Presence of *Ambystoma californiense* within Pools of Varying Macroinvertebrate Diversity

Bryson H. Marks

ABSTRACT

Amphibian populations worldwide are declining, and habitat loss is a major factor. Although research has focused on monitoring and preserving *Ambystoma californiense* populations, no quantitative studies have been conducted to determine whether macroinvertebrate composition affects presence of *A. californiense* within vernal pools. Therefore, I examined macroinvertebrate community structure and relevant abiotic factors *A. californiense* require in 22 vernal pools within the San Joaquin Valley, California. Macroinverterbates were sampled using standardized net sweeps (6 m^2 area), subampled to 500 individuals, with organisms identified to order and family. I used t-tests, and nonmetic multidemsional scaling (NMS) ordination to determine whether macroinvertebrate communities and abiotic factors predict the presence or absence of *A. californiense*. Neither pH, dissolved oxygen, turbidity, nor conductivity differed significantly between *A. californiense* present and absent pools ($p > 0.05$). NMS analysis indicated that conductivity, pH, and emergent vegetation were the three abiotic factors most associated with changes in the macroinvertebrate community. I observed no significant correlation between macroinvertebate community structure and salamander presence (A = 0.0003, p = 0.41). If evidence continues to suggest that benthic macroinvertebrate communities are not predicting of *A. californiense* pond use, then research resources should be allocated to investigating abiotic factors.

KEYWORDS

California tiger salamander; macroinvertebrate; vernal pool; amphibian; wetland; Central Valley

INTRODUCTION

Amphibian populations worldwide are declining due to anthropogenic impacts, especially habitat loss and alteration (Purrenhage & Boone, 2009; Alford & Richards, 1999; Collins & Storfer, 2003; Stuart, Chanson, Cox, Young, Rodrigues, Fischman, & Waller, 2004). In California, populations of the California tiger salamander (*Ambystoma californiense*) have also declined due to habitat loss caused by conversion of habitat to agriculture, urbanization, and other anthropogenic activities (Barry & Shaffer, 1994). Encroachment of non-native predators has also contributed to population declines (Ryan, Johnson, & Fitzpatrick, 2009). Consequently, *A. californiense* is listed as threatened in the Central Valley and endangered in Sonoma and Santa Barbara counties under the Endangered Species Act (USFWS, 2009). The *A. californiense* inhabits its full historic range, but is becoming increasingly rare (Trenham, Shaffer, Koenig, & Stromberg, 2000). A greater understanding of the influence of macroinvertebrate composition on *A. californiense* presence within pools may be a valuable source of information for future habitat restoration efforts.

Previous and current research has focused on monitoring and preserving *A. californiense* populations through habitat restoration, conservation, and species movement patterns (Barry & Shaffer, 1994; McCamman, 2010; Trenham et al., 2000; Vollmar, 2009). Much research has been done on *A. californiense* habitat communities and the way they are utilized by *A. californiense*. For example, mesocosms lacking habitat structure resulted in species unevenness, with low community-level response by species to experimental treatments in pond communities (Purrenhage and Boone, 2009). In addition to aquatic conditions, understanding upland habitats is crucial for managing *A. californiense* habitats (Trenham and Shaffer, 2005). *A. californiense* movement and interpond dispersal patterns have been analyzed to understand subpopulations and sink habitats (Trenham, Koenig, & Shaffer, 2001).

Many studies have also been conducted on feeding habits of ambystomatids, indcluding *A. californiense*. In one case, the stomach contents of barred tiger salamanders (*Ambystoma mavortium*) included remains of coleopterans, hemipterans, homopterans, hymenopterans, and orthopterans (Smith, Gray, & Quarles, 2004). Similarly, another case observed the stomach contents of tiger salamanders and found plankton, ostracods, mollusks, hemipterans, and other invertebrates (Denoël, Whiteman, & Wissinger, 2006).

