Alaska eDNA Workshop

April 1, 2019 Anchorage & Juneau

eDNA Primer & USFWS Perspectives

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eDNA

- What is eDNA?
- Why use eDNA and if you do WHEN?
- How do we collect & detect eDNA?
- Considerations for using eDNA:
  - USFWS eDNA Readiness Questionnaire
  - USFWS eDNA Best Management Practices Draft
- Questions

What is eDNA?

Kellie Carim 2016 ppt.

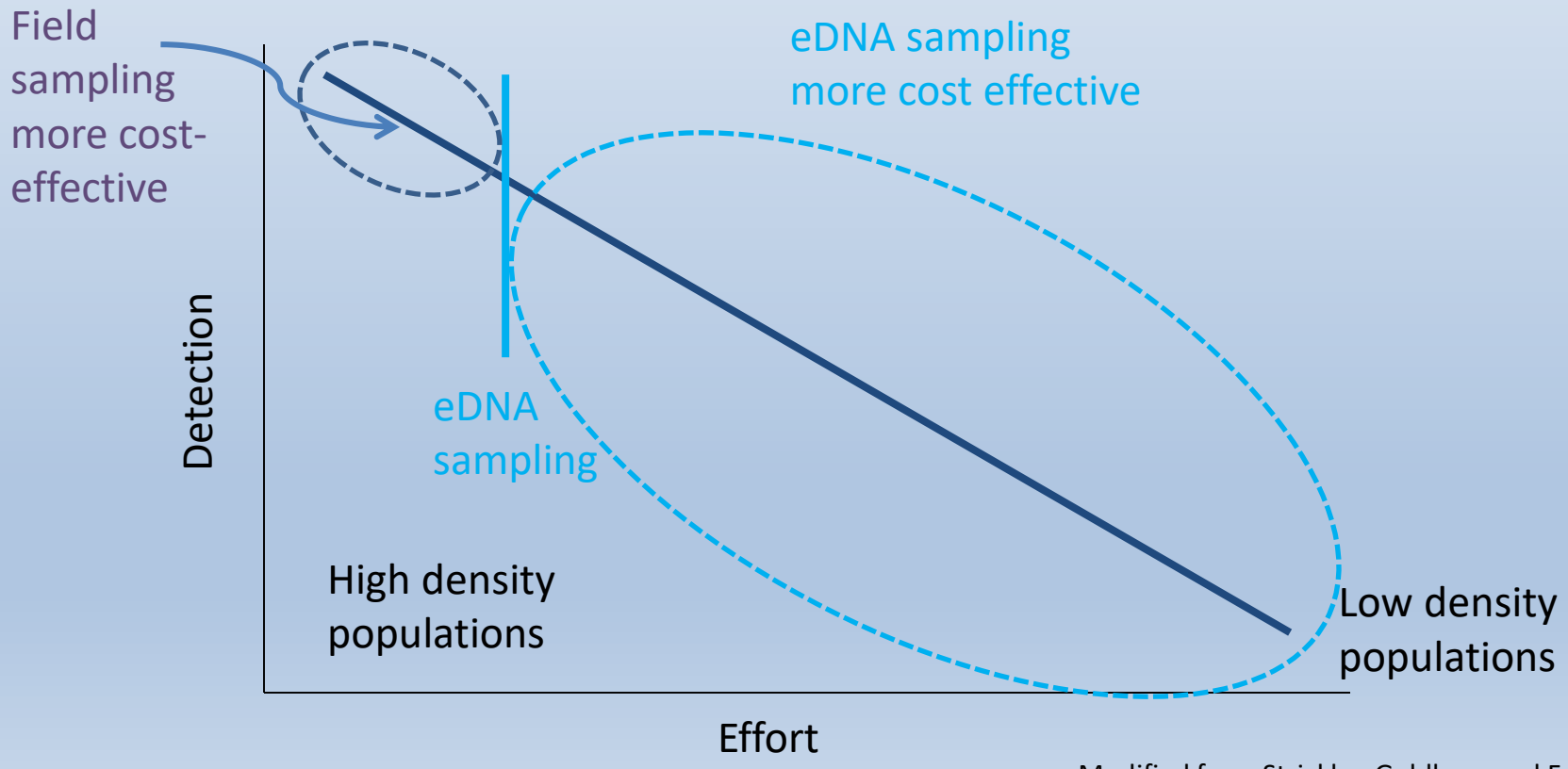
Why use eDNA?

