



UCDAVIS
ANIMAL SCIENCE



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

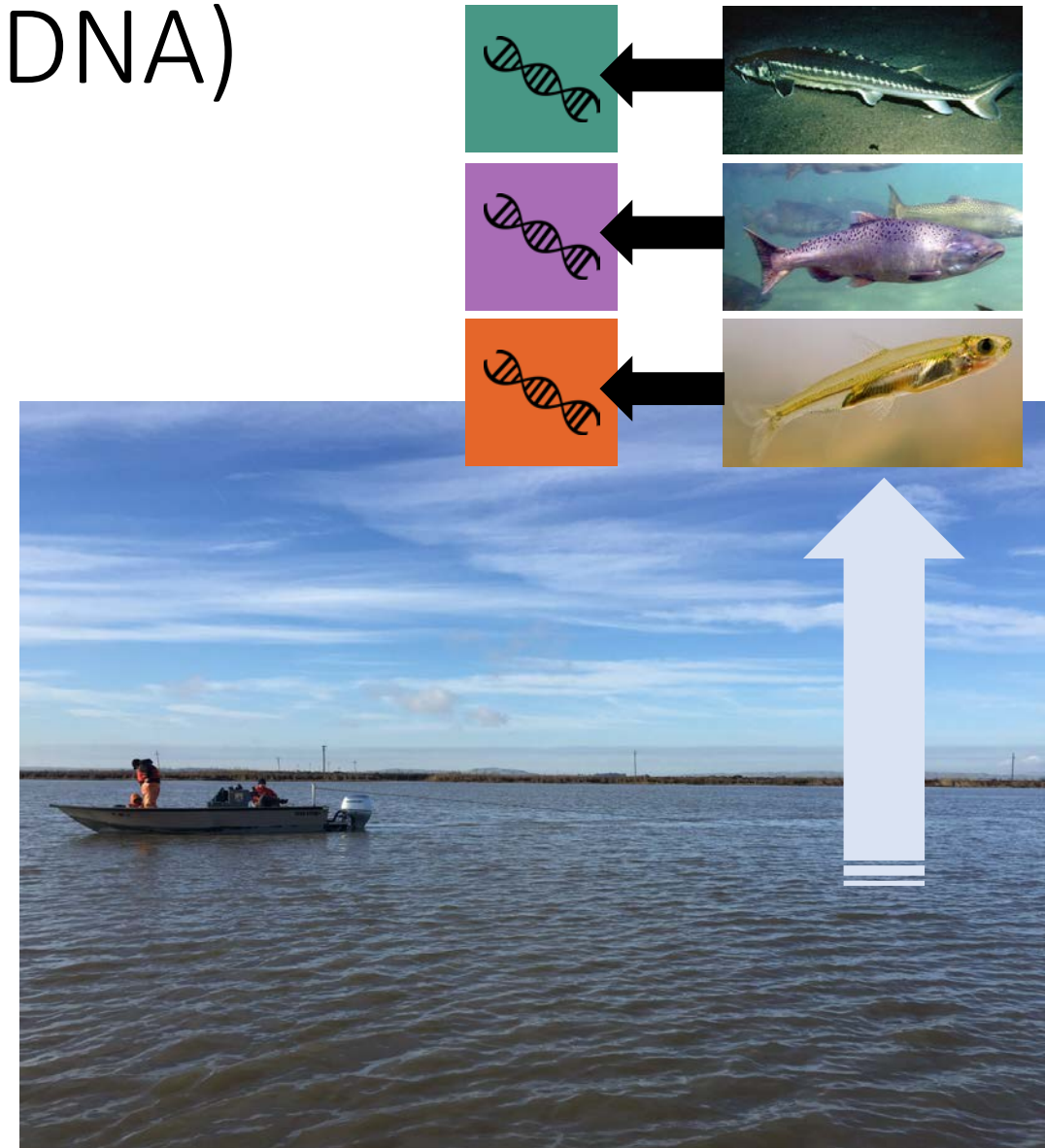
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¹UC Davis Genomic Variation Lab (GVL)

²California Department of Water Resources (DWR)