A. californiense adults and larvae both feed on aquatic insects as well as zooplankton, snails, and tadpoles (Barry & Shaffer, 1994; McCamman, 2010). Anderson (1968) found that *A. californiense* larvae feed primarily on tadpoles, cladocerans, copepods, and snails; however, he did not study the dietary habits of adult *A. californiense*. Although *A. californiense* diets have been examined by many researchers, no studies have been conducted to determine whether macroinvertebrate community composition is associated with the presence or absence of *A. californiense* within vernal pools. Understanding which habitat features have the strongest influence on wetland fauna is critical if we are to properly manage amphibian habitat (Purrenhage & Boone, 2009). Knowing which food sources are required by *A. californiense* is important in understanding habitat preference.

In this study, I measured a number of biotic and abiotic factors to test if *A. californiense* preferred pools with particular attributes at vernal pools in California's Central Valley. In particular, I aimed to determine 1) if macroinvertebrate composition and abundance predicted the presence of *A. californiense* within pools, 2) what invertebrate communities were most commonly present in pools that supported *A. californiense* larvae, and 3) what abiotic factors most significantly predicted the presence of *A. californiense*. I hypothesized that macroinvertebrate family composition will be correlated to the presence or absence of *A. californiense* within pools according to their known dietary needs. Specifically, I expected that the presence and relative abundance of aquatic macroinvertebrate taxa preferred by the ambystomatids researched by Smith et al. (2004) and Denoël et al. (2006) would result in *A. californiense* presence, while the absence of these families within pools will result in *A. californiense* absence within those pools.

METHODS

Study system

This study was conducted on one preserve and one privately owned ranch in the eastern San Joaquin Valley: Stone Corral in Tulare County, California (Latitude 36º 26' 32.99" N; Longitude 119º 18' 29.57" W), and Lazy K Ranch in Madera County, California (Latitude 37º 09' 45.87" N; Longitude 120º 09' 26.62" W). Both ranches have upland grassland habitats characterized by a Mediterranean climate and contain perennial and seasonal pools formed by winter rains. Using a Trimble GPS unit, Vollmar Consulting had already delineated all vernal pools on the two sites and recorded presence or absence of *A. californiense* within pools in April 2010. This information was used to identify pools that did and did not support *A. californiense* and sample the macroinvertebrate communities within both classes of wetlands.

Data collection

I collected macroinvertebrates from vernal pools to assess macroinvertebrate composition. In May 2010 I sampled 22 vernal pools at the two sites, 11 from each ranch. Nine pools had *A. californiense* present, nine pools lacked *A. californiense*, and four pools lacked *A. californiense* but contained the salamander when Vollmar Consulting collected data in April. At each pool I took 20 standardized 1-meter long sweeps with a 500 µm dip net. I elutriated collected samples on-site with a 500 micron sieve. The collected macroinvertebrate samples were preserved in Nalgene containers with 95% ethanol. I measured water temperature, salinity, pH, and dissolved oxygen in each pool using a YSI 556 professional handheld multiparameter instrument (Korfel, Mitsch, Hetherington, & Mack, 2010). I measured turbidity using a Hach 2100 turbidity-meter. Pool perimeter and area was measured with a handheld Trimble GPS unit. Maximum pool depth was measured with a PVC pipe bearing hash marks at one-inch intervals, and emergent and submergent vegetation cover were assessed visually.

I selected 15 reference pools from the twenty sampled pools. In the laboratory, I randomly subsampled each reference pool's macroinvertebrate samples to a fixed count of 500 organisms and identified them to the taxonomic resolution of order, with additional resolution for Corixidae (family) and Notonectidae (family). Four samples were sent to EcoAnalysts, Inc. for analysis. I used a 30.5 x 35.5 cm Caton-type tray, divided into 5 x 5-cm sections, for subsampling. I sampled at least three of the 42 total 5 x 5-cm sections, and each of these was randomly subsampled using a 5-cm square Petri dish divided into 16 subsections (Carter & Fend, 2005).

