

# Designing monitoring programs for aquatic species using environmental DNA

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# Challenges of monitoring aquatic species

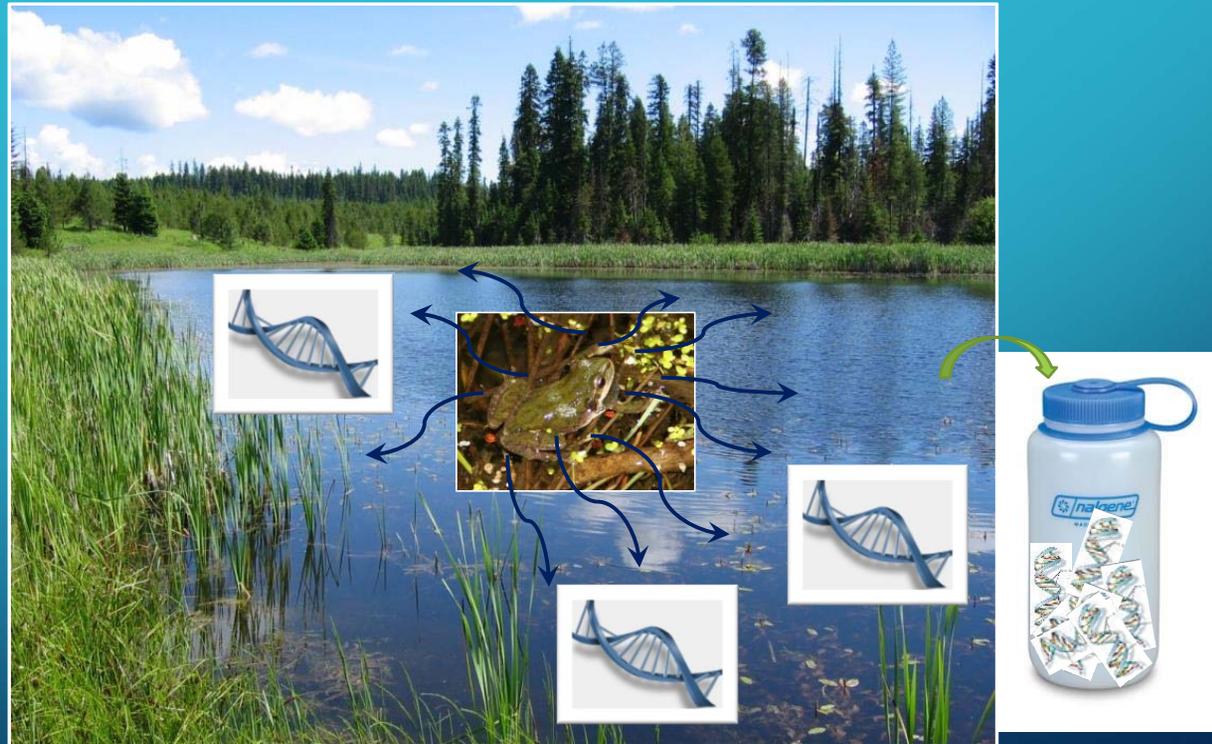
Surveys can be:

- difficult
- destructive
- time-consuming

And still may miss species that are present

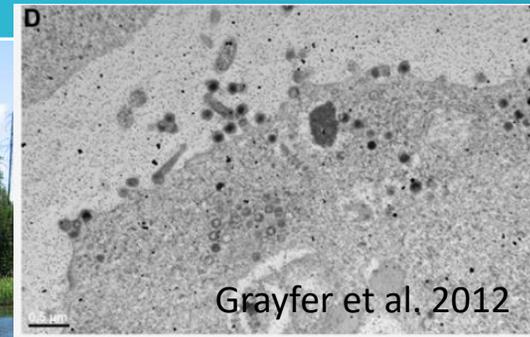
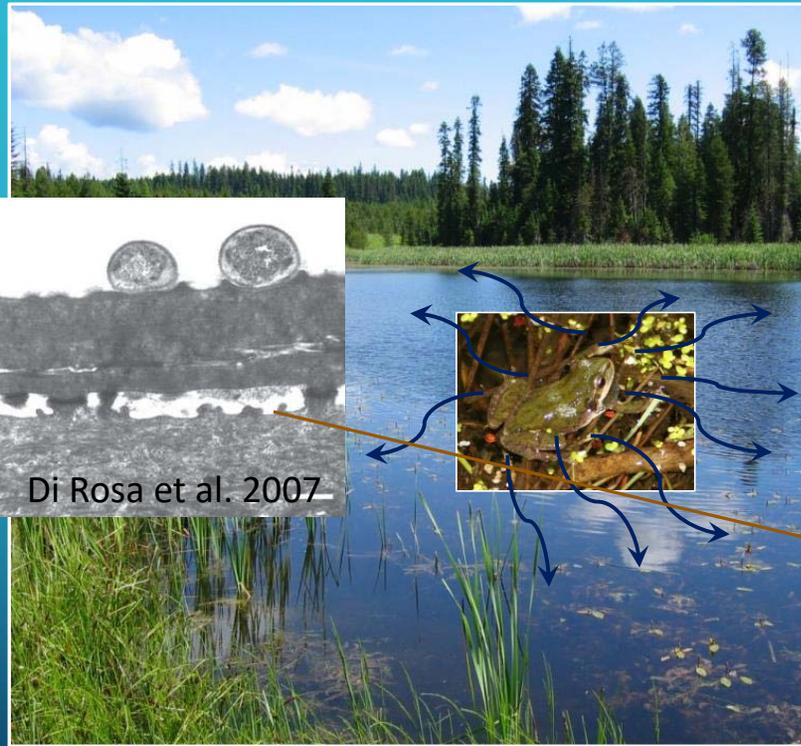
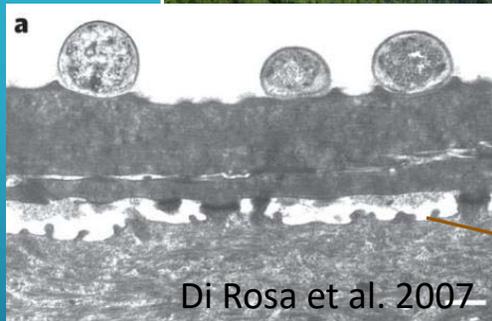