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**

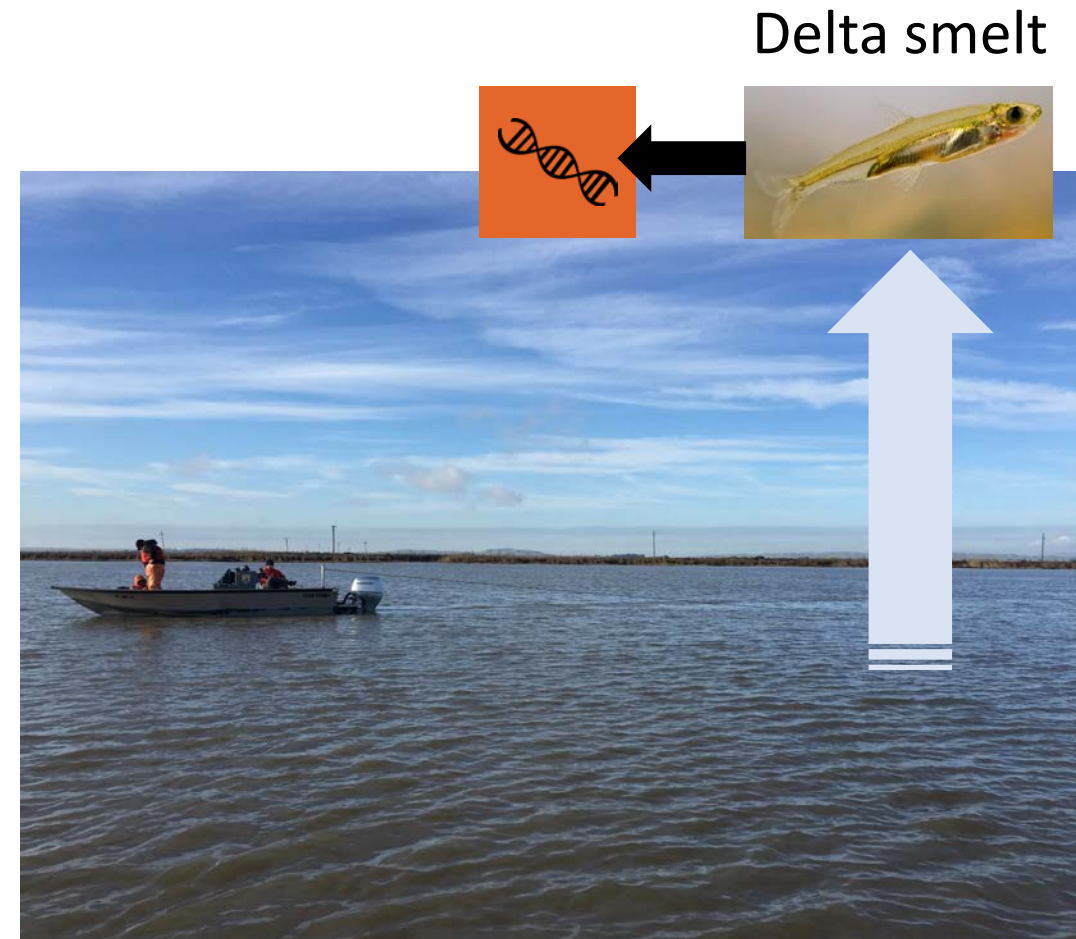


Photo: DWR (Delta smelt)

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

Judit Vörös^{1,2*}, Orsolya Márton³, Benedikt R. Schmidt^{4,5}, Júlia Tünde Gál¹, Dušan Jelić⁶



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

Philip Francis Thomsen^{1*}, Peter Rask Møller², Eva Egelyng Sigsgaard², Steen Wilhelm Knudsen², Ole Ankjær Jørgensen^{3,4}, Eske Willerslev^{1,5,6*}



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

Irene Adrian-Kalchhauser^{1*}, Patricia Burkhardt-Holm^{1,2}

eDNA: Advantages



Photo: USFWS

- Detect rare/cryptic species^{1,2,3}
- No morphological identification
- No sampling-related mortality
- Less disturbance of habitat/populations⁴
- Ability to sample sites not accessible by trawl
- Same gear for all life stages
- Large-scale implementation

