Development of eDNA tools for California Tiger Salamanders (*Ambystoma californiense*) in Vernal Pools on the Santa Rosa Plain

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Background

- The California tiger salamander (CTS), *Ambystoma californiense*, is an endangered species of mole salamander
- Reproduces in ephemeral vernal pool habitat on the Santa Rosa plain.
- Dipnet sampling traditionally used for surveying populations
- Environmental DNA (eDNA) sampling is an emerging and less invasive method of detecting species presence from water or soil samples.
- We sought to design molecular markers that could be used to detect CTS in water samples taken from ponds.

Figure 1. a) A vernal being sampled via dip-netting on the Santa Rosa plain and b) a California tiger salamander larva.

Methods

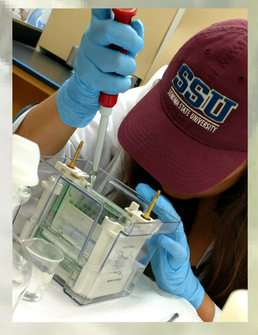
- DNA was extracted from a known sample using a Qiagen DNeasy Blood and Tissue Kit
- DNA (10ul or 5ul of extratcted sample) was also added to 50ml of water and then filtered through a 25mm glass fiber semipermeable membrane w/ a vacuum
- Variety of PCR reaction conditions were tested varying MgCl2, annealing temperature, extension time, and bovine serum albumin use were tested.
- PCR reaction results initially run a 2% agarose gel until consistent appearance of products occurred
- Product size was estimated using 10% polyacrylamide gels with a 100 base pair standardized ladder (Fisher)

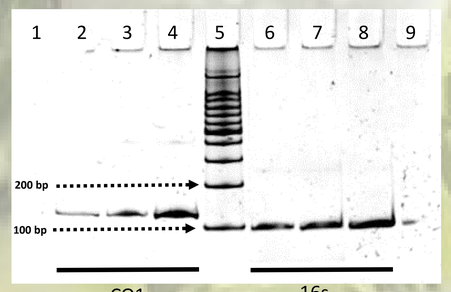
Primer Design

- Primers were designed for the cytochrome oxidase I (COI) gene and the 16s rRNA gene.
- Sequences were compared from the NCBI database for multiple populations of CTS and closely related species in the genus *Ambystoma*.
- Candidate sites for primer design were selected for one or more mutations that distinguished CTS from other species.
- Final primer design conducted using Primer3 software following standard optimization for thermal stability, dimerization, and binding affinity.
- Final primer sets chosen were selected to amplify products of 130 base pairs for COI and 99 base pairs for 16s rRNA, which could allow for detection of small fragments of DNA.

Preliminary Results

- COI primers produced products of approximately 130 bp in size from extracted tissue as well as from diluted and filtered DNA.
- 16s rRNA primers produced products of approximately 99 bp in size from extracted tissue as well as from diluted and filtered DNA.





- Figure 3. a) SSU student Jazmin Morales loads a polyacrylamide gel and b) PCR products on the gel with COI in lanes 2-4, 100 bp ladder in lane 5 and 16s rRNA in lanes 6-8.

16s sequence

CCTGGATTACTCCGGTCTGAACTCAGATCACGTAGGACTTTAATCG
TTGAACAAACGAACCTTTAATAGCTGCTGCACC**ATTTGGGTGTCCT**
GATCCAA

CO1 sequence

TGTTGACACACGGGCATATTTTACATCCGCTACAATAATTATTGCCA
TTCCAACTGGGGTAAAAGTATTTAGCTGATTAGCAACTATACACGG
AGGGGCAATTAA**ATGAGATGCAGCAATACTATGGGCC**

- Figure 2. Depicts the known CTS sequences associated with each primer set for COI and 16s rRNA. Primer sequences are indicated in bold.

Discussion

- Designed primers consistently amplified products that had the expected sizes for both COI and 16s rRNA, suggesting a possible positive result of detection
- Amplification of DNA diluted in water, then filtered and extracted from filters resulted in appropriate sized PCR products suggesting that filtration method may be appropriate.

Additional Work Needed

- Prior to using these primers for eDNA samples, DNA sequencing products is necessary to demonstrate that product is CTS.
- In addition, the primers need to be run with DNA from other taxa known to occur in the vernal pool system to ensure that they are species specific
- If species specificity is confirmed, eDNA samples that were collected from Alton and Engel preserves in Santa Rosa, CA can be filtered and amplified to detect presence of CTS in each pool.
- Results of eDNA screening can be compared to dip net sampling results taken at the same time as eDNA sampling.

Acknowledgements

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