# Environmental DNA (eDNA)



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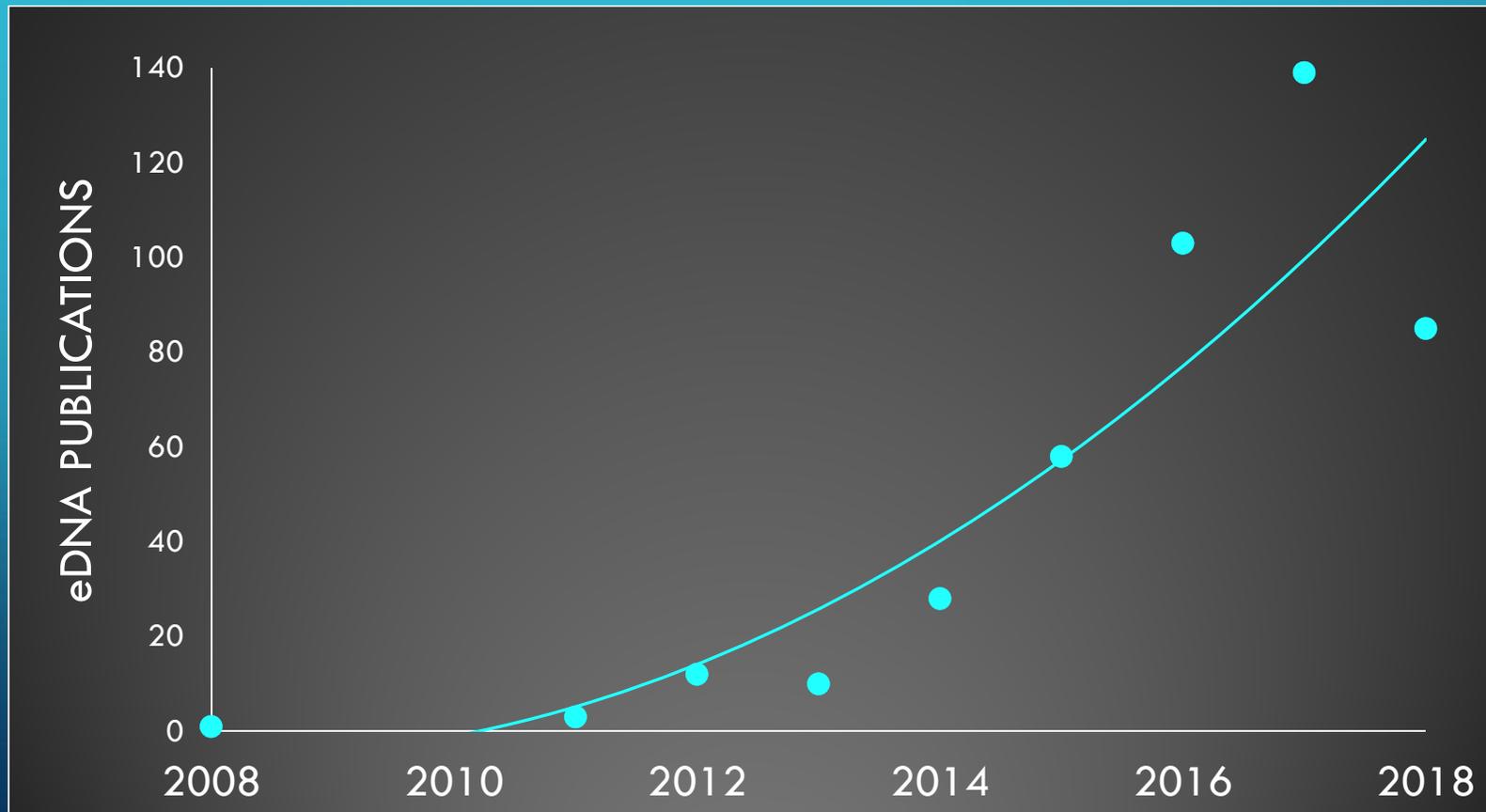
Bd



