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science for a changing world

eDNA Monitoring Research

- USGS round-robin assay evaluation
- USGS streamgage eDNA sampling

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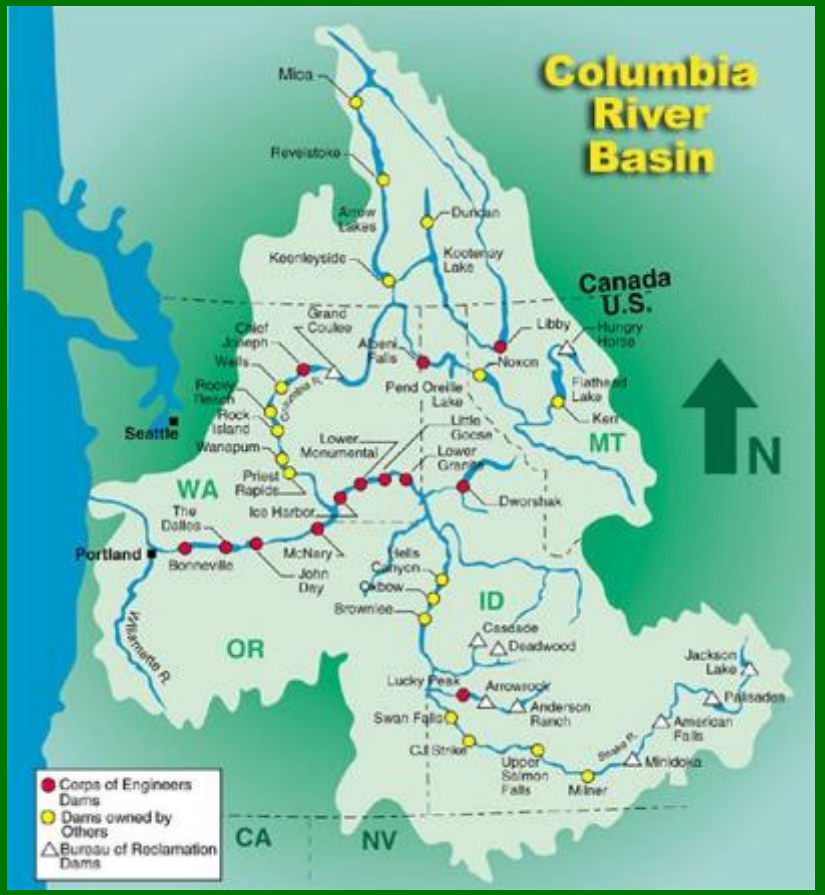
Need

1. How to sample?
2. Where to sample?



Round-robin evaluation of eDNA assays

eDNA is powerful early detection tool but…

Uncertainty associated with eDNA results

- Indirect method that detects DNA
  - DNA presence ≠ Species presence
- Often impossible to corroborate with direct methods
- Newer science with checkered early history

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Towards reducing uncertainty of eDNA results…

Identify lab-based protocols that provide repeatable & reproducible results.

- Lab environment can be totally controlled
- Repeatable: same sample & conditions = same results
- Reproducible: same sample, different conditions same result
- Cornerstone good science & diagnosis
  - confidence that initial results not obtained by chance or errors.

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Approach, 5 assays & 4 labs

- Public, probe-based quantitative PCR assays
  1. DRE16S: zebra & quagga
  2. DRE2: zebra
  3. ZebCOI: zebra
  4. ZebCYT: zebra
  5. DREQM: quagga
- Four USGS labs
  - Boise, ID; Bozeman, MT; LaCrosse, WI; Seattle, WA

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Approach

With mussels

- Seneca Lake (ZM & QM)
- Lake Michigan (ZM & QM)
- San Justo Res (ZM)
- Lake Mead (QM)