……..Advantages of eDNA

- ★ Non-destructive
- ★ Highly sensitive – higher detection probabilities
- ★ Multi-species detections (including pathogens)
- ★ Reduced need for taxon-specific field training
- ★★ Reduced permitting requirements
- ★ Samples (filters) can be stored/analyzed later.

When do we use it?

When is eDNA sampling more efficient than standard field surveys?



Modified from Strickler, Goldberg and Femier ppt.

eDNA Dichotomy

Single species 1-3

Multi species

Confirm presence/absence?

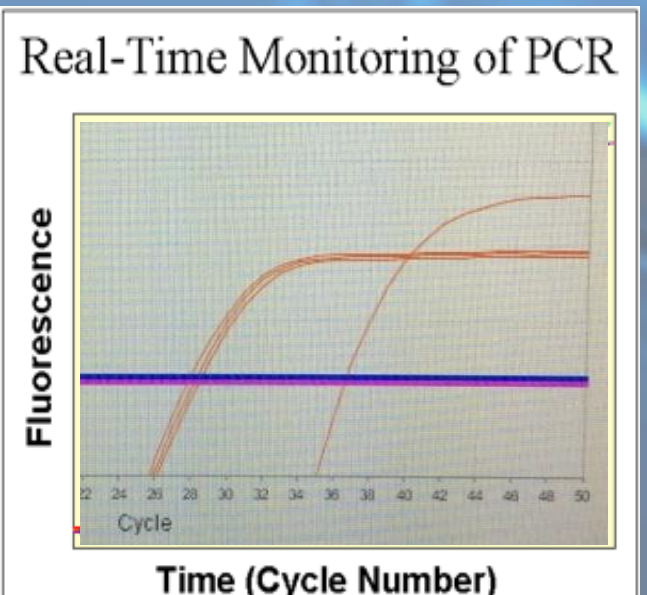
Estimate relative abundance/biomass?

How do we detect eDNA?

- DNA extraction (get DNA out of whatever cells happen to be in the water you collected)

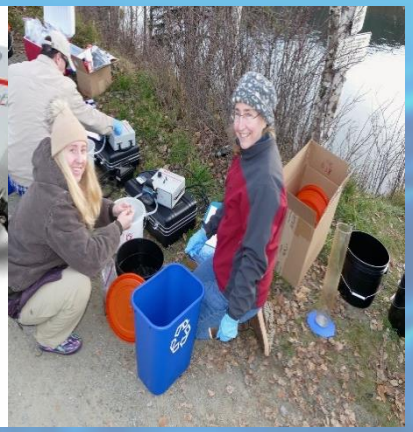
Single Species Approach
*Quantitative PCR (qPCR)
*Less time & $$

Multiple Species Approach
*DNA Sequencing Metabarcoding
*Higher time & $$ investment (Bioinformatics)



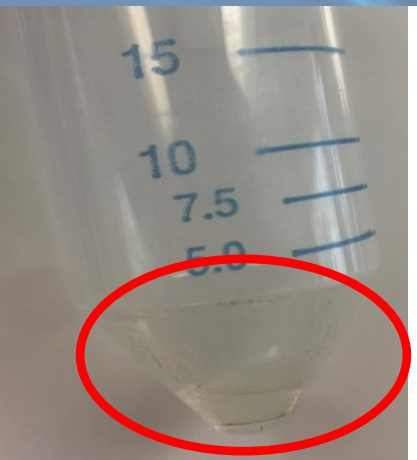
How to collect eDNA water samples?

