The Viability of using eDNA to Detect the Presence of Species in a Warm Water Ecosystem

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Abstract

One of the fundamental issues in biology is detection to study organisms. One method for detecting the presence of organisms involves examining environmental DNA (eDNA) samples, the DNA left behind by an organism living in a specific environment. In order to test the effectiveness of this sampling approach, we designed molecular tools for species identification of two common species, the Bullfrog (*Lithobates catesbeianus*) and the non-native crayfish (*Precambarus clarkii*). Swamp Crayfish (*Procambarus clarkii*). We developed new molecular tools to be used on both species. We tested these tools on water samples obtained from different regions from the Colusa National Wildlife Refuge (Figure 1). We used filtration, DNA extraction, polymerase chain reaction, and agarose gel electrophoresis to determine if the molecular marker system, based on DNA bar-coding regions cytochrome oxidase I was a viable option for sampling presence/absence of these species in the Colusa region.

Background

- eDNA methodology assumes that species can be detected from environmental samples such as water samples or soil samples.
- eDNA can be less invasive, more sensitive, less expensive and more time efficient than traditional survey methods (Deiner et al. 2015).
- Bullfrogs and Red Swamp Crayfish are invasive species that can impact native species found in the Colusa National Wildlife Refuge through predation and competition.

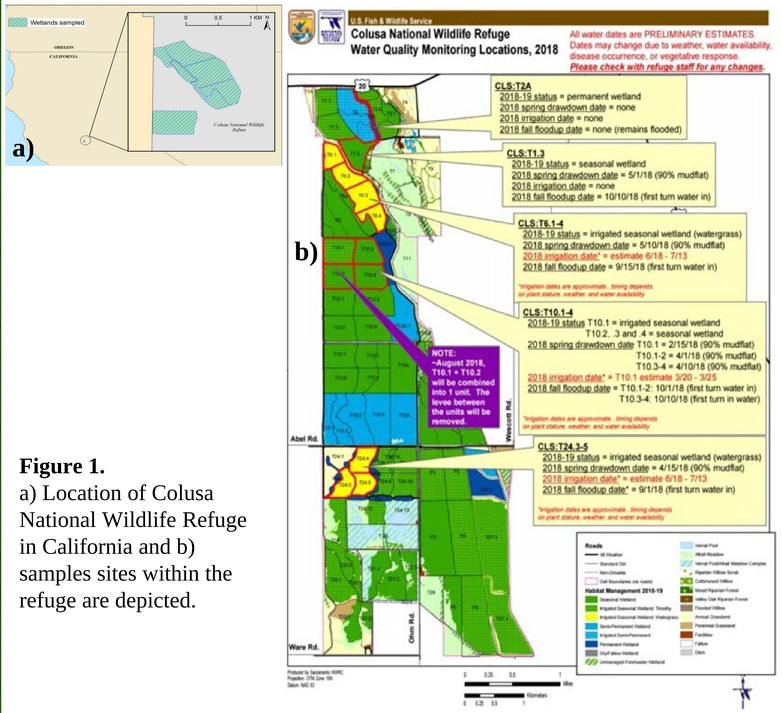

Figure 1.
a) Location of Colusa National Wildlife Refuge in California and b) samples sites within the refuge are depicted.

Objectives

- Develop genetic barcoding markers from the cytochrome oxidase I gene that identify presence of invasive bullfrog (*L. catasbeianus)* and crawfish (*P. clarkii*).
- Determine if DNA can be detected from environmental DNA samples using newly developed marker.

Methods

- **Water Sample collection:** Collected 41 water samples from Colusa County, California (1L in total per sample)
- **Water sample filtration:** DNA from water samples was filtered through a 25mm glass fiber membrane w/ a vacuum pump
- **DNA extraction**: DNA was extracted from filter paper w/ a Qiagen DNeasy Blood and Tissue Kit
- **Optimization:** Custom primers (Figure 3 and 4) were designed for the bullfrog & crayfish using DNA sequence data from GenBank with primer design software primer 3. We used a Target sequence length less than 100bp to target potentially fragmented DNA.
- **Polymerase chain reaction (PCR):** 4 ul of extracted DNA sample was added to a 25ul reaction containing 10mM of each primer, 10mM of dNTP. An optimal Thermocyler protocol was determined through experimentation and an annealing temperature of 55C was used.
- **Gelectrophoresis:** PCR product was run in either a 2% agarose gel or a 10% polyacrylamide gel to visualize PCR results.

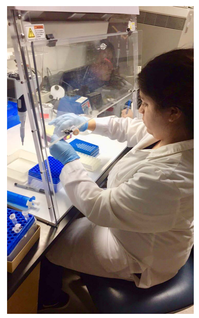

Figure 2. Shows a) SSU student, Jessica Pena, conducting polymerase chain reaction experiment in the SSU Core DNA Facility and b) SSU students, Veronica Nabor, Laura Perez, and Jessica Pena, running gel electrophoresis.

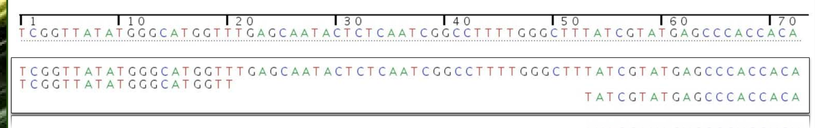

Figure 3. DNA sequence of *L. catesbeianus* F and R primers designed for a product of 72 bp from the cytochrome oxidase I locus

Figure 4. DNA sequence of *P. clarkii* F and R primers designed for a product of 63 bp from the cytochrome oxidase I locus

Results

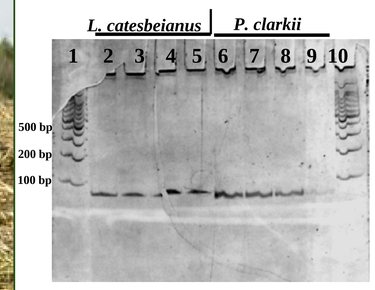

Figure 5. Polyacylamide gel of amplified DNA from bullfrogs and invasive crawfish. Lanes 2-5 include 3 environmental samples and one positive control (lane 5) for *L. catesbeianus.* Lanes 6-9 include 1 positive control (lane 6) and three environmental samples from *P. clarkii. Lanes 1 & 10 are 100 bp ladder*

- All 41 environmental DNA samples produced PCR products of equivalent size to positive controls for each species examined
- Products using primers designed for *L. catesbeianus* were the expected 72 bp in size.
- Products using primers designed for *P. clarkii* were the expected 63 bp in size.

Figure 6. shows a) invasive *L. catesbeianus* (bullfrog) and b) *P. clarkii* (non-native crawfish)

Discussion

- Using environmental DNA from water samples we detected the presence of DNA in those samples.
- In both species, the correct product size was produced based on primers designed from the COI region.
- If the markers turnout to be species specific, then all 41 ponds appear to harbor evidence of presence of both bullfrogs and crawfish.
- Additional analyses are needed to confirm that the products produced are indeed strictly related to the targeted species (via DNA sequencing and showing negative results using DNA from other species known to occur.

References

Deiner, K., Walser, JC., Machler, E. et. al, (2014) Choice of capture and extraction methods affects detection of freshwater biodiversity from environmental DNA. Elsevier 183, 53-63.

Acknowledgements

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