No mussels, spiked with ZM + QM DNA

- Yellowstone River
- Jackson Lake
- Columbia River

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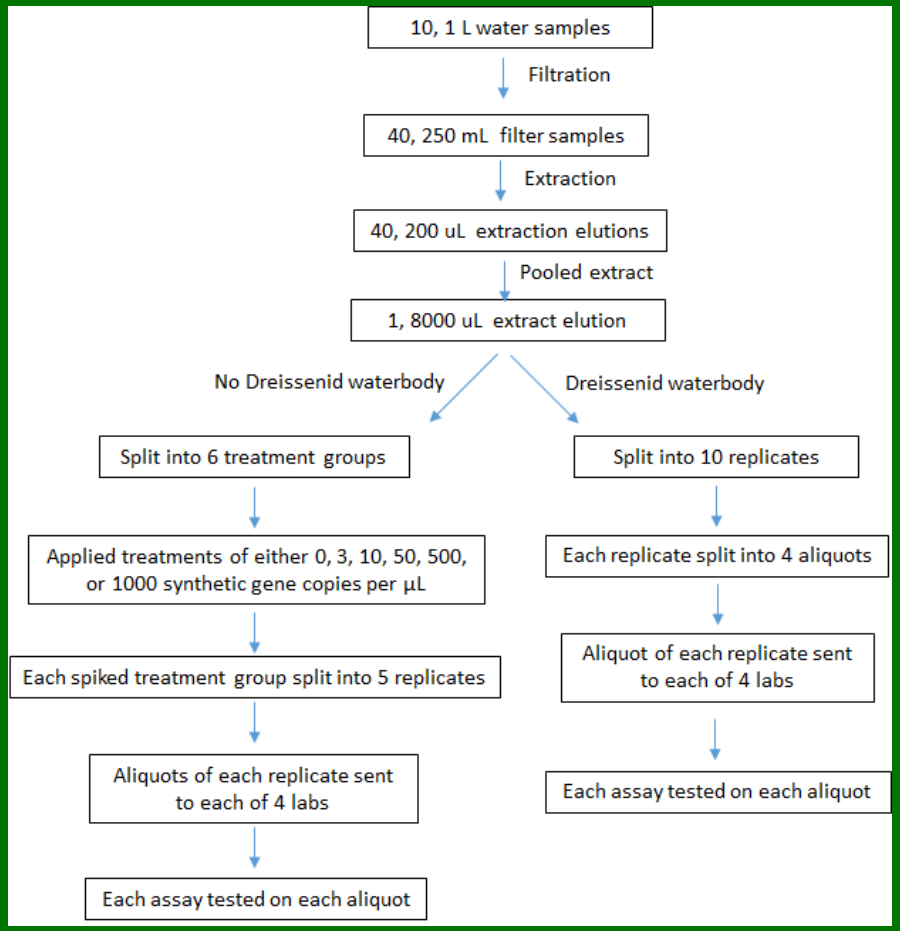
Approach

- Partners collected samples using SOP
- Double-blind
  - Sample prep
  - Sample analysis
- Identical samples
- 2832 samples, 8497 qPCR reps



Repeatability ~ 90%

Likelihood that two identical samples analyzed under the same conditions (same assay or same lab) will yield same result

- 1 = results are the same

Assay	Mean	CI95
DRE16S	0.92	0.03
DREQM	0.93	0.02
DRE2	0.85	0.03
ZEBCOI	0.91	0.03
ZEBCYT	0.93	0.03
Lab	**Mean**	**CI95**
1	0.93	0.03
2	0.92	0.03
3	0.92	0.03
4	0.86	0.04

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Reproducibility ~ 0.90

Likelihood that two identical samples analyzed under different conditions (assay or lab) will yield same result

- 1 = results are the same

	Mean	CI95	
Assays	0.92	0.01	
Labs	0.92	0.01	
Assay	**DRE16S**	**ZEBCOI**	**ZEBCYT**
DRE2	0.90	0.90	0.90
ZEBCYT	0.93	0.93	
ZEBCOI	0.93		
DREQM	0.95		
Lab	**4**	**3**	**2**
1	0.89	0.93	0.94
2	0.90	0.93	
3	0.87		

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Conclusions & Next Steps

- High probability (~90%) that identical samples analyzed under the same or different assays or labs will yield the same result
- Dreissenid mussel eDNA: increase confidence / decrease uncertainty in results
- Benchmark for new assays and /or labs
- Next steps: evaluate eDNA field sampling protocols