Statistical analysis

Macroinvertebrate community composition

To determine whether macroinvertebrate communities and abiotic factors predict the presence of *A. californiense*, I used four statistical analyses. First, I conducted Welch two-sample t-tests on four abiotic factors (pH, dissolved oxygen, turbidity, conductivity) between *A. californiense*-present and *A. californiense*-absent pools (R, 2009; R Commander, 2009). I then used PC-ORD software to search for clustering based on the relative composition of macroinvertebrate communities for all 15 reference sites and to see what biotic and abiotic variables were associated with difference in macroinvertebrate community structure (McCune & Grace, 2002). I used non-metric multidimensional scaling (NMS) ordination with Sørenson/Bray Curtis distance measurements on log transformed relative abundance data. I used multi-response permutation procedures (MRPP) with Sørenson/Bray Curtis distance measurements of the ordination in order to test if there were differences between macroinvertebrate communities of ponds inhabited by *A. californiense* and ponds lacking *A. californiense* (McCune & Grace, 2002). Groups were defined by presence or absence of *A. californiense.*

RESULTS

Comparison of abiotic factors

Using Welch two-sample t-tests, I found that neither pH, dissolved oxygen, turbidity, nor conductivity were significantly different between pools inhabited by *A. californiense* and pools not inhabited by *A. californiense* (Table 1).

Table 1. Welch two-sample t-tests for four abiotic factors. None of the abiotic factors showed significant differences between *A. californiense* and non-*A. californiense* pools.

Factor	t	D. F.	p-value
pH	1.202	13.26	0.2504
DO %	0.5946	10.601	0.5646
Turbidity	-0.3183	13.517	0.7551
Conductivity	1.3983	7.83	0.2004

I conducted NMS with a Sørenson/Bray Curtis distance measure with two axes and 50 runs with real data. Stress and instability were low (8.03540 and < 0.0001, respectively). I found that conductivity, pH, and emergent vegetation were the three abiotic factors most associated with changes in the macroinvertebrate community (Table 2, Figure 1). MRPP results indicated that there was no difference between macroinvertebrate communities of ponds inhabited by *A. californiense* and ponds lacking *A. californiense*. The p-value was not significant (0.41), and *t*-value was very low (-1.00).

Table 2. Weighted NMS r-squared values for abiotic factors. The r-squared values for each factor were weighted to determine which factors are most associated with changes in the macroinvertebrate community. For each variable, values from Axis 1 were multiplied by the percent variance explained by Axis 1, and values from Axis 2 were multiplied by the percent variance explained by Axis 2. The products for each variable were then summed and divided by the total percent of variance explained by the ordination to determine association. Conductivity, pH, and emergent vegetation are most strongly associated with changes in the macroinvertebrate community.

Conductivity	0.058
pH	0.049
Emergent Vegetation %	0.049
Temperature	0.021
Area	0.017
Max Depth	0.008
Dissolved Oxygen %	0.008
Turbidity	0.008
Submergent Vegetation %	0.008