Filter onsite

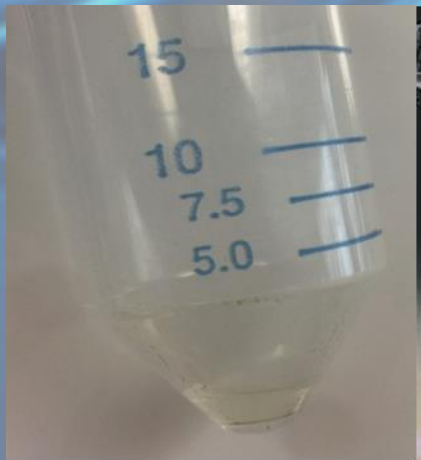


Filter in lab

Centrifuge

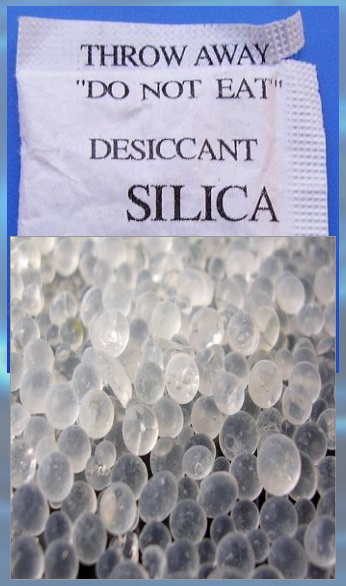


Precipitate



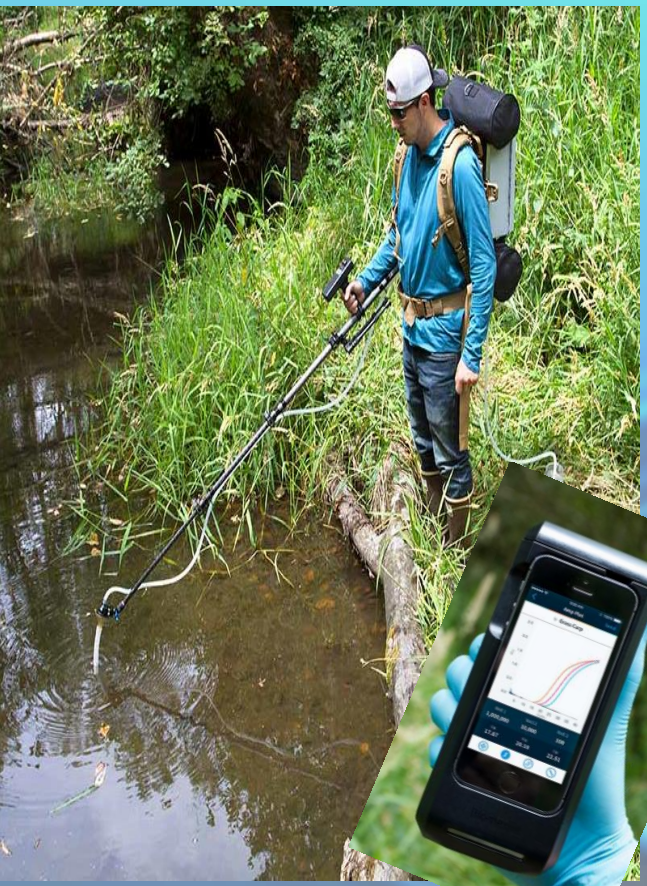
How to preserve filters post processing water samples?

