

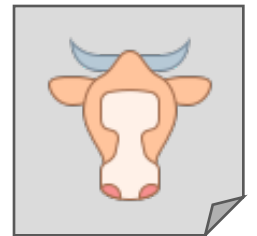
Using environmental DNA to monitor vernal pool organisms in California

Shannon Rose Kieran, Joshua Hull, Kristy Deiner, Amanda Finger

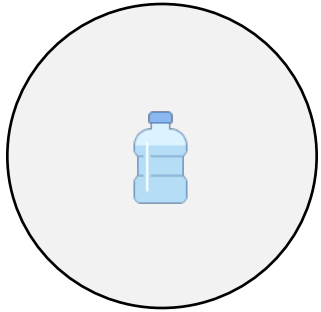
University of California, Davis

March 21, 2018

Environmental DNA is a new sampling technique



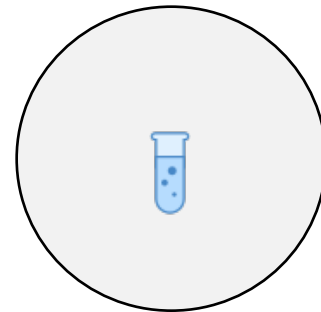
The four steps of eDNA analysis



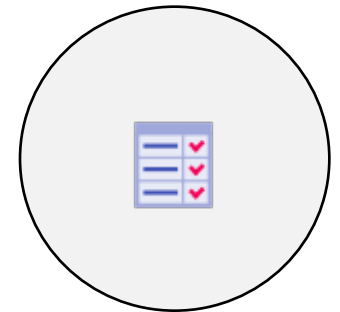
collection



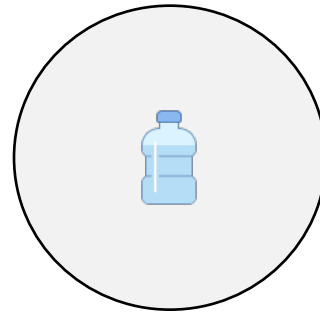
filtration



extraction



analysis



collection

Genetic material is left behind in the environment from an organism's normal activity



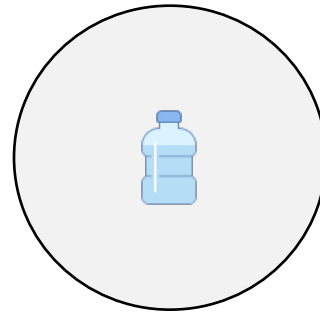
All organisms in an environment will have genetic material represented in an eDNA sample



Activities like predation, reproduction, movement, injury, and shedding all release DNA



Plant, animal, and bacterial DNA are all present



collection

Many environments can be sampled for eDNA, but water is the most common

water



soil

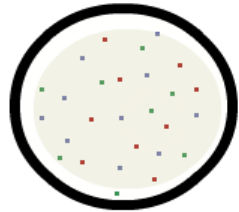
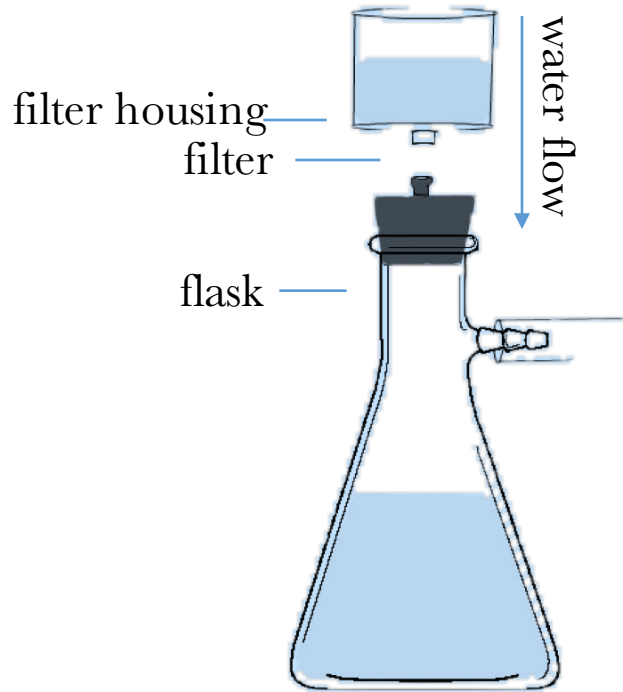


ice





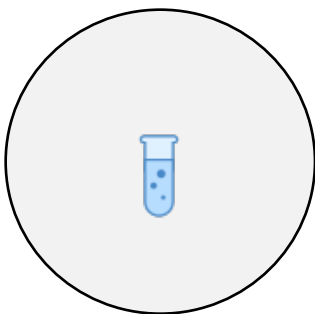
filtration



cellulose nitrate filter

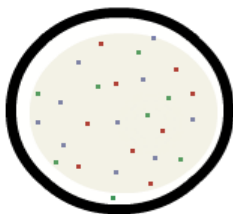
*0.45 μm pore size
47 mm diameter*



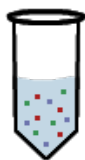


extraction

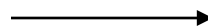
Extraction is carried out in a clean laboratory, but in most other ways the eDNA sample is treated like any other