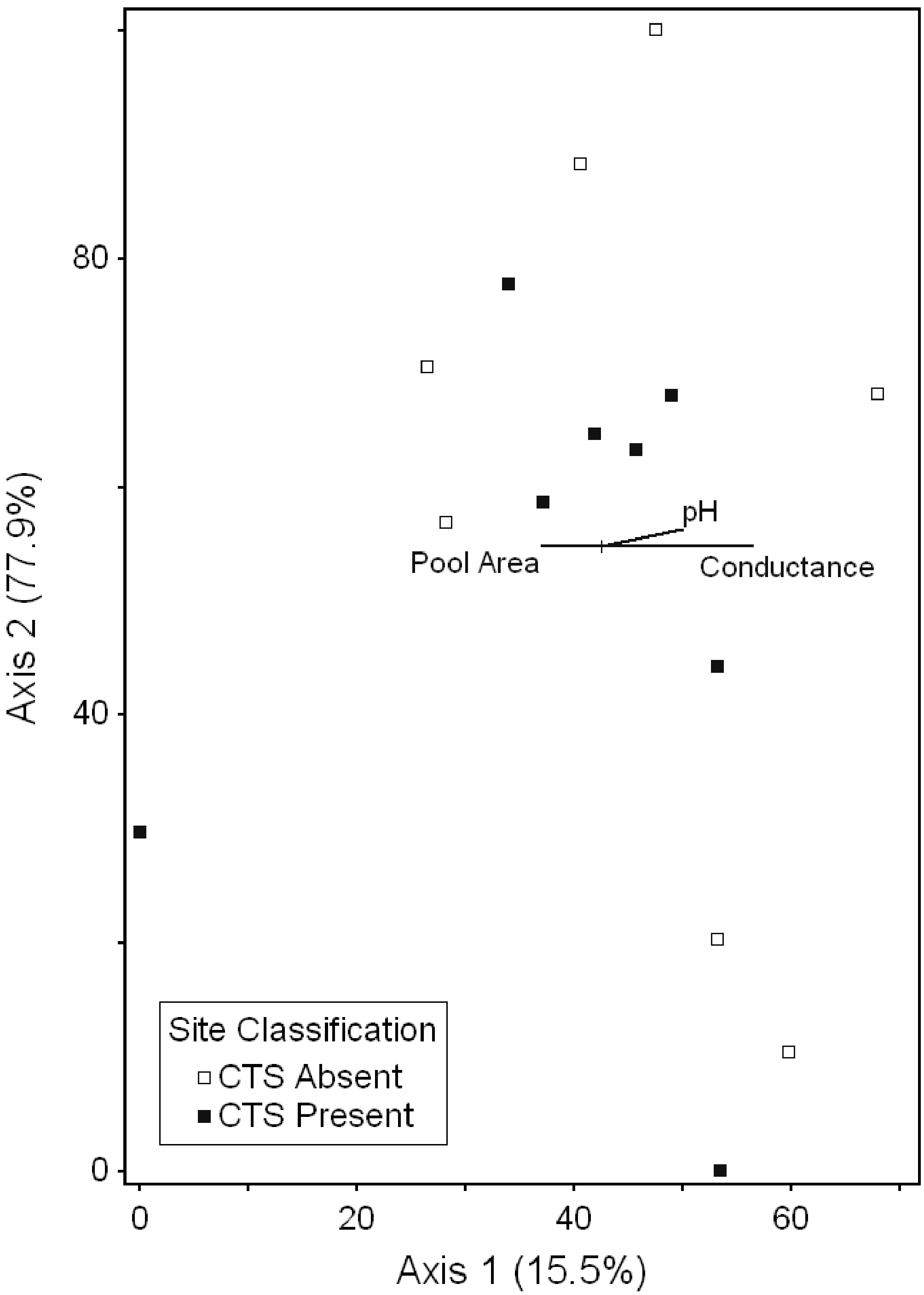

Figure 1. Stressor ranking and identification in NMS ordination of 15 sites (Final stress = 8.03, Instability = 0). Axes 1 and 2 explain 15.5% and 77.9% of the variability in the data. The lengths of vectors show the association with the macroinvertebrate community. MRPP showed no significant difference in macroinvertebrate communities between *A. californiense* present sites and *A. californiense* absent sites (t = -1.00, p = 0.41).

DISCUSSION

Predicting *A. californiense* presence of ponds could help predict suitable habitat for a cryptic species. This is especially valuable because even suitable ponds do not always support a breeding population. Therefore, I investigated whether physical habitat, water chemistry, and diet (macroinvertebrate community) were associated with *A. californiense* presence.

Welch two-sample t-tests indicated that neither pH, dissolved oxygen, turbidity, nor conductivity differed significantly between *A. californiense* present and *A. californiense* absent pools. Conductivity, pH, and emergent vegetation are the three abiotic factors most associated with changes in the macroinvertebrate community. Shaffer and Trenham (2005) state that larvae are sedentary on the bottom of wetlands when not feeding, and that larvae seek cover in vegetation when disturbed. This is in agreement with the NMS ordination result that emergent vegetation was a major variable correlated with *A. californiense* presence. Perhaps *A. californiense* adults preferentially select pools with greater amounts of vegetation.

It was surprising that dissolved oxygen content was not significantly different between pools with and pools without *A. californiense*. However, this is not necessarily an indication that this factor is unimportant. The results suggest that pools without *A. californiense* may have sufficient dissolved oxygen levels for *A. californiense* survival, while some other biotic or abiotic factor is the limiting agent.

Past studies have determined that *A. californiense* larvae feed primarily on cladocerans, copepods, ostracods, amphipods, and corixids (Anderson, 1968; Shaffer & Trenham, 2005). However, this study showed that macroinvertebrate composition had no significant association with *A. californiense* presence. One explanation for this discrepancy may be that *A. californiense* might have eaten preferred macroinvertebrates, and therefore I found less of those taxa. Or, perhaps *A. californiense* are feeding generalists and avoid being significantly impacted by variations in macroinvertebrate communities.