Freezer -20 or -80 C



Ethanol, sodium acetate

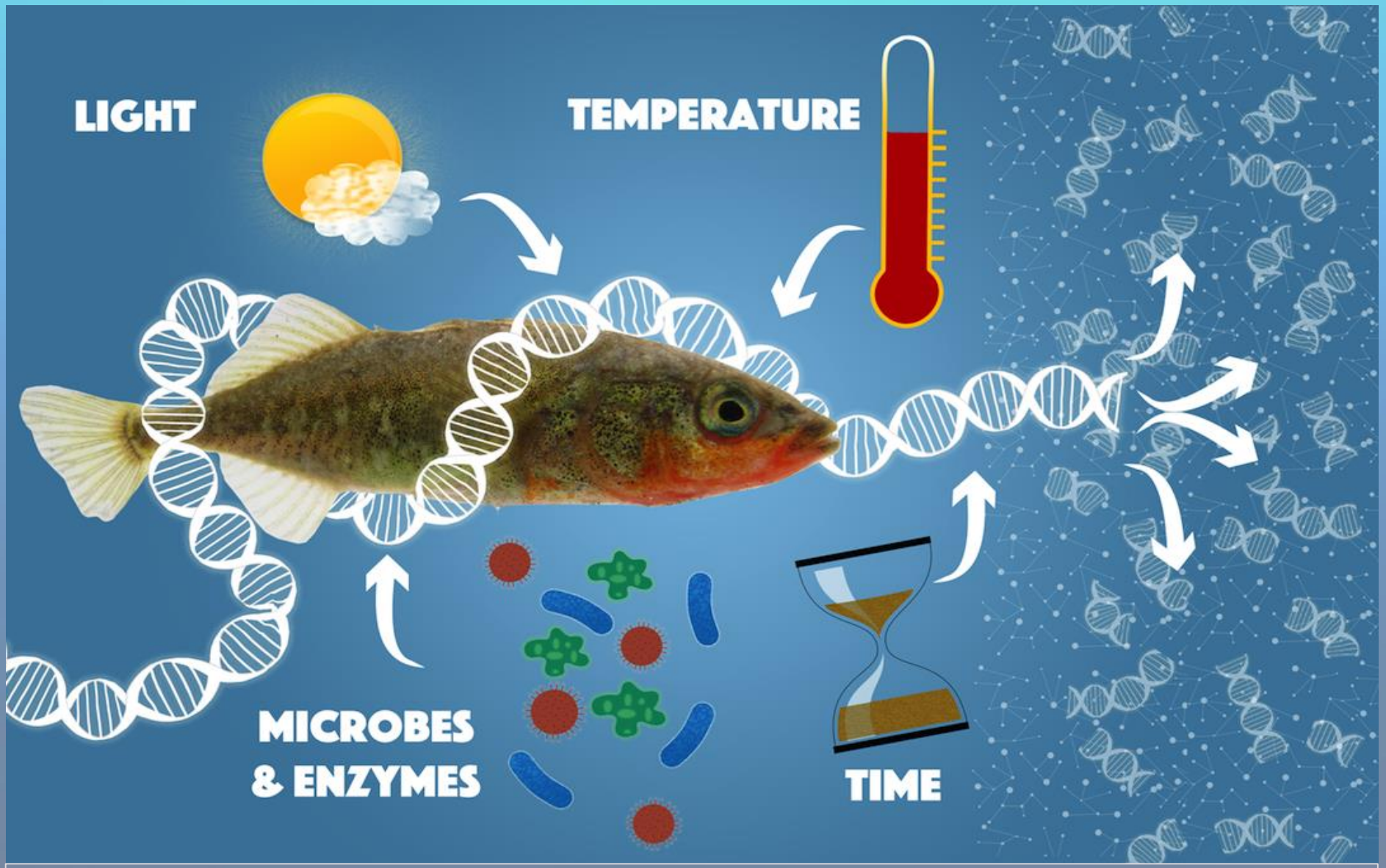
How will we collect and detect eDNA as technology changes?



Smith-Root ANDe eDNA sampling backpack with the Biomeme device to achieve a complete eDNA sampling and detection system.

Sepulveda et al. 2018 comparing traditional methods to Biomeme handheld qPCR machine that hooks up to your phone.

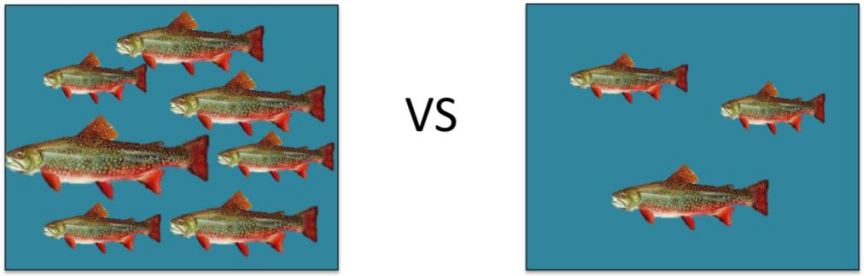
Considerations



https://fishbio.com/field-notes-/the-fish-report/true-or-false-challenges-of-edna-species-detection