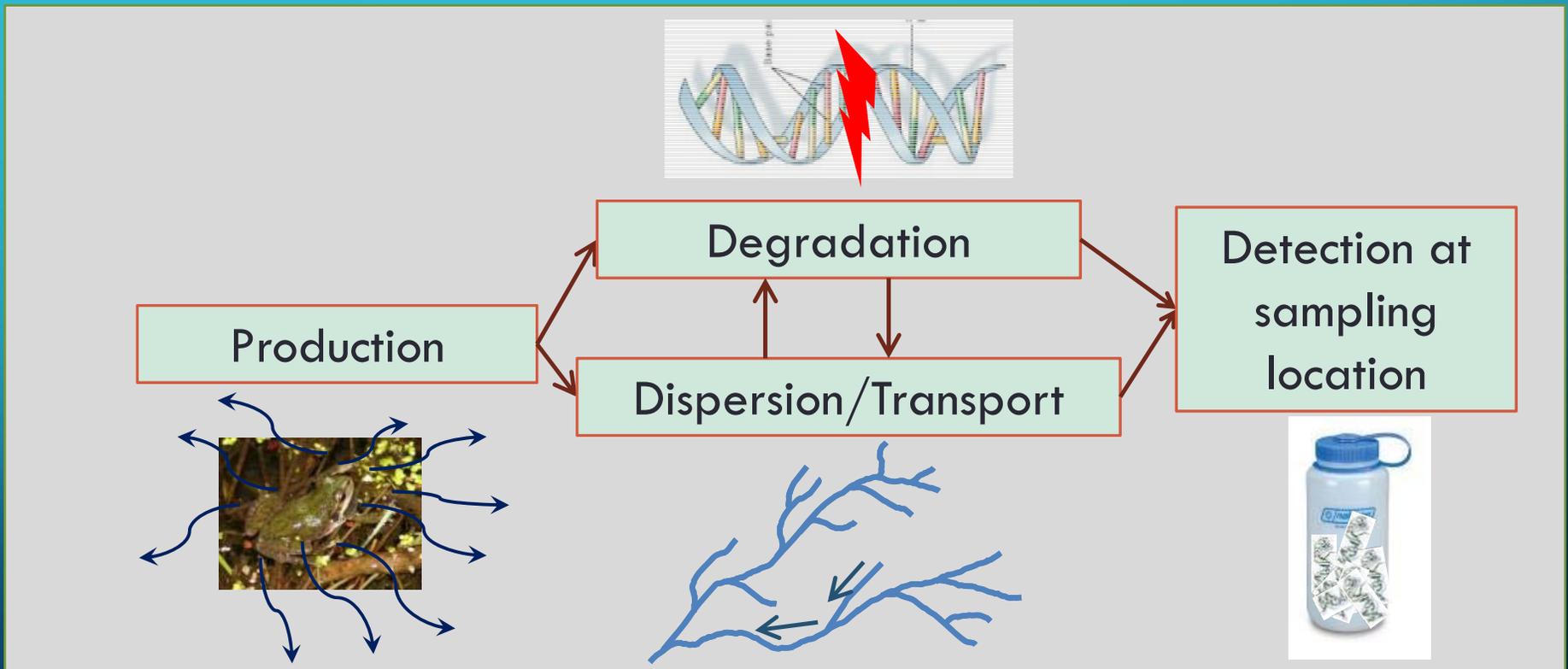
Iridovirus



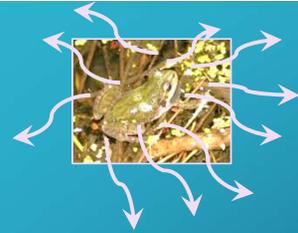
# eDNA detection of macro-organisms



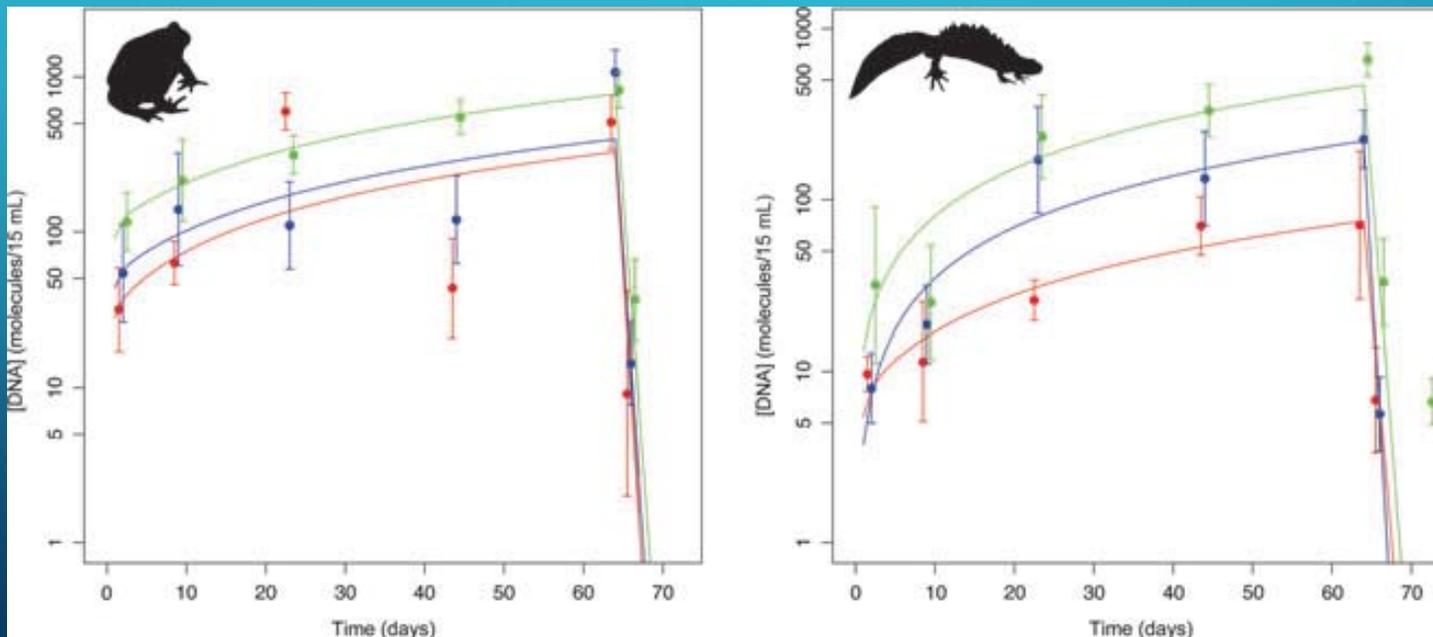
# What limits eDNA detection?



# eDNA production



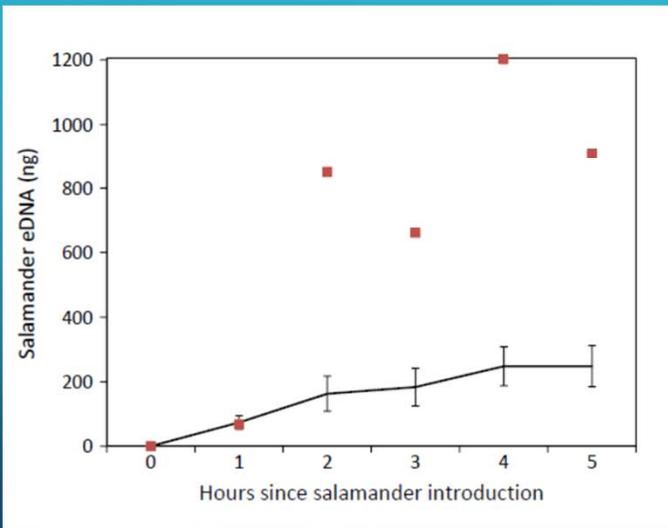
- Could be affected by:
  - Metabolism, stress, water chemistry, population density...