Limitations

This study met with several difficulties that may be addressed and fixed in future studies. The experimental design should be revised: because vernal pools are seasonal, the timing of data collection is critical (Holland & Jain, 1981). I collected my data in early May 2010; during this time, many of the pools on my study sites were dry or drying, reducing sample size. It is possible that late sampling affected the benthic macroinvertebrate compositions within each pool and impacted my results. If data had been collected at a more optimal point in time (e.g. February) then results may have more adequately addressed my hypothesis. My sampling methods were similar to Korfel et al. (2010), and they reported possible bias due to small sample size and data from only one season.

Another potential issue with the macroinvertebrate data was related to preservation. After I collected macroinvertebrate samples, I stored them for three months before beginning analysis. Because my samples contained too much mud, many specimens had decayed by the time I analyzed them; this may bias my results toward decay-resistant taxa.

Future directions

Because ecological systems are dynamic, it is possible that the macroinvertebrate communities within pools at the time of sampling had changed from their compositions at the time of *A. californiense* breeding and pond selection. The sampled macroinvertebrates may not accurately reflect the communities preferred by adult breeding *A. californiense*. To address this issue, I recommend sampling multiple times over the hydroperiod in order to detect changes in macroinvertebrate communities over time.

While this study analyzed how macroinvertebrtaes predict the selection of breeding ponds by *A. californiense*, future studies should focus on macroinvertebrate community changes over time within the hydroperiod of the pools. This would allow analysis of predator/prey relationship between *A. californiense* and benthic macroinvertebrates. Research should determine if *A. californiense* presence determines the invertebrate communities observed, or if the communities present determine the presence of *A. californiense*. My research has suggested that the macroinvertebrate community does not show significant associations with *A. californiense* presence. Future research may focus on water chemistry and other abiotic factors in amphibian-wetland interactions.

Broader implications

Knowledge of *A. californiense* habitat requirements is important in order to properly manage *A. californiense* habitat. This study suggests that benthic macroinvertebrates would not be good indicators for *A. californiense* suitable habitat. While it may be possible that *A. californiense* does not select breeding ponds based on macroinvertebrate community, I suggest further research before making this conclusion. Perhaps other biotic and abiotic factors have a stronger determination on pond selection. If evidence continues to suggest that benthic macroinvertebrate communities are not predicting of *A. californiense* pond use, then research resources should be allocated to investigating abiotic factors.

ACKNOWLEDGEMENTS

I would like to thank John Vollmar and the employees of Vollmar Natural Lands Consulting, Professor Vince Resh and Kevin Lunde of Resh laboratory for their invaluable help and support in pursuing my research. The tasks I undertook were both physically and mentally demanding, and I could not have completed this project without their guidance. I would also like to thank Patina Mendez, Lara Roman, Kurt Spreyer, and Seth Shonkoff for guiding me through sixteen months of learning to properly research, analyze, write, and rewrite in order to complete this paper.

REFERENCES

Alford, R. A., & Richards, S. J. (1999). Global Amphibian Declines: A Problem in Applied Ecology. *Annual Review of Ecology and Systematics, 30,* 133-165.

Anderson, J. D. (1968). A Comparison of the Food Habits of *Ambystoma macrodactylum sigillatum, Ambystoma macrodactylum croceum,* and *Ambystoma tigrinum californiense. Herpetologica, 24,* 273-284.

Barry, S. J., & Shaffer, H. B. (1994). The status of the California Tiger Salamander (*Ambystoma californiense*) at Lagunita: A 50-year Update. *Journal of Herpetology, 28,* 159-164.

Carter, J. L., & Fend, S. V. (2005). Setting limits: The development and use of factor-ceiling distributions for an urban assessment using macroinvertebrates. *Effects of Urbanization on Stream Ecosystems, 47*, 179-191.

Collins, J. P., & Storfer, A. (2003). Global amphibian declines: sorting the hypotheses. *Diversity and Distributions, 9,* 89-98.