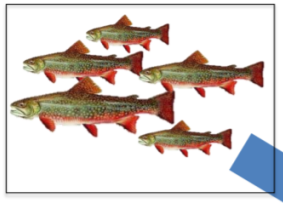
Considerations

What limits eDNA detection:
Density, abundance and biomass



We expect:
More biomass = More eDNA

What limits eDNA detection: Proximity



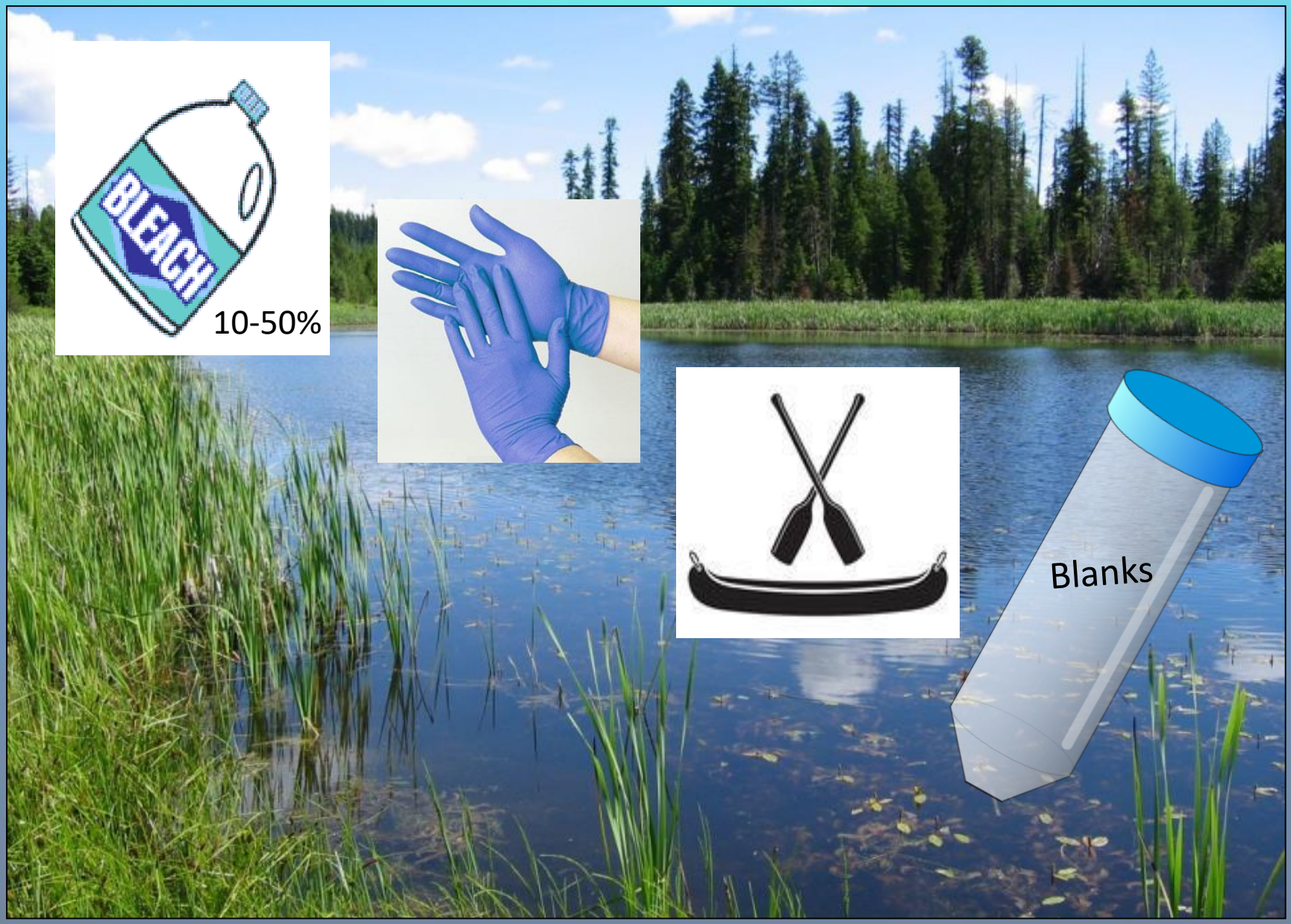
5 caged brook trout
Average biomass in cage=104g
(Range= 68.2g - 168.1g)

Brook Trout detected in
100% of eDNA samples

Jane et al. 2015

Kellie Carim 2016 ppt.