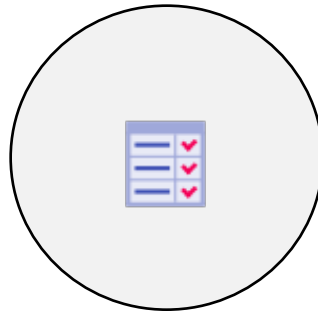
cellulose nitrate filter



process with
Qiagen DNeasy
Blood and Tissue Kit

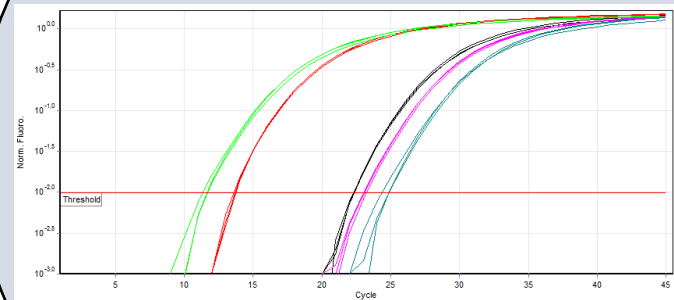


Treat for inhibition
with the Zymo One-
Step
PCR Inhibitor
Removal Kit



analysis

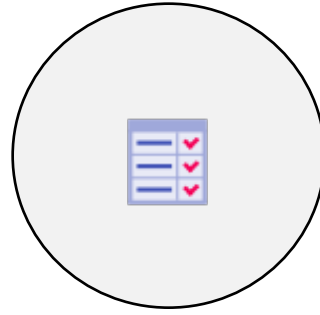
There are two common ways to analyze eDNA samples: Single-species targeted qPCR assays and multi-species community metabarcoding



qPCR analysis results

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.....
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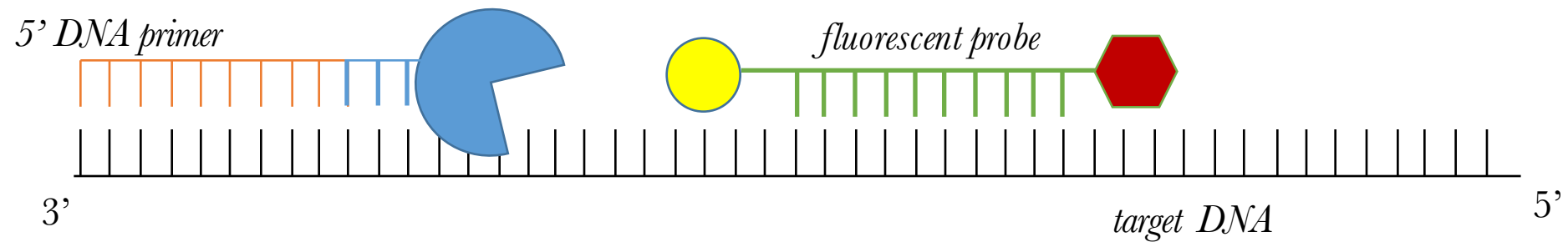
Metabarcoding results



analysis

Single-species qPCR assays test for the presence of one or a few species in an environment

Two PCR primers and a fluorescent probe combine to create a specific, sensitive test that can pick a target sequence out of an eDNA sample and amplify it