Denoël, M., Whiteman, H. H., & Wissinger, S. A. (2006). Temporal Shift of Diet in Alternative Cannibalistic Morphs of the Tiger Salamander. *Biological Journal of the Linnean Society, 2006*, 373-382.

Fox, J., Andronic, L., Ash, M., Boye, T., Calza, S., Chang, A., Grosjean, P., Heiberger, R., Kerns, G. J., Lancelot, R., Lesnoff, M., Ligges, U., Messad, S., Maechler, M., Muenchen, R., Murdoch, D., Neuwirth, E., Putler, D., Ripley, B., Ristic, M., & Wolf, P. (2009). *Rcmdr: R Commander.* R package version 1.5-4. http://CRAN.R-project.org/package=Rcmdr

Korfel, C. A., Mitsch, W. J., Hetherington, T. E., & Mack, J. J. (2010). Hydrology, Physiochemistry, and Amphibians in Natural and Created Vernal Pool Wetlands. *Restoration Ecology, 18,* 843-854.

McCamman, J. (2010). A Status Review of the California Tiger Salamander. California Department of Fish and Game. Retrieved March 30, 2010 from http://www.dfg.ca.gov/wildlife/nongame/publications/docs/CTS-StatusEvaluation.pdf.

McCune, B., & Grace, J. B. (2002). *Analysis of ecological communities*. Gleneden Beach, OR: MjM Software Design.

Purrenhage, J. L., & Boone, M. D. (2009). Amphibian Community Response to Variation in Habitat Structure and Competitor Density. *Herpetologica, 65,* 14-30.

R Development Core Team (2009). R: A language and environment for statistical computing. *R Foundation for Statistical Computing, Vienna, Austria.* ISBN 3-900051-07-0. http://www.R-project.org.

Ryan, M. E., Johnson, J. R., & Fitzpatrick, B. M. (2009). Invasive hybrid tiger salamander genotypes impact native amphibians. *Proceedings of the National Academy of Sciences, 106,* 11166-11171.

Sacerdote, A. B., & King, R. B. (2009). Dissolved Oxygen Requirements for Hatching Success of Two Ambystomatid Salamanders in Restored Ephemeral Ponds. *The Society of Wetland Scientists, 29,* 1202-1213.

Shaffer, H. B., & Trenham, P. C. (2005). *Ambystoma californiense* Gray, 1853. In Michael Lannoo (Ed.), *Amphibian Declines: The Conservation Status of United States Species*, 605-608. Canada: University of California Press.

Smith, L. M., Gray, M. J., & Quarles, A. (2004). Diets of Newly Metamorphosed Amphibians in West Texas Playas. *The Southwestern Naturalist, 49*, 257-263.

Stuart, N. S., Chanson, J. S., Cox, N. A., Young, B. E., Rodrigues, A. S. L., Fischman, D. L., & Waller, R. W. (2004). Status and Trends of Amphibian Declines and Extinctions Worldwide. *Science, 306,* 1783-1786.

Trenham, P. C., Shaffer, H. B., Koenig, W. D., & Stromberg, M. R. (2000). Life History and Demographic Variation in the California Tiger Salamander (*Ambystoma californiense*). *Copeia, 2000,* 365-377.

Trenham, P.C., Koenig, W. D., & Shaffer, H. B. (2001). Spatially Autocorrelated Demography and Interpool Dispersal in the Salamander *Ambystoma californiense. Ecology, 82,* 3519-3530.

Trenham, P.C., & Shaffer, H. B. (2005). Amphibian Upland Habitat Use and its Consequences for Population Viability. *Ecological Applications, 15,* 1158-1168.

U.S. Fish and Wildlife Service. (2009). Species Account, California tiger salamander (*Ambystoma californiense*). Retrieved from http://www.fws.gov/sacramento/es/animal_spp_acct/california_tiger_salamander.pdf

Vollmar, J. (2009). Proposal to Conduct Breeding, Movement and Population Dynamics Studies in the Eastern Central Population of the California Tiger Salamander (*Ambystoma californiense*). Prepared by Vollmar Consulting, Berkeley, CA, for Department of the Interior Bureau of Reclamation, U.S. Fish and Wildlife Service.