eDNA: Fish detection

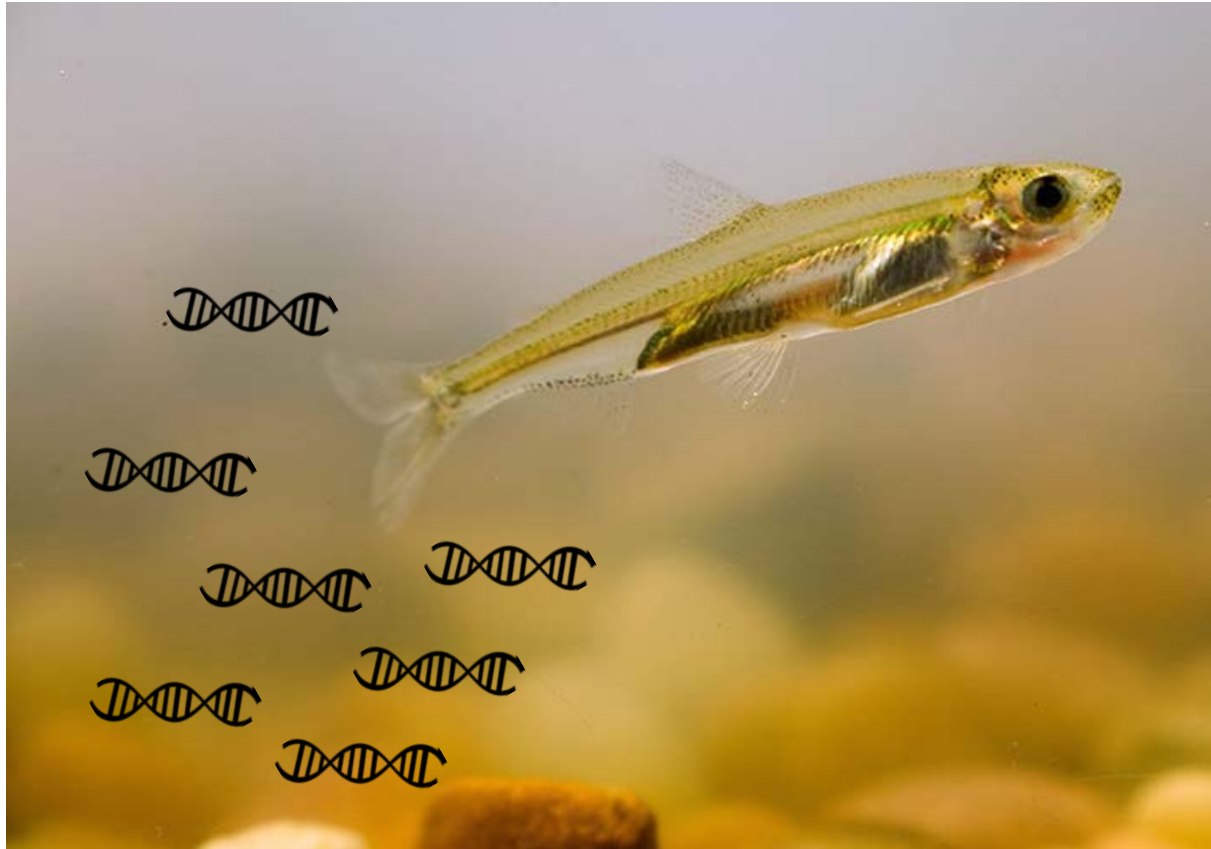


Photo: DWR

- 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) assay⁵

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 samples⁶



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

Delta Smelt detection using eDNA: Protocol considerations

- Detection method: species specific
Delta smelt qPCR assay⁵ ✓
- 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

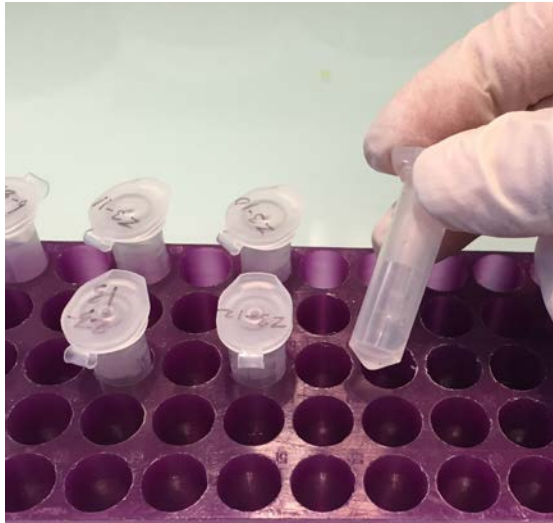


Protocol development



More control

More realistic



DNA dilutions



Tank experiments



Field experiments

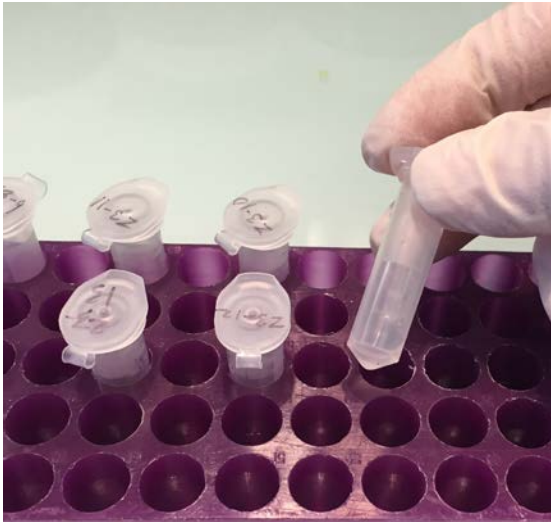


Field sampling

Protocol development



More control



DNA dilutions



Tank experiments

- Serial DNA dilutions 2016-2017
 - Consistent detection at delta smelt DNA concentration of $0.1 \text{ pg}/\mu\text{l}$
- Tank experiments at Fish Conservation and Culture Lab (FCCL) Upcoming
 - eDNA shed rate
 - eDNA decay rate

