Detection of California tiger salamanders (*Ambystoma californiense*): comparing eDNA and traditional net-based survey methods

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Introduction

Figure 1. California tiger salamander adult.
PC: C. Wyckoff

Figure 2. California red-legged frog adult.
PC: A. Calhoun

The California tiger salamander (*Ambystoma californiense*, CTS) is a cryptic fossorial species that faces multiple threats: habitat destruction, hybridization with invasive barred-salamanders (*Ambystoma mavortium*), and climate change. This study compares the sensitivity of CTS environmental-DNA (eDNA) detection to traditional net-based surveys, patterns of eDNA signal intensity, seasonality of CTS detection, and the relationship between aquatic vegetation and net-based detection. Field surveys were conducted on the Santa Lucia Preserve and Palo Corona Regional Park, using an integrated technique of visual surveys, net-based surveys, and eDNA sampling to assess perennial stock ponds for the presence of CTS. The eDNA experimental design includes a negative control (known CTS-negative pond), three known positive controls (reliable CTS-positive ponds), several ponds of unknown status, and uses detection of California red-legged frogs (*Rana draytonii*, CRLF) as a proxy for eDNA sensitivity. Preliminary results show increased sensitivity of eDNA compared to net-based surveys. Application of these methods to other sites could increase confidence in presence-absence detection, reduce sampling efforts and impacts, and improve conservation protections of vulnerable habitats.

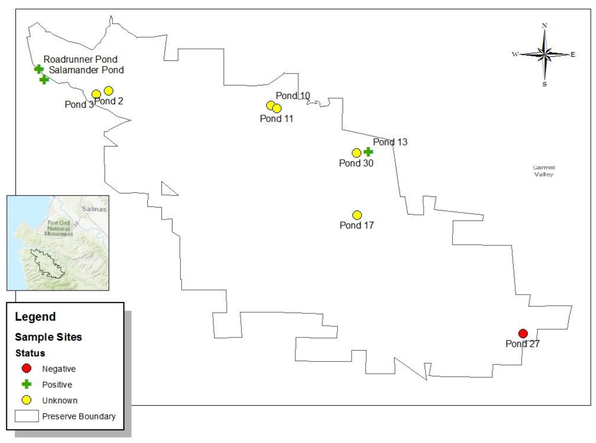

Figure 3. Map of Santa Lucia Preserve and Palo Corona Ranch Regional Park ponds.

Methods

Field monitoring and sampling included a combination of survey methods to measure sensitivity and specificity of the assay:

- Visual & vegetation surveys
  - Recorded emergent & surrounding vegetation percentages
  - Counted herpetofauna eggs, larvae, and adults

Methods cont.

- Net-based surveys
  - Sampled for 30 minutes or until pond was fully seined
  - Counted herpetofauna captured in nets

Figure 4. Santa Lucia Conservancy staff seining Palo Corona pond.

Figure 5. Dr. Wyckoff using a dip-net at Pond 3.

- eDNA sampling & processing
  - Goldberg Lab's (Washington State University) protocol was utilized
  - Longitudinal study with bi-weekly sampling occurred for 2 years
  - Pond 27 was sampled monthly as a negative control
  - Cross-contamination was prevented by single-use equipment
  - 250mL of water from each pond was filtered through a 5.0 micron polyethersulfone membrane filter

Figure 6. Sample collection at Pond 30.
PC: C. Wyckoff

Figure 7. On-site eDNA filtration equipment.
PC: C. Wyckoff

- Quantitative polymerase chain reaction (qPCR)
  - Filtered DNA was tested at Goldberg Lab using qPCR amplification and visualization techniques