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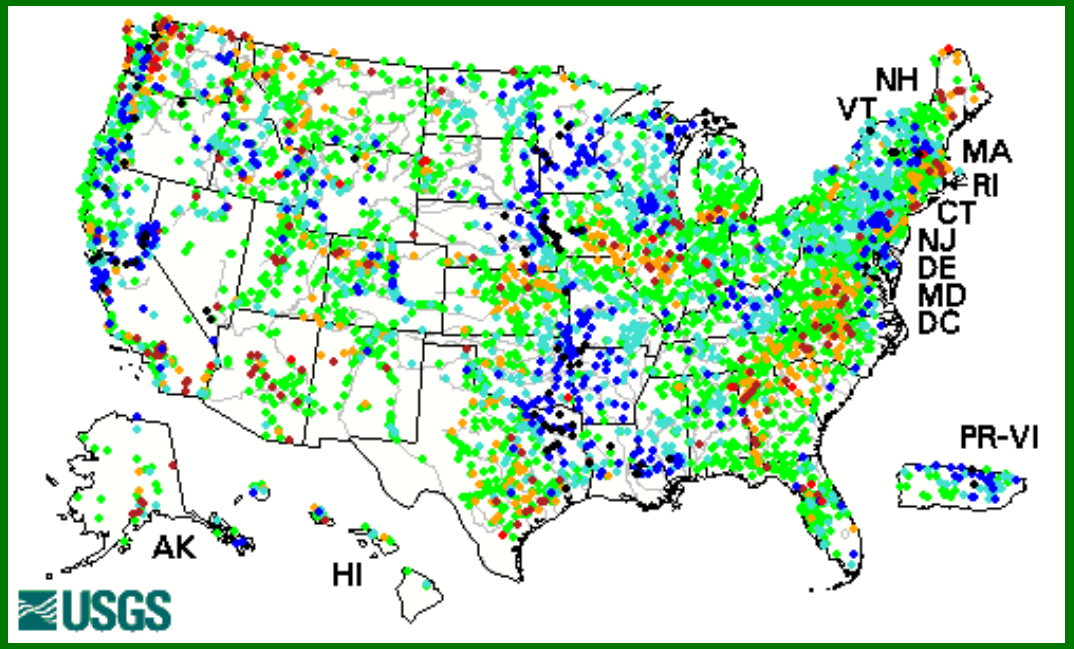
USGS Streamgage eDNA sampling

- 10,000+ USGS streamgages
- USGS hydro techs visit ~ monthly
- Incorporate eDNA sampling into visits
  - Cheap and effective way to help *Safeguard the West*?



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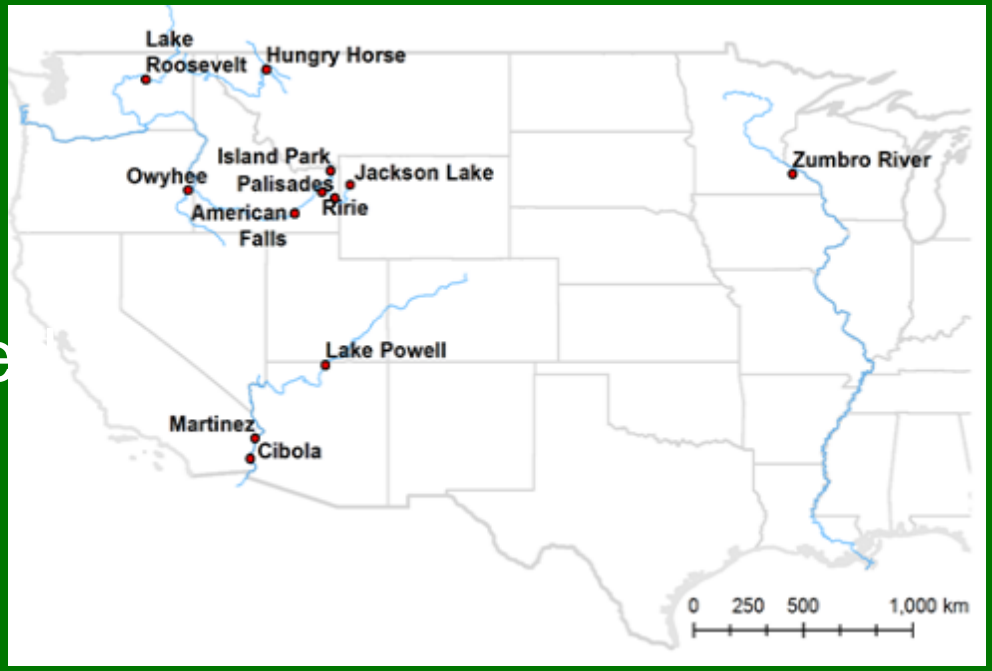
USGS streamgages below reservoirs

- Reservoirs
  - invasion hotspots
  - critical infrastructure
- USGS gages often locate below reservoirs
- eDNA at gages
  - Mussel (-), vulnerable
  - Mussel (+)



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Objectives

1. eDNA surveillance for dreissenid mussels
2. Additional cost of incorporating eDNA into USGS streamgage program
3. Are streamgages adequately located for bio-surveillance of upstream reservoirs?
   - What influences increase detection rates?

