Development and evaluation of an environmental DNA (eDNA) protocol to monitor wild delta smelt

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Environmental DNA (eDNA)

• Genetic methods for detecting species in water or soil
• Does not directly sample the organism
• Species-specific and community approaches

Photos: DW Gotshall (green sturgeon), J Katz (Chinook salmon), DWR (Delta smelt)
Environmental DNA (eDNA)

- Genetic methods for detecting species in water or soil
- Does not directly sample the organism
- **Species-specific** approach:
  - Advantages for monitoring
    - Cost per sample: lower
    - Processing time: shorter

Photo: DWR (Delta smelt)
eDNA: Monitoring application

Surveying rare or cryptic species with traditional methods requires substantial field effort.

Surveying Europe’s Only Cave-Dwelling Chordate Species (Proteus anguinus) Using Environmental DNA

Environmental DNA from Seawater Samples Correlate with Trawl Catches of Subarctic, Deepwater Fishes

An eDNA Assay to Monitor a Globally Invasive Fish Species from Flowing Freshwater

eDNA: Advantages

- Detect rare/cryptic species\(^1,2,3\)
- No morphological identification
- No sampling-related mortality
- Less disturbance of habitat/populations\(^4\)
- Ability to sample sites not accessible by trawl
- Same gear for all life stages
- Large-scale implementation

eDNA: Fish detection

- Fish shed: mucus, waste, skin, scales
  - Cellular
  - Extracellular
- Particulates filtered from water
- Total DNA extracted from sample
- Delta smelt eDNA detected using quantitative PCR (qPCR) assay\textsuperscript{5}

\textsuperscript{5} Baerwald et al. 2011

Photo: DWR
Field sampling

Collect water ➔ Filter ➔ Extract DNA from filter

3 x 1L per tow
eDNA + negative controls
What can go wrong?

• Low detection rate / no detection
  • Optimize assay for sensitivity
  • Understand impact of environmental factors (e.g. variable flows, turbidity)
• Contamination
  • Manage contamination in the field (right) and cross-contamination between samples\textsuperscript{6}

Field sampling for fish (top) and eDNA (left) in the San Francisco Estuary with USFWS

\textsuperscript{6} Brandl et al. 2014
Delta Smelt detection using eDNA: Protocol considerations

- Detection method: species specific
  Delta smelt qPCR assay

- Detection protocol (risk of false negatives)
  Current project

- Contamination (risk of false positives)
  Current project
Detection using eDNA: Protocol considerations

• Detection method: species specific

• Detection protocol (risk of false negatives)

• Contamination (risk of false positives)
  • Identify steps most likely to cause contamination
  • Field methods, equipment
  • Infrastructure: clean lab
  • Negative controls

Field sampling with USFWS
Protocol development

- DNA dilutions
- Tank experiments
- Field experiments
- Field sampling

More control  More realistic
Protocol development

- Serial DNA dilutions **2016-2017**
  - Consistent detection at delta smelt DNA concentration of 0.1 pg/µl
- Tank experiments at Fish Conservation and Culture Lab (FCCL) *Upcoming*
  - eDNA shed rate
  - eDNA decay rate

More control

DNA dilutions

Tank experiments
Protocol development

- Field Experiment: **May 2017**
  - How far away is eDNA detectable?
  - How long is eDNA detectable?
- Field Sampling: **Jan-Feb 2017**
  - Concurrent with USFWS Enhanced Delta Smelt Monitoring (EDSM)
Field sampling with EDSM (USFWS)

Delta smelt catch

- LSR13
- SMB53
- HB52
- SBM62
- HB6
- RV3
- RV51

Legend:
- 0
- 1
- 2
- 3
- 8
eDNA detection

Threshold for detection
eDNA detection

Threshold for detection

Delta Smelt DNA not detected in eDNA samples
eDNA detection

Delta Smelt DNA not detected in eDNA samples
eDNA detection

Delta smelt DNA standard from fish tissue (1 ng/µl)

Delta Smelt DNA standard from fish tissue (0.1 pg/µl)

Threshold for detection

Delta Smelt DNA not detected in eDNA samples
eDNA detection

Delta smelt DNA standard from fish tissue (1 ng/µl)

Delta Smelt DNA detected in eDNA sample

Delta Smelt DNA not detected in eDNA samples

Threshold for detection

RFU (10^-3)

Cycles

Amplification
Summary

- Species-specific approach
- Significant advantages for monitoring of Delta smelt
- Maximize detection, minimize contamination
- Experiments and field sampling
- Challenges and limitations
- Promising preliminary data
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Field sampling with USFWS