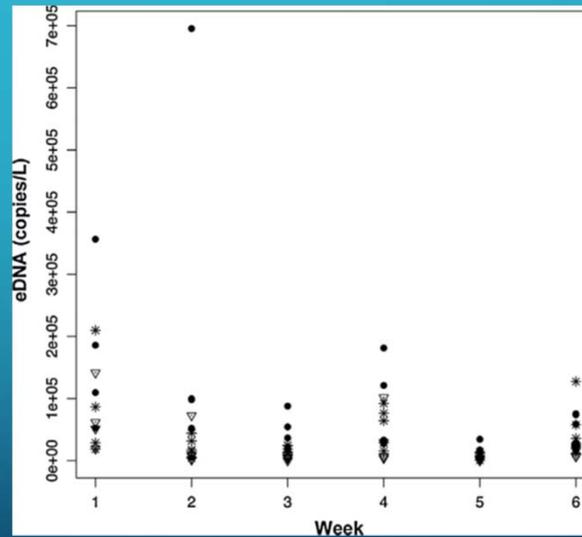
# eDNA production



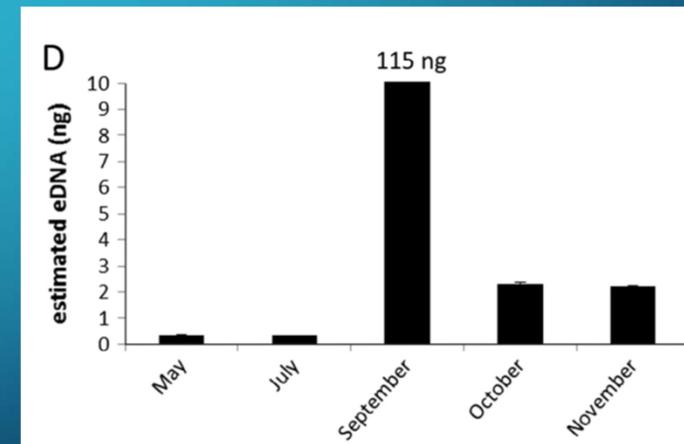
- ▶ Varies widely across individuals and within individuals across time