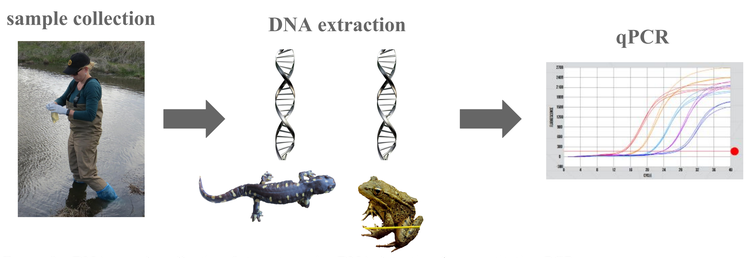

Figure 8. eDNA sample collection & target species DNA detection for quantitative-PCR.
PC: C. Wyckoff

Results & Discussion

- eDNA demonstrated higher sensitivity in detection of CTS than traditional net-based surveys
  - All 3 positive controls were confirmed positive for CTS with eDNA
  - eDNA CTS present at 3 of the 6 unknown status ponds when net surveys determined species absence
  - Our negative control stayed constant throughout the study

Results & Discussion cont.

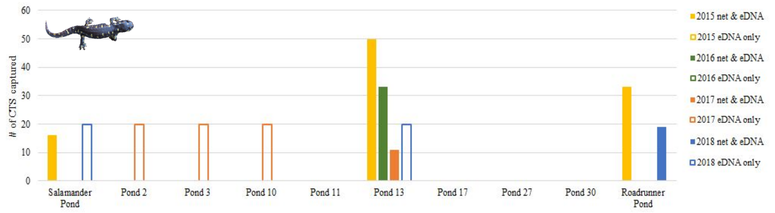

Figure 9. Comparison of method sensitivities for detecting CTS.

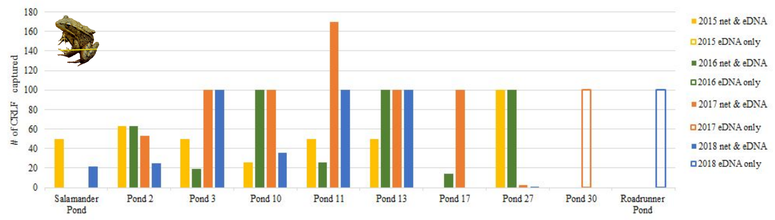

Figure 10. Comparison of method sensitivities for detecting CRLF.

- CTS signal intensity shows strong correlation with increases in rainfall

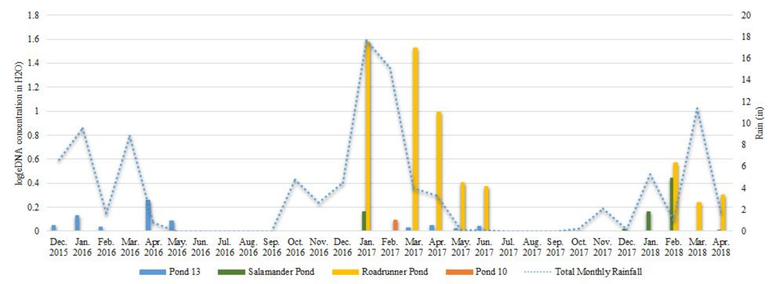

Figure 11. CTS eDNA concentration in H2O and rainfall comparison.

- Correlation of increased aquatic vegetation affecting net-based survey detection success

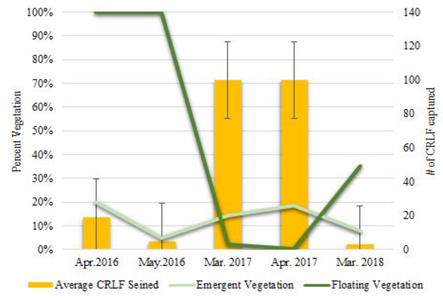

Figure 12. Preliminary analysis for Pond 3.

Figure 13. Pond 11 with heavy vegetation.
PC: C. Wyckoff

Future Work

- Calculate sensitivity & specificity for both methods
- Publish results in a peer-reviewed journal
- Improve management plan for CTS on the Santa Lucia Preserve
- Share & collaborate with other agencies to improve CTS detection statewide

Acknowledgments

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