Approach

- Hydro-techs trained in SOP
  - 3 – 10 water samples per visit
  - May – September 2018
  - Shipped/analyzed at USGS LaCrosse, WI
- Target species
  - Dreissenid mussels
    - Likely all no-detects… hard to learn
  - Kokanee salmon & Yellow perch
    - Reservoir ‘obligates’ at varying abundance
    - Used these results to learn

Results

1. Dreissenid mussel DNA
   - Mussel pos. waters: all samples positive
   - Mussel neg. waters: no sample met criteria for being scored as positive
2. Additional costs:
   - $400 – 500 for three samples at a streamgage
   - Additional 2 hr of hydro-tech time
3. Streamgages adequately located
   - Kokanee and yellow perch DNA: yes

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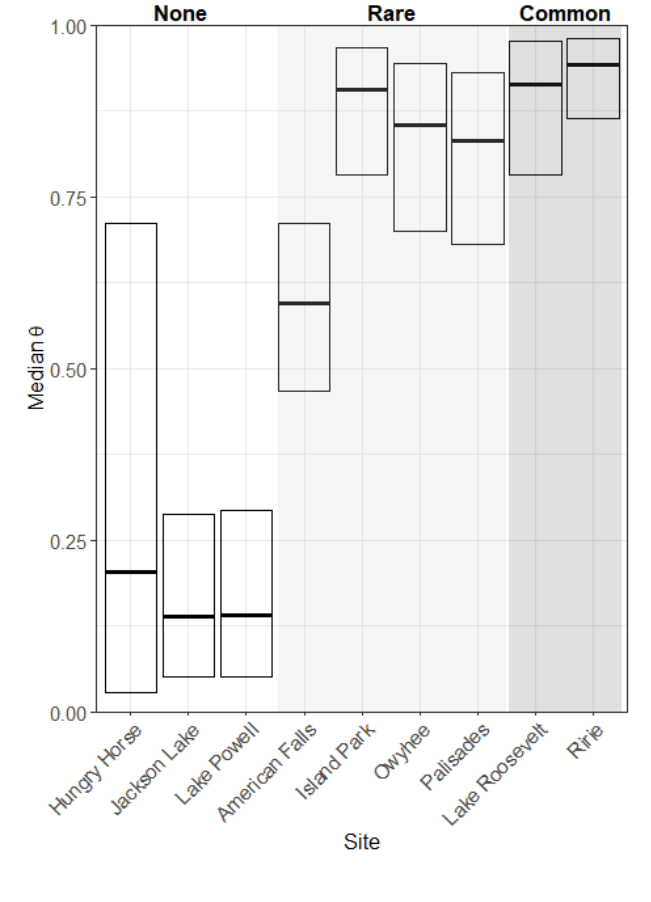
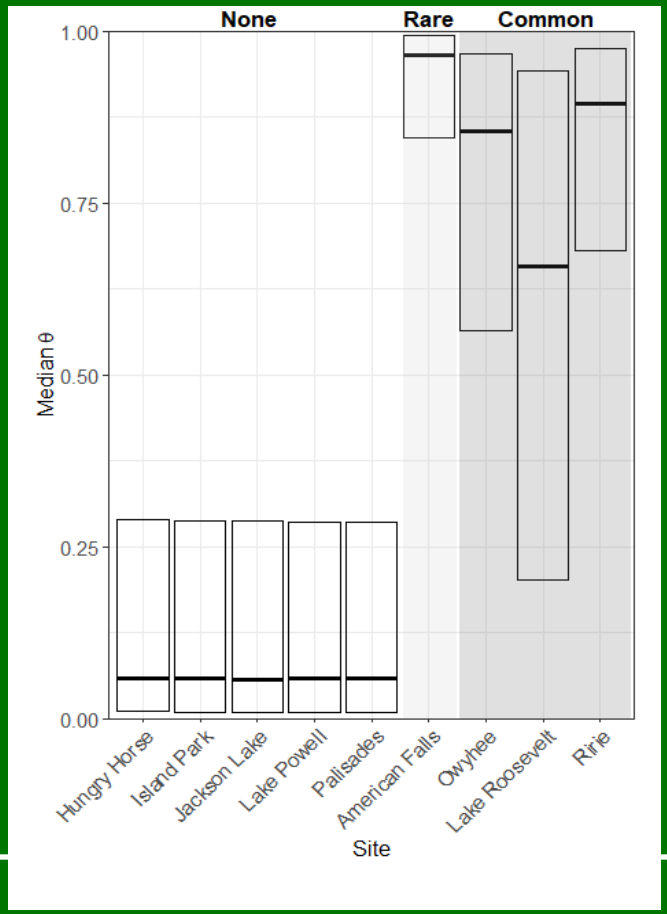
Results: detection rate in 1 sample