Pilliod et al. 2014

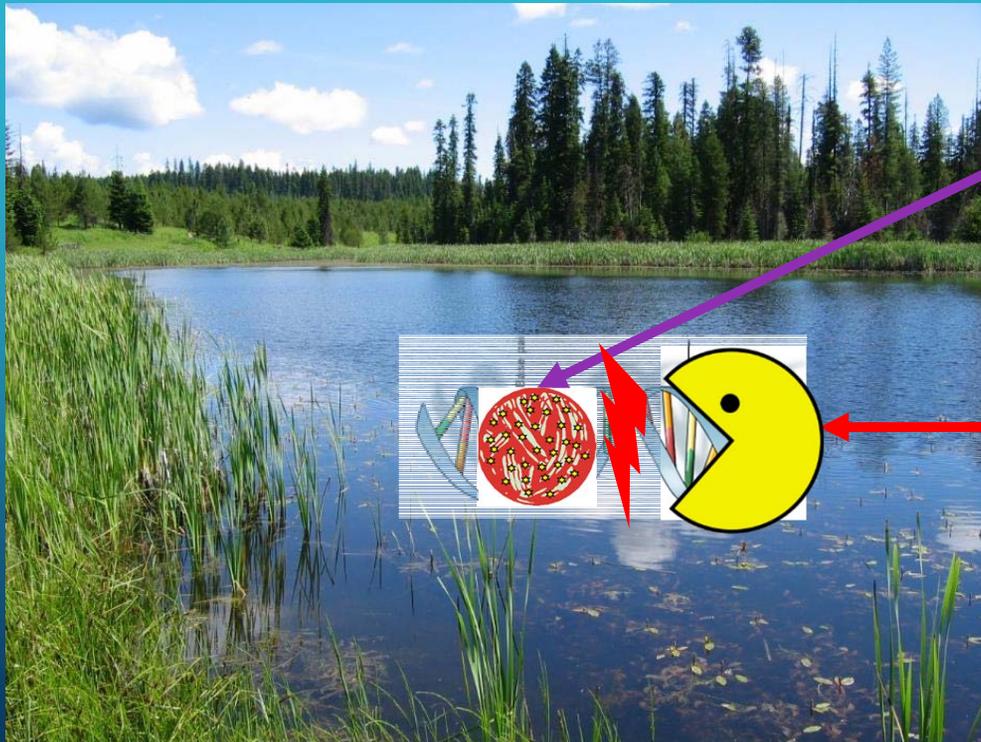


Klymus et al. 2015

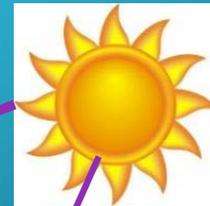


Spear et al. 2015

# eDNA degradation



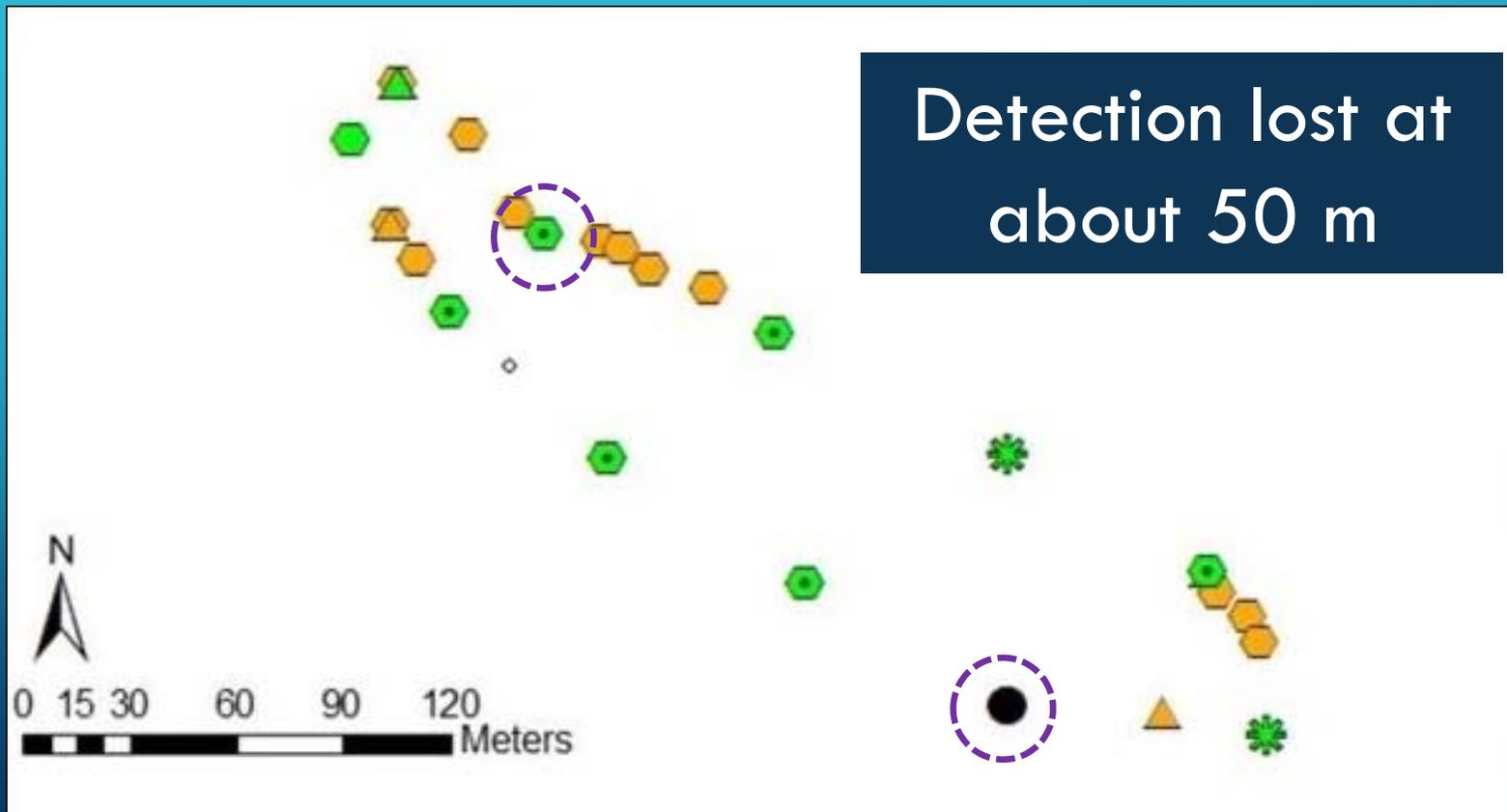