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)

More realistic

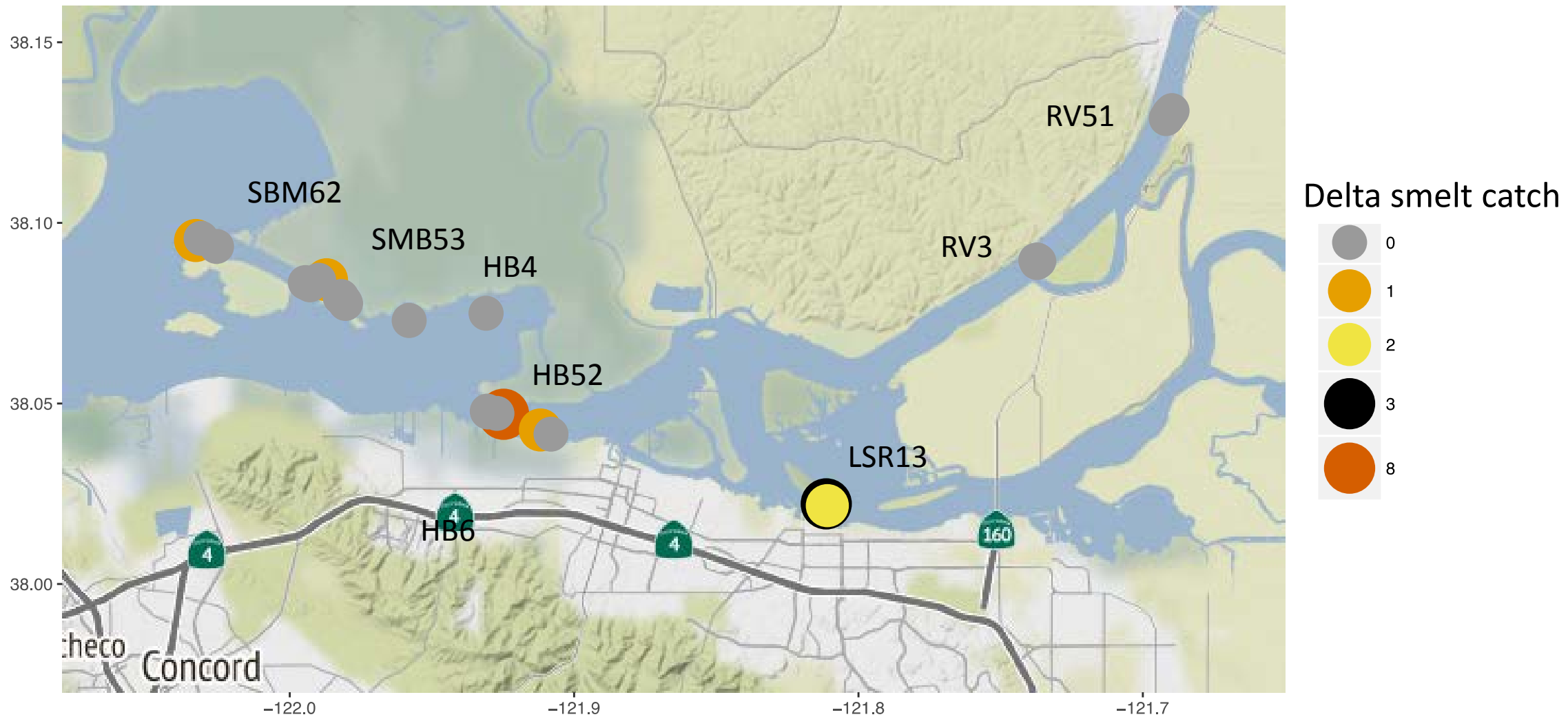


Field experiments