Kokanee



Yellow perch



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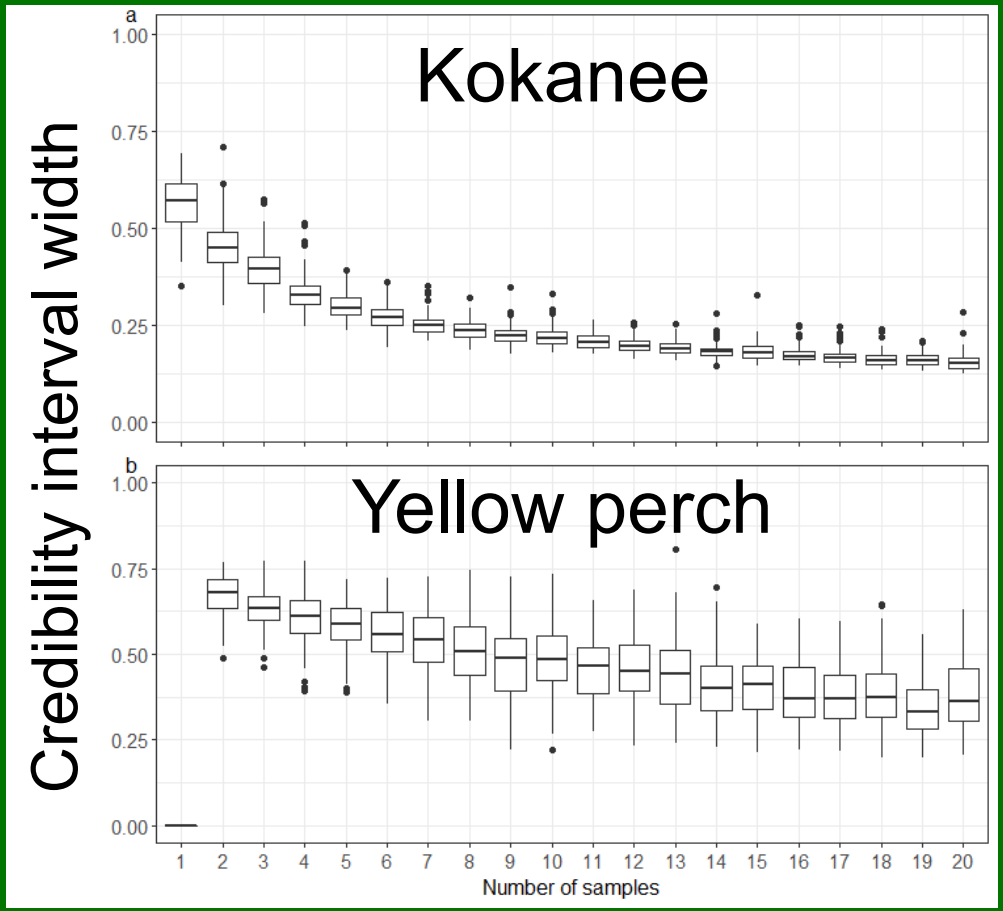
"Preliminary Information-Subject to Revision. Not for Citation or Distribution."

Results: how many samples?

Kokanee
- diminishing returns > 6

Yellow perch
- Less certain, but likely > 10



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Take homes

Streamgage eDNA sampling

- always detected mussel DNA when mussels are present
- sensitive even when fish at low abundance
- cost-effective

Next steps

- What are we missing by limiting to streamgage?
  - Send USGS hydro techs to reservoirs rather than, or in addition to, streamgage?