UV



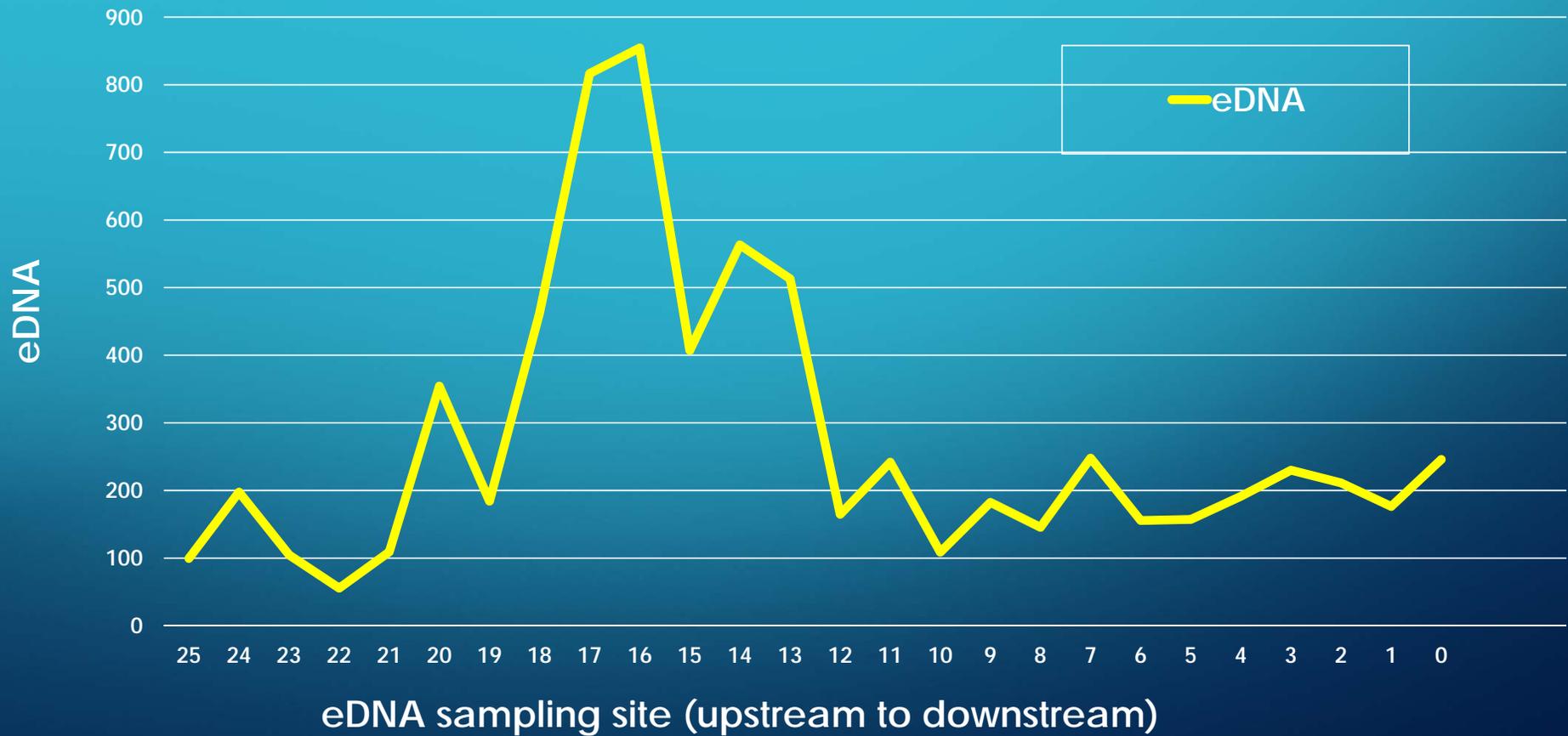
Increased activity

endonucleases/  
exonucleases

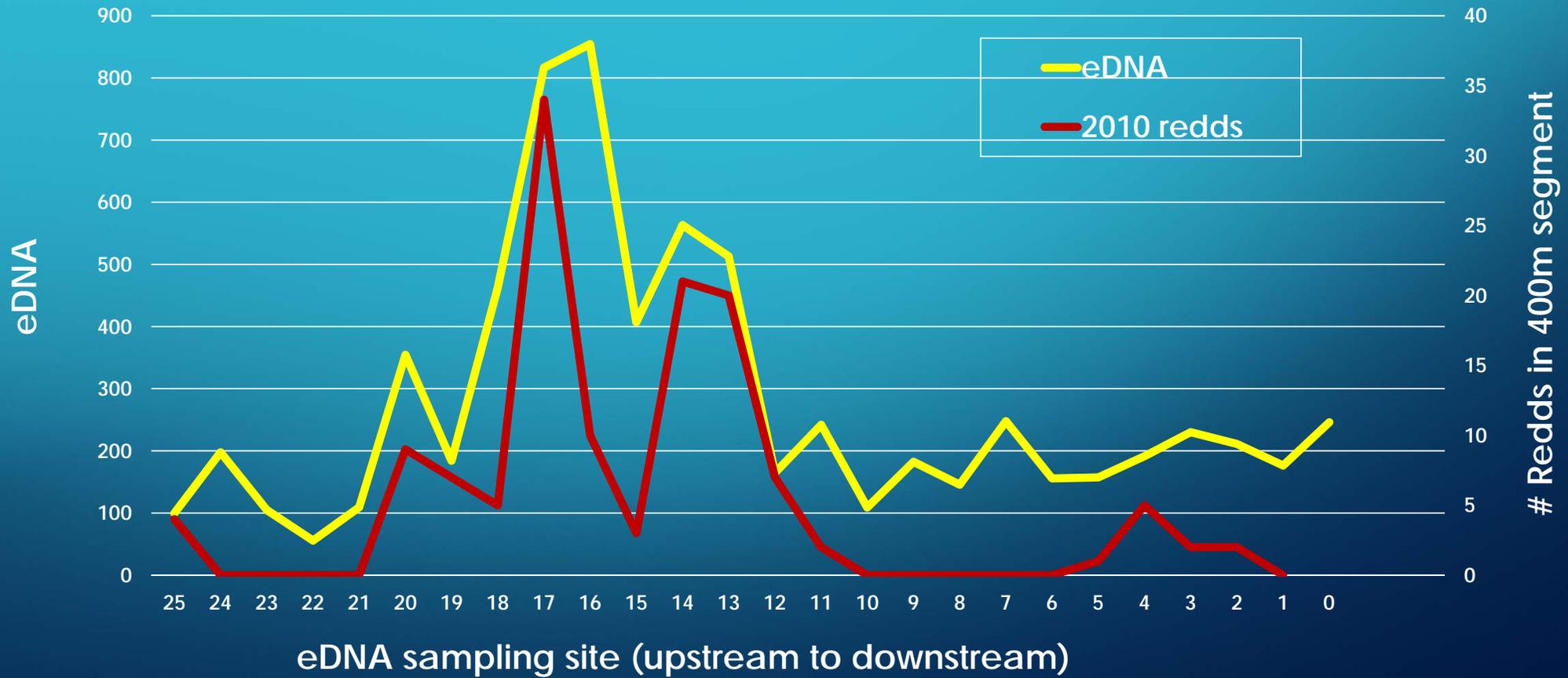
# Limits of eDNA dispersion



# Limits of eDNA dispersion



# Limits of eDNA dispersion



# eDNA in Practice

- Very sensitive, accurate tool for species detection
- Works better for some species and systems than others
- ▶ Need for systematic implementation of eDNA methods