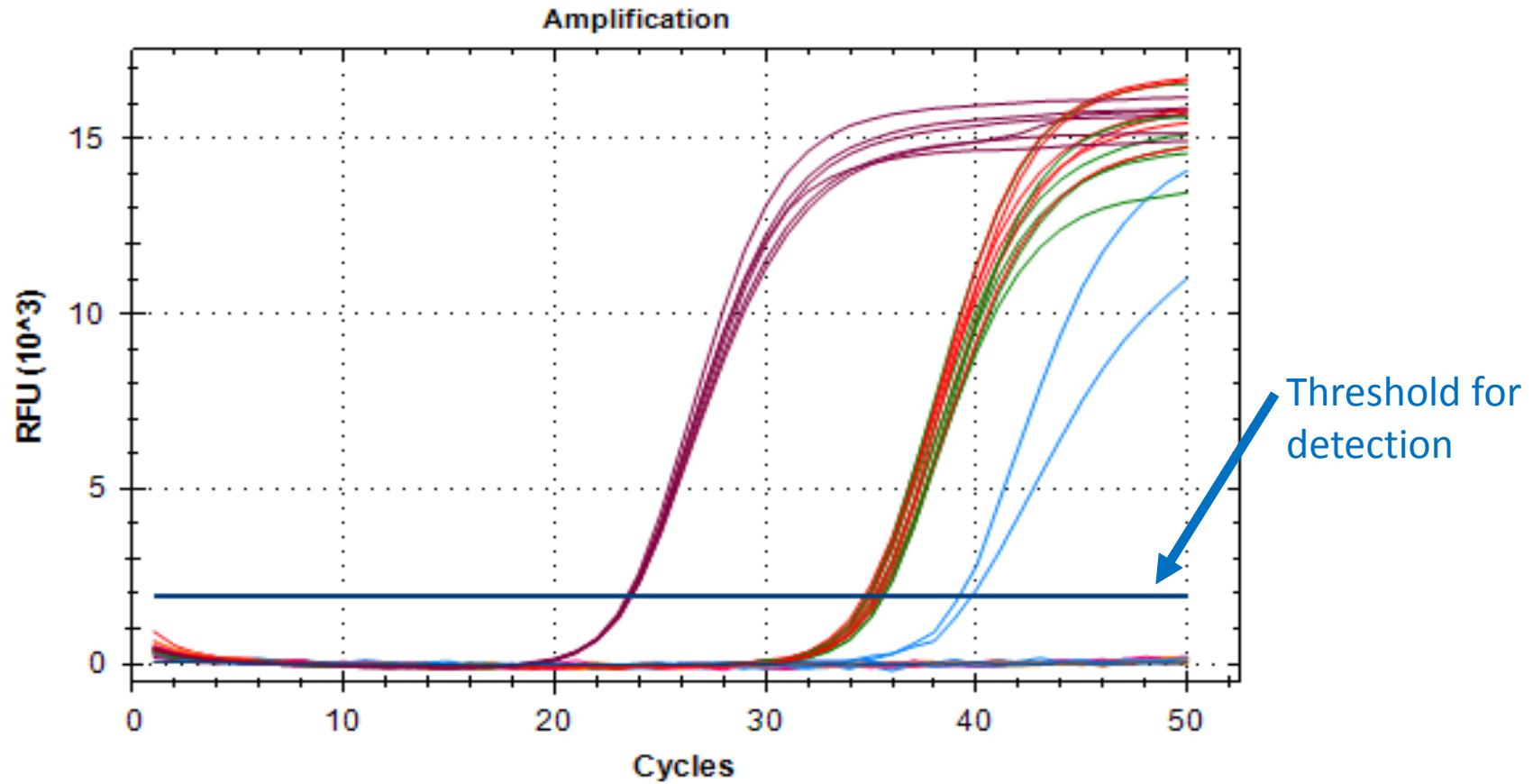


Field sampling

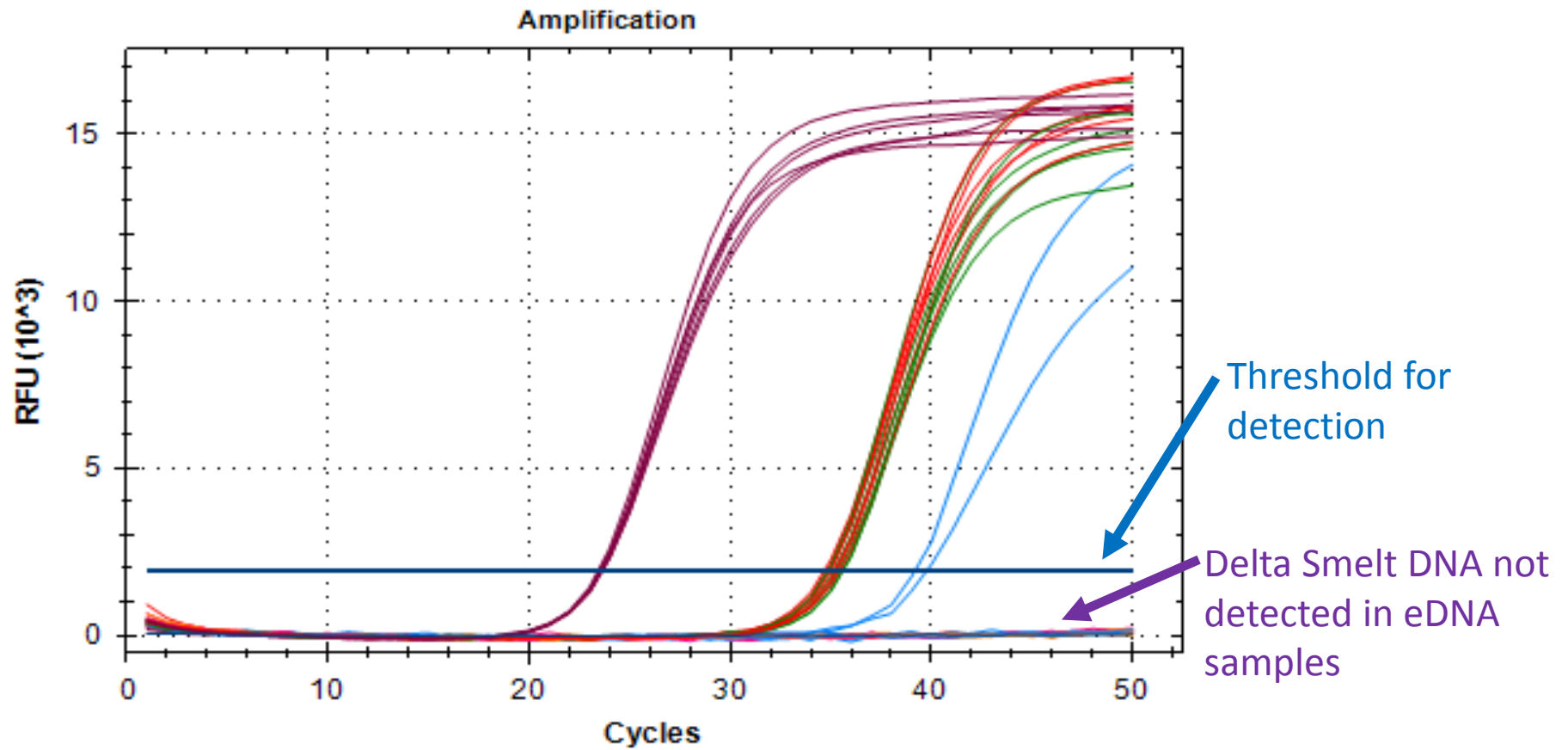
Field sampling with EDSM (USFWS)