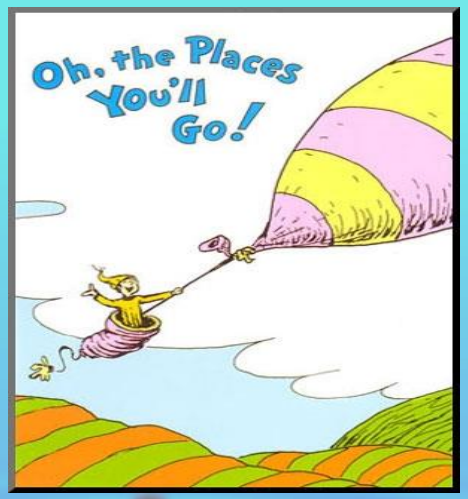
eDNA's biggest foe? contamination

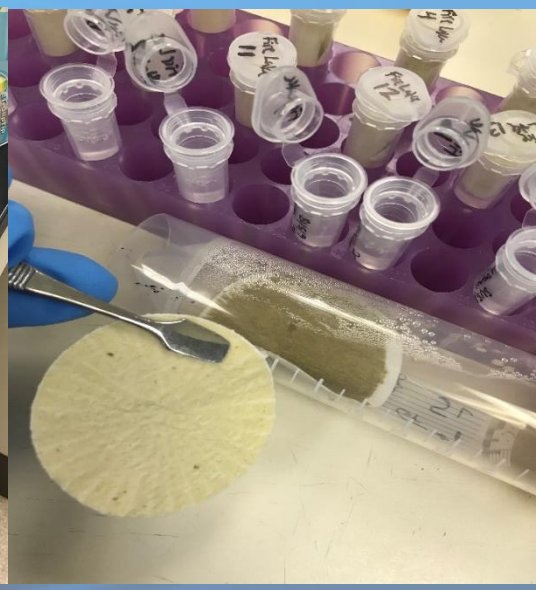


Are you eDNA ready?

USWFS Questionnaire (Draft) Mgmt. Agency Perspective 7 USFWS labs

1. **Have you defined your study objective?**
    a. Is eDNA the best approach to use? It may not applicable to all target species in all ecosystems. Logistics or cost may also affect the ability to use eDNA.
2. **Do you have a study and sampling design?**
    a. Have you considered how many samples you need to adequately address your objectives within the desired level of uncertainty? Have you considered the temporal and spatial scale of your study and what is needed to meet your objective?
    b. Does your partner genetics lab have capacity to process that number? Do you have funding for that many samples?
3. **Once you get an eDNA result, how will it be interpreted and what will you do next?**
    a. Will these results be used for management decisions?
        i. If yes, have you considered pairing eDNA with additional traditional field methods?
    b. Will your results trigger litigation? (QAQC will save you!!)
4. **How many species are you looking for?**
5. **Do you have a partner genetics lab available?**
    a. Has that lab done any eDNA in the past?
    b. Has your partner genetics lab designed and validated eDNA markers before?
    c. Has your partner lab been working on the target species in any other genetic studies? I.e. is their lab full of that target DNA?
    d. Does this lab have the dedicated space needed to conduct eDNA work? Can they make arrangements to get something to meet minimum requirements?


Oh, the Places
You'll
Go!



