A Comparison of eDNA sampling Methodology to Dipnet sampling of the California Tiger Salamander (*Ambystoma californiense*) in Vernal Pools of the Santa Rosa Plain

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INTRODUCTION

Can we detect presence and absence of California Tiger Salamander (CTS) via environmental DNA (eDNA)?

The California Tiger Salamander (*Ambystoma californiense*) is an endangered species that reproduces annually in vernal pools. Detection of species using eDNA methods, rather than directly using the invasive net dip sampling method, can reduce impacts on sensitive species and increase the power of field surveys for rare and elusive species and aid in conservation (U.S. Fish and wildlife Service, 2017)

METHODS

SAMPLE COLLECTION → FILTRATION → EXTRACTION

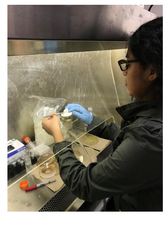
SAMPLE COLLECTION

- Multiple ponds were sampled from four preserves in Sonoma County
- 1L water samples taken from each pond
- Prior to collection: water bottles washed with 50% bleach and rinsed 3x's with DIH_2O
- Sterile Plastic booties and gloves were donned at each site to avoid contamination between pools
- A 500mL control sample of DIH_2O was taken at each pond

Larval CTS

FILTRATION

- Filtration was conducted within 24 hours of collection
- Mixed cellulose filter: acetate and nitrate
  - pore size: 3 microns
  - diameter: 47 mm
- Filtration time and volume was recorded
- Negative control sample was processed the same time as the test samples
- Membranes were transferred into 1mL of ethanol for preservation

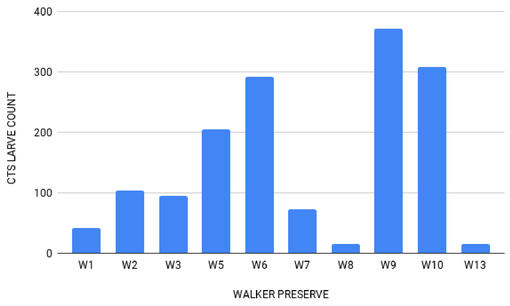


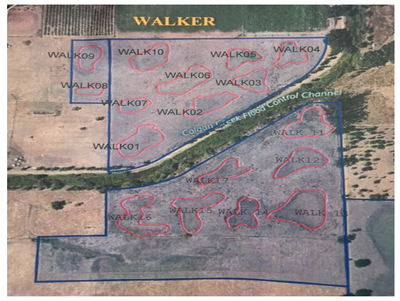
EXTRACTION

- eDNA Extraction from Filters (stored in ethanol) using the Qiagen DNeasy Blood and Tissue kit
- Filters were air dried
- An extraction control was performed to ensure clean protocols
- Stored in refrigerator until PCR analysis

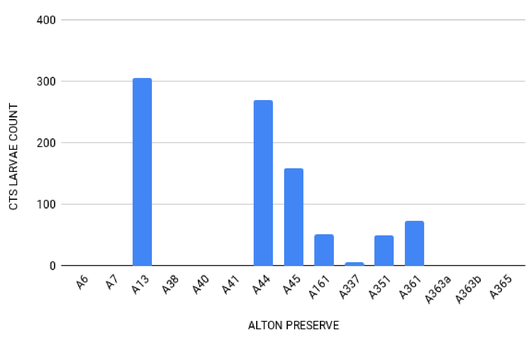
RESULTS

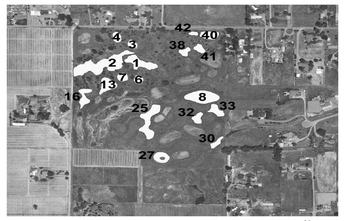
CTS Larval Dip-net counts

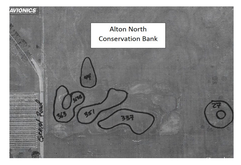




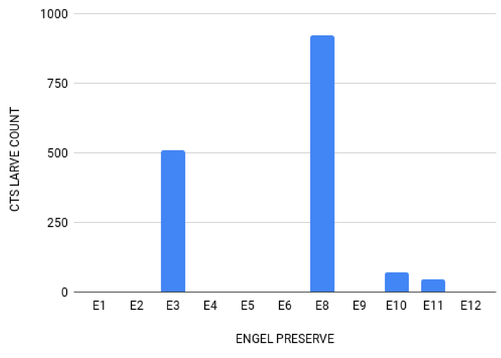
Walker Preserve

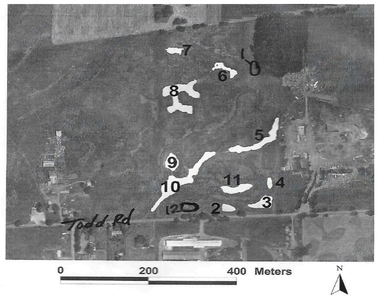




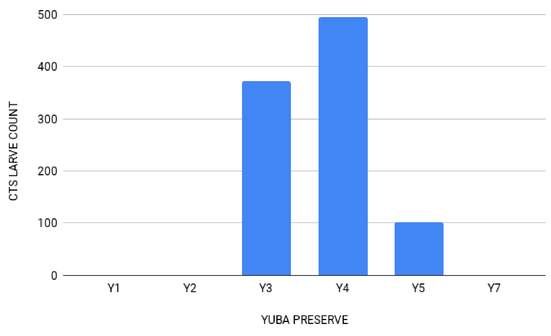


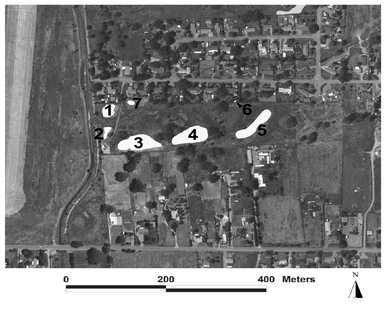
Alton Preserve





Engel Preserve





Yuba Preserve

Frog Song Vernal Pool, Cotati CA

DISCUSSION

To date, we have effectively collected eDNA samples from 34 pools amongst 4 preserves in the Santa Rosa Plain. We have used sterile techniques to transfer these samples to filters in the lab for preservation and future analyses. We have used standard techniques to extract DNA from these filters and from a known CTS tissue sample to use as a positive control. Using primers designed by Dr. Caren Goldberg's lab at Washington State University, we have begun PCR analysis which will continue in order to evaluate the effectiveness of the qPCR approach. This innovative survey methodology can count and conserve CTS most efficiently by shortening field time, eliminating CTS stress, and minimizing disturbance of vernal pool habitats.

ONGOING ANALYSES

To complete this project, we are analyzing extracted eDNA samples with qPCR to determine the concentration of target species DNA in water samples by creating a standard curve and estimating relative levels of initial DNA. We expect to obtain eDNA amplification in the places where larvae were found via dip-netting. In addition, by using a qPCR approach we may be able to determine that the concentration of eDNA parallels the results of dip-netting survey results.

CTS Sequence

Forward Primer

GACCAGATCTGAGGACTTTTATTGTAGAGTGCCTTACTTCCCTTG
AGGCGCCACTGGTTAAAATCTATGGGCACGG
CTTGAAGACTCATTCATCAATTGGATCGAACGGGTACCTGGCGGC
TGC **Reverse Primer**

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