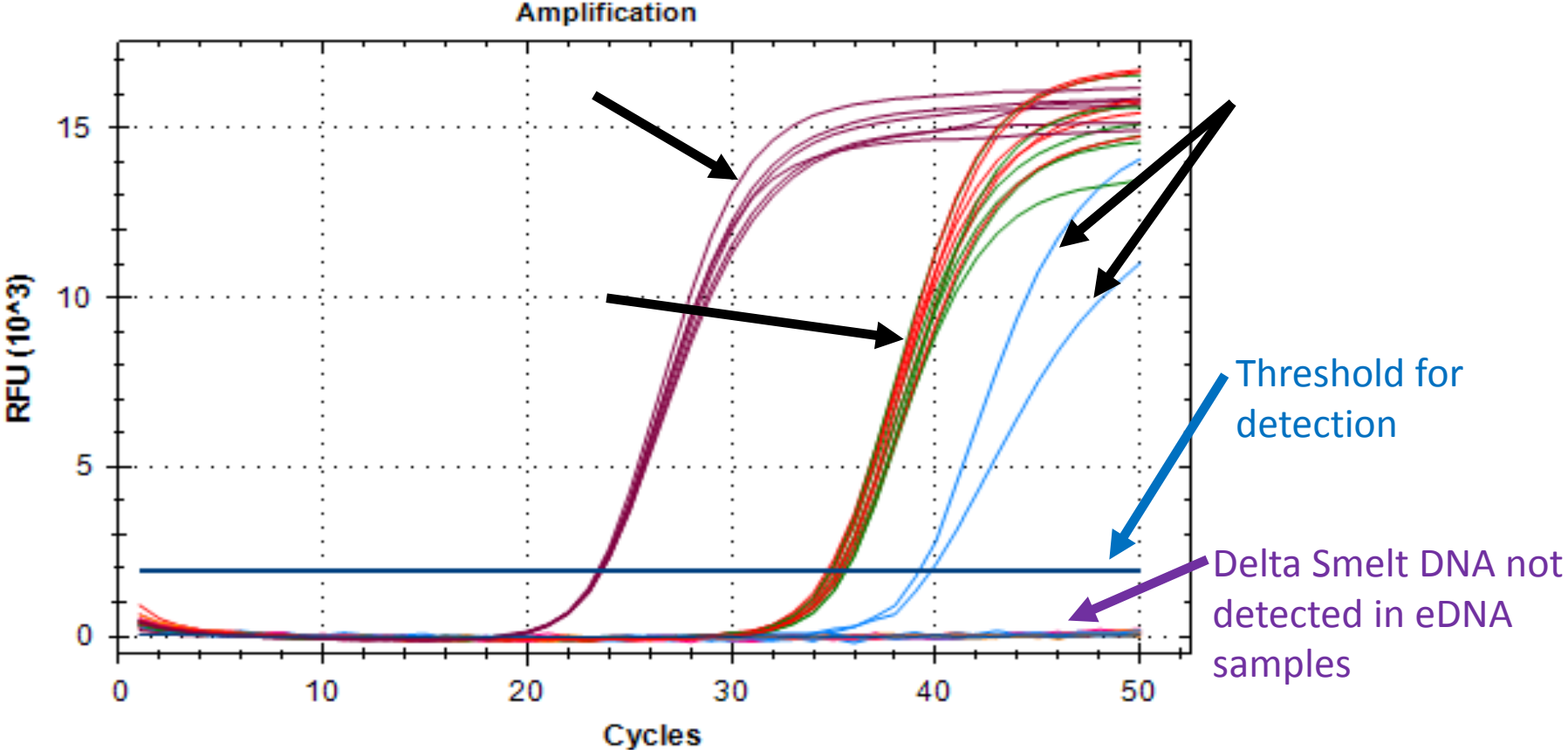
eDNA detection



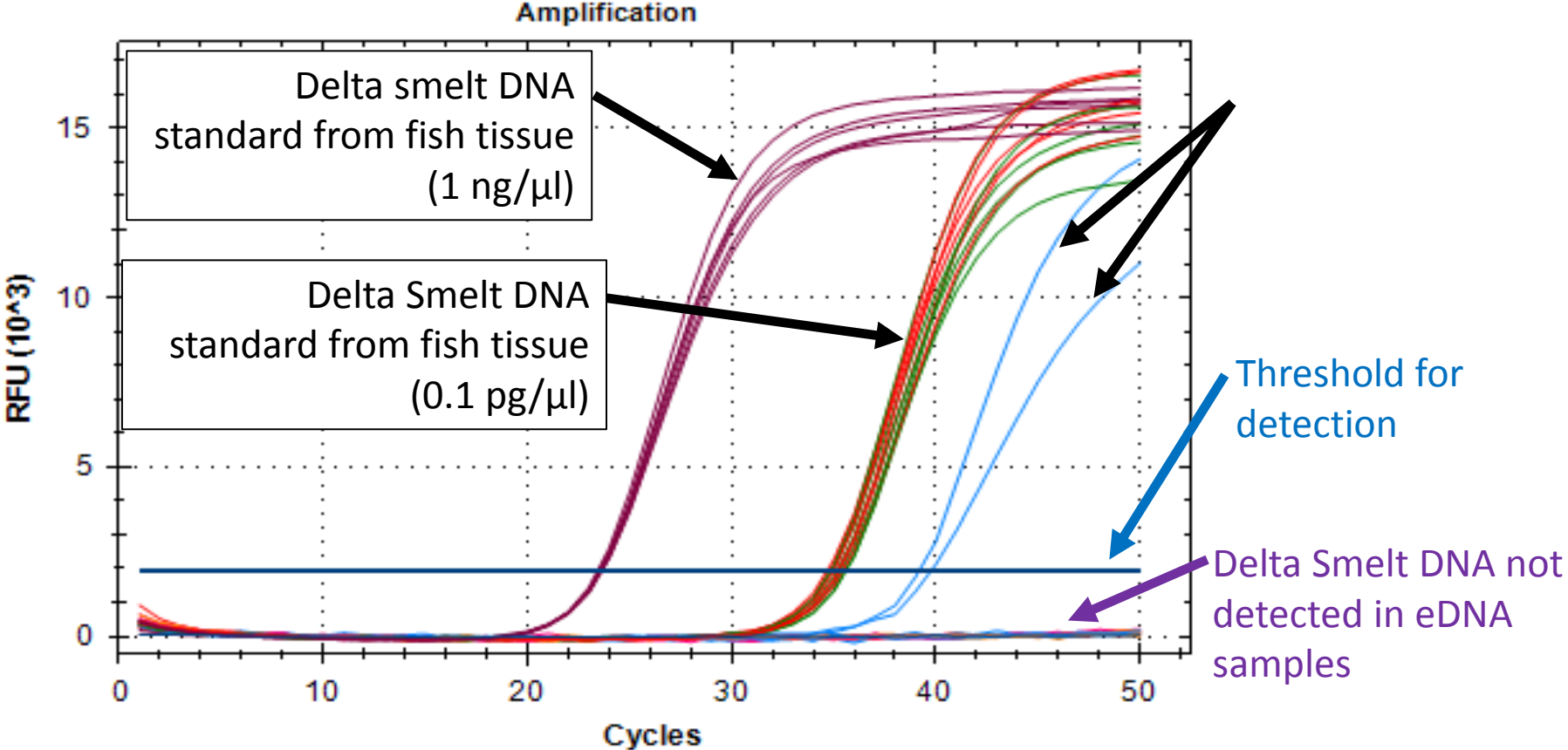