6. Is there an eDNA marker available?

a. If yes, is it validated in your geographic area?
    i. Does it detect your target species and only your target species? Is it specific in your region? (e.g. Takahara et al. bluegill marker finds all *Lepomis*, but since only bluegill are invasive there, it was good enough for them).
    ii. Has sensitivity been described? Is it adequate to meet your study objective?
    iii. If no, is there reference data available to create one? Is it good quality reference data for your geographic area (GenBank is full of bad data)?

7. Have you considered sampling medium and detection probability?

a. Water, Sediment, Fecal, Gut?
b. Is there any safety concern for your field or lab biologists? E.g. superfund site contaminants, or human health pathogens. Are you putting other biota at risk by spreading non-targets (white-nosed syndrome, New Zealand mud snails).

8. Have you considered life-history characteristics of your target organism that would influence eDNA detection?

a. DNA may be shed differently at different life stages, DNA may degrade faster in summer, when is spawning season, does your target hibernate or migrate? etc.

9. Have you considered how your target ecosystem structure and function affect eDNA detection?

10. Are you working in streams/rivers or ponds and lakes, or on land or ocean?

11. Have you considered how remote your sampling sites are? And does your genetics lab understand the limitations this places on your sampling logistics?

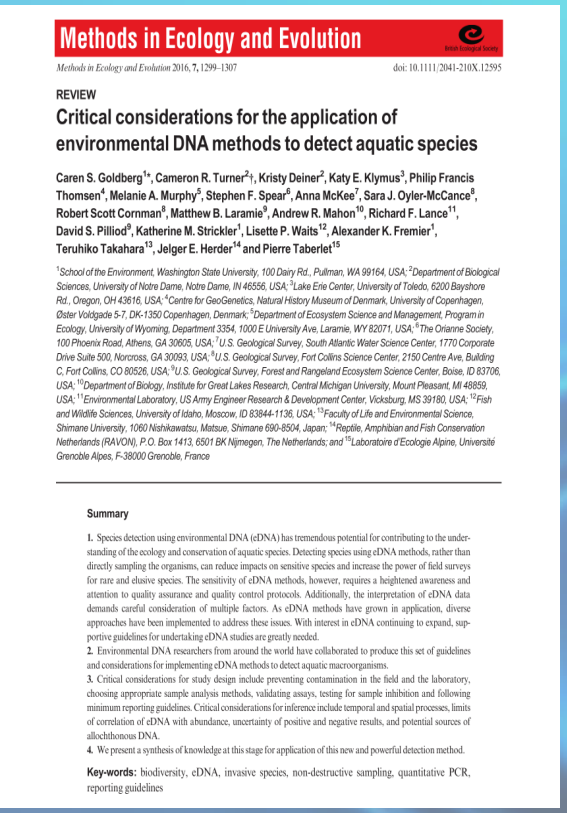
If you have questions or concerns about any of these questions, there is further detailed information in our BMP document (in development) that also has references and resources. It may be helpful to ask a statistician or biometrician for help with study design, sample numbers, etc. (depending on study and your ?s).

Is eDNA a stand-alone tool?

- Depends on study objective and managers use of results. Typically, UFWS supports eDNA as only one tool in the toolbox, and in conjunction with or in support of other field methods.
- Most studies start out using eDNA + several other field sampling tools to confirm eDNA results and/or test relationships of eDNA and species presence/absence.

- **USFWS Regional (across 7 labs) BMP in draft…but key things that we can not stress enough…that will give your project the best chance of success!!**
  - Ensure eDNA study design accounts for species life history
  - Use/develop robust markers for your species of interest (Probe based qPCR is the minimum standard for molecular detection for single species eDNA assays).
  - Count on the time and $$ for (pilot study) field validation in your region
    What is the limit of detection **(LOD)** for your marker(s)?
  - Use a lab with dedicated eDNA space
  - Mitigate contamination from field to lab (be aware of vectors…upwelling)
  - Include replicates & blanks in sampling and in molecular steps @lab (replicates help define “positives” while blanks serve as neg. controls to help address concerns with false positives)
  - Make sure to define a “positive hit”…. what is the biological meaning of said hits?