Testing eDNA in California's vernal pools

Self-contained, still water

Many species of interest to conservationists

Large ongoing management and monitoring already underway



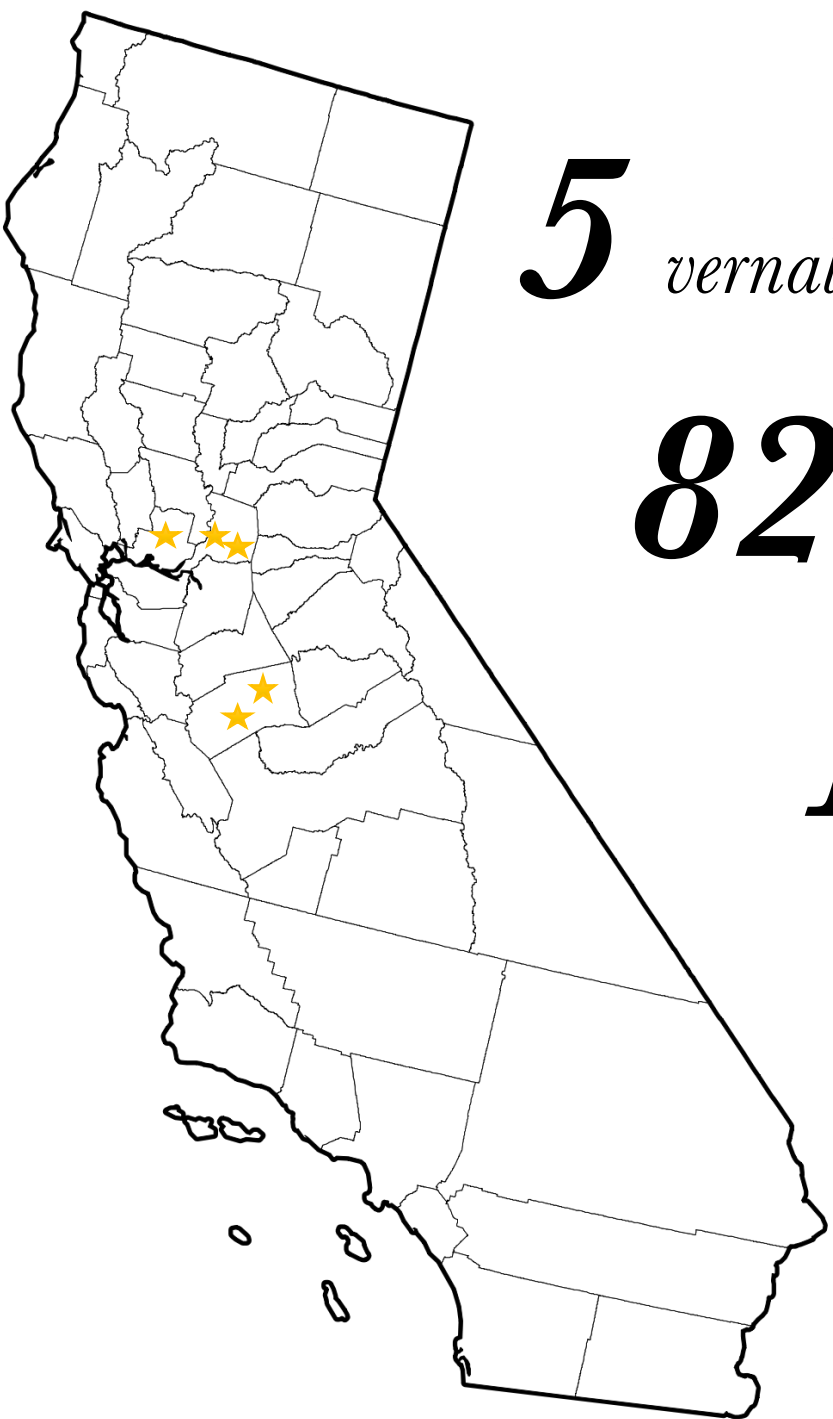
Vernal pool tadpole shrimp
Lepidurus packardii



California Tiger Salamander
Ambystoma californiense



Vernal Pool Fairy Shrimp
Branchinecta lynchi

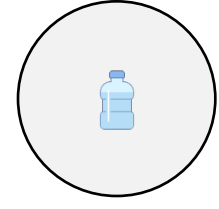


5 *vernal pool complexes*

82 *vernal pools sampled at least once*

130 *eDNA & dip-net sampling events*

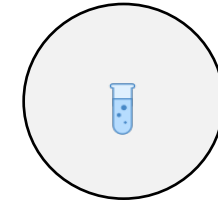
520 *filters processed and analyzed*



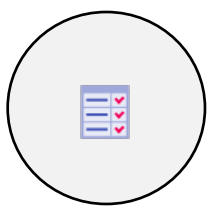
collection



filtration



extraction



analysis

5 Single species qPCR assays &

Multispecies metabarcoding at the cytochrome oxidase I gene

Initial results suggest:

100% detection of California Tiger Salamander when larvae are present

84% detection of California Tiger Salamander before larvae hatch

96% detection of Vernal Pool Tadpole Shrimp while they swim

75% detection of Vernal Pool Tadpole Shrimp after they die off, before dry-down

Acknowledgements

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