eDNA detection



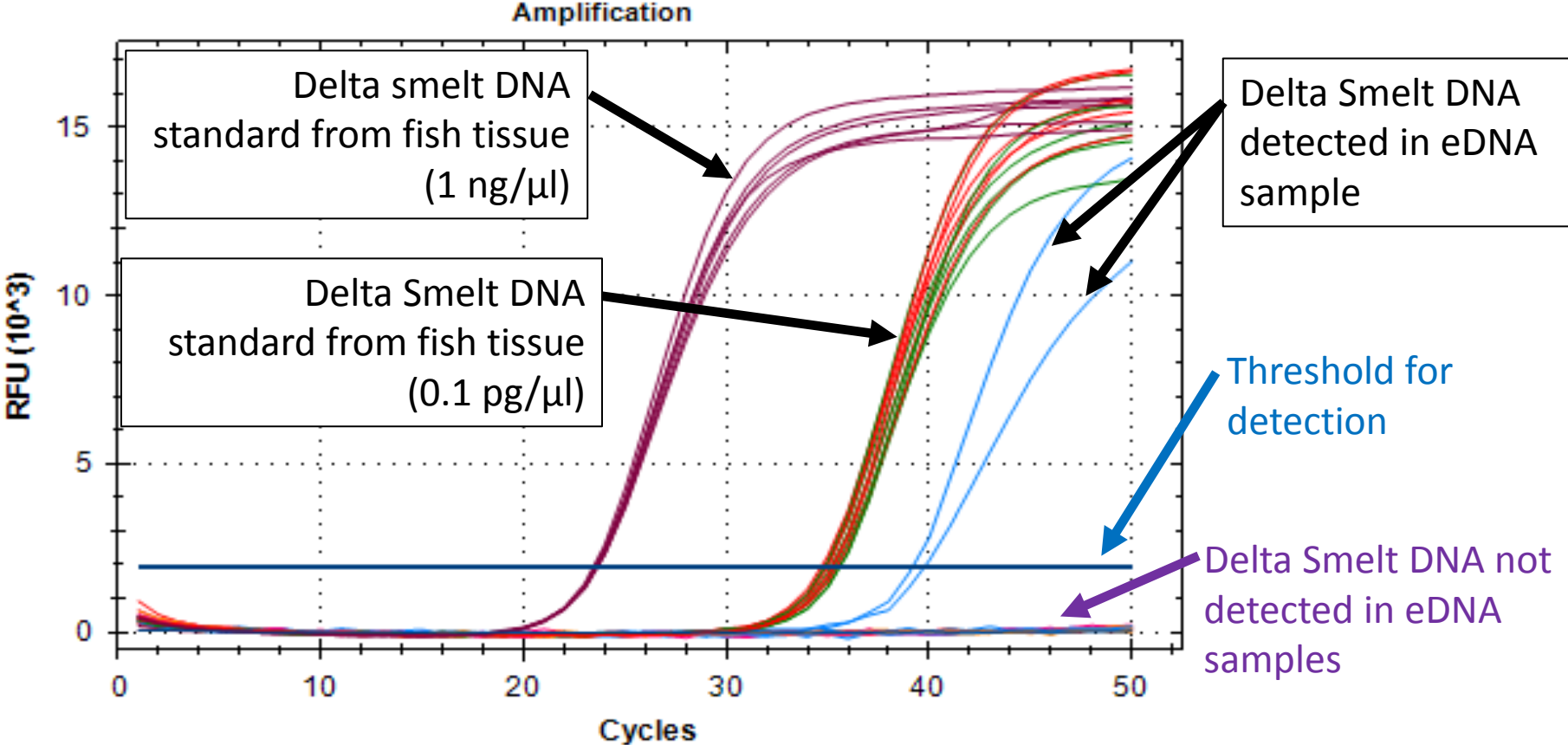
eDNA detection



eDNA detection



eDNA detection



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



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