Resources



Methods in Ecology and Evolution

Methods in Ecology and Evolution 2016, 7, 1299–1307 doi: 10.1111/2041-210X.12595

REVIEW

Critical considerations for the application of environmental DNA methods to detect aquatic species

Caren S. Goldberg[1]*, Cameron R. Turner[2]†, Kristy Deiner[2], Katy E. Klymus[3], Philip Francis Thomsen[4], Melanie A. Murphy[5], Stephen F. Spear[6], Anna McKee[7], Sara J. Oyler-McCance[8], Robert Scott Cornman[8], Matthew B. Laramie[9], Andrew R. Mahon[10], Richard F. Lance[11], David S. Pilliod[9], Katherine M. Strickler[1], Lisette P. Waits[12], Alexander K. Fremier[1], Teruhiko Takahara[13], Jelger E. Herder[14] and Pierre Taberlet[15]

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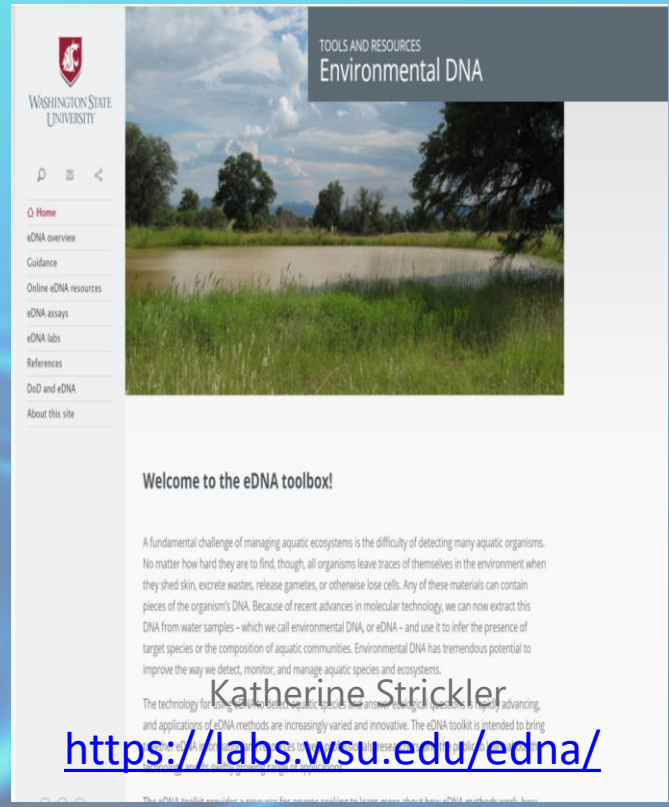
Summary

1. Species detection using environmental DNA (eDNA) has tremendous potential for contributing to the understanding of the ecology and conservation of aquatic species. Detecting species using eDNA methods, rather than directly sampling the organisms, can reduce impacts on sensitive species and increase the power of field surveys for rare and elusive species. The sensitivity of eDNA methods, however, requires a heightened awareness and attention to quality assurance and quality control protocols. Additionally, the interpretation of eDNA data demands careful consideration of multiple factors. As eDNA methods have grown in application, diverse approaches have been implemented to address these issues. With interest in eDNA continuing to expand, supportive guidelines for undertaking eDNA studies are greatly needed.
2. Environmental DNA researchers from around the world have collaborated to produce this set of guidelines and considerations for implementing eDNA methods to detect aquatic macroorganisms.
3. Critical considerations for study design include preventing contamination in the field and the laboratory, choosing appropriate sample analysis methods, validating assays, testing for sample inhibition and following minimum reporting guidelines. Critical considerations for inference include temporal and spatial processes, limits of correlation of eDNA with abundance, uncertainty of positive and negative results, and potential sources of allochthonous DNA.
4. We present a synthesis of knowledge at this stage for application of this new and powerful detection method.

Key-words: biodiversity, eDNA, invasive species, non-destructive sampling, quantitative PCR, reporting guidelines



Katherine Strickler

https://labs.wsu.edu/edna/

USGS, NOAA, USFS, NPS, USACE, Universities, others and soon USFWS have sites where eDNA work is consolidated in some form (gives idea of studies in progress that are not yet published---bridge that gap ☺)

Thank you

Questions