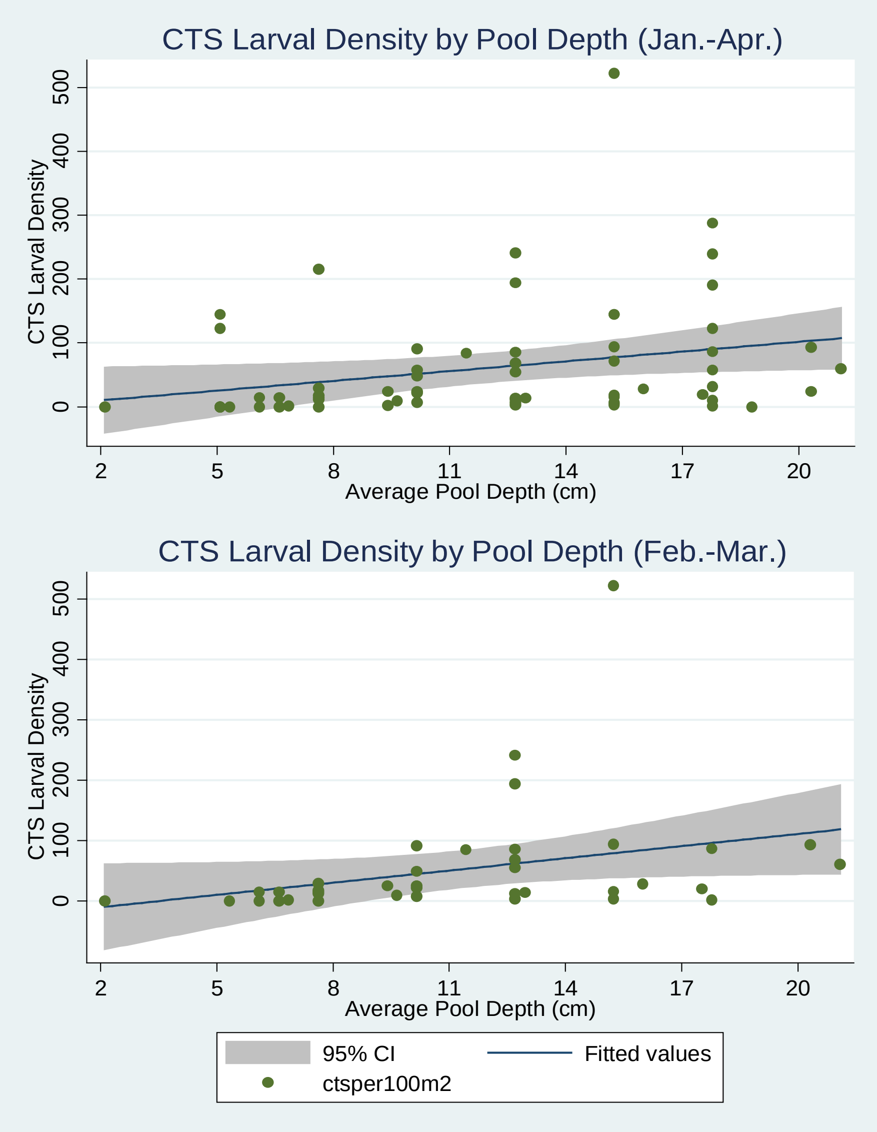

Figure 4. CTS larval density as a function of pool depth with 95% confidence bands for all data and peak periods

It is unclear what specific influence pool depth may have for larvae, but there may be a relationship between pool depth and hydroperiod that may influence larval habitat selection. Though pool area was not related with larval density by itself, it was related with larval density after accounting for pool depth, such that larval density increased as pool area decreased. This suggests that perhaps larvae may occur in higher densities in smaller pools as they dry.

Input for future survey efforts

This study provides several methodological insights for future survey efforts. First, though purposeful sampling made sense to document mitigation credits at this site, future efforts would benefit from a sampling protocol that was probabilistic (e.g., using random or stratified random sampling) to enable inference to a population of pools. Second, while there is certainly value in assessing larval density, an even more basic question pertains to larval and metamorphic occupancy, to help identify pond features related with habitat selection. A stratified approach is likely warranted to capture a range of variation and sampling considerations across pools of differing types, depths, or areas. Given the potential range in size and subsequent range in volume of water sampled, careful consideration is also warranted to both standardize and/or account for different effort applied in different pools. Finally, while the data considered here lacked sufficient replication to use occupancy analysis to account for larval detection, future efforts could be applied to account for potential heterogeneous detection probabilities. Given potential intra-annual differences in growth of larvae, there would also be some benefit in standardizing the timing of sampling to characterize “peak” larvae compared with larvae closer to the stage of metamorphosis.

In addition to sampling considerations, this study also suggests several additional covariates of interest. Our original intent was to account for upland characteristics, though the fine-scale elevational microtopography (2-4 feet) at this site was unexpected and will require additional methodological work to enable use of available digital elevation models or remote sensing imagery. Given the importance of water temperature, this study also suggests potential importance of other water quality parameters. Finally, given the interplay between pond area, pond depth, and pond type, this study suggests the importance of other, potential confounding variables that may include soils, ground squirrel burrow density, or other upland characteristics. The challenge will be collecting sufficient data that enables teasing apart the many potential predictor variables.

Acknowledgements

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