# Framework for applying eDNA methods

Defining objectives



Deciding when to use eDNA methods



Selecting an eDNA approach



Developing an eDNA sampling design



Implementing adaptive sampling



Integrating eDNA and field methods

## Defining objectives



- Occupancy (species presence)
- Population estimation
- Disease status/physiological condition
- Single species or multispecies detection
- Temporal and spatial scales

# Defining objectives

## eDNA inference

### • eDNA *can* tell us:

- Recent target species presence
- Amount of eDNA in a sample
- Pathogen presence
- Presence of potential hybridizing non-native species

### • eDNA *can't* tell us:

- Age structure
- Reproductive status
- Population size
- Disease status
- Presence of hybrid individuals
- Presence of non-target species (qPCR)



U.S. Air Force Photo  
Heide Couch

## Deciding when to use eDNA methods



When is eDNA is most useful?

*Target species are  
difficult to detect*

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- elusive
- rare/low density
- difficult to identify

*Conventional survey methods are  
problematic*

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- low detection rates
- expensive
- require extensive training
- destructive

## Deciding when to use eDNA methods



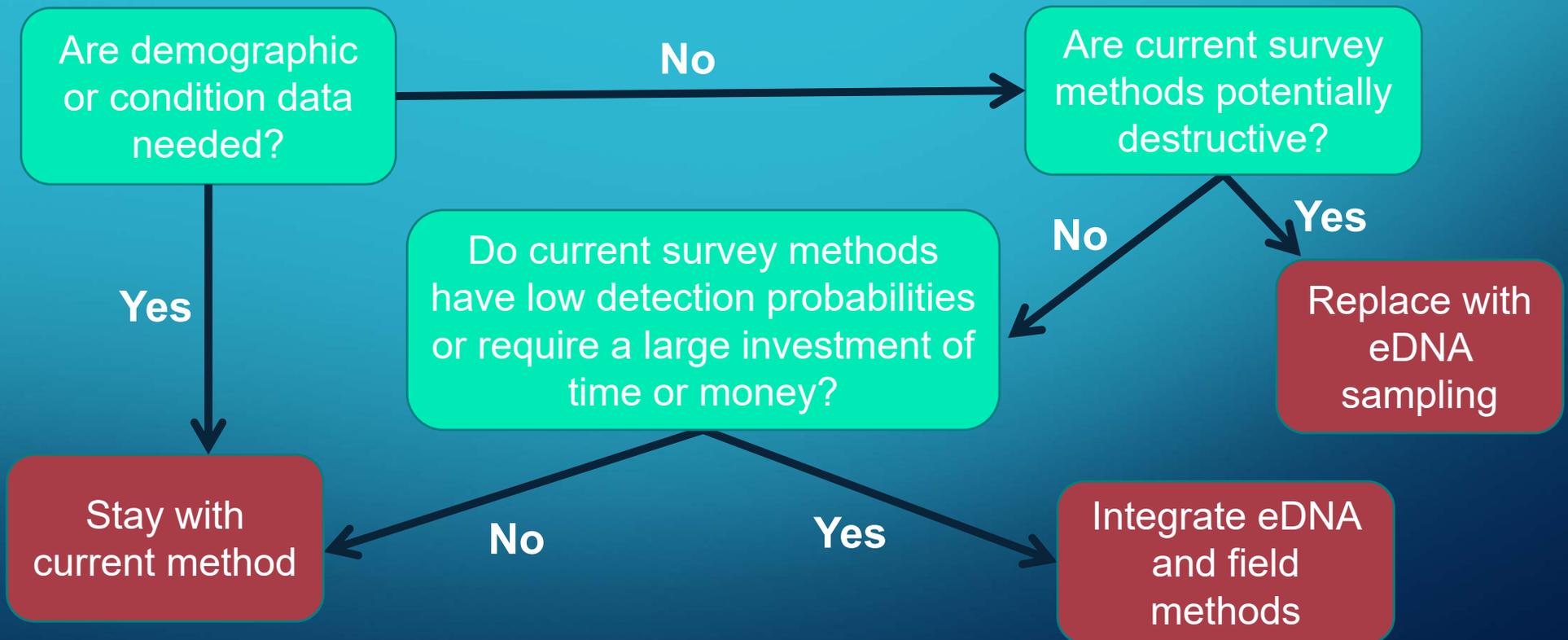
When is eDNA is most useful?

*Community-level or system-level information is needed*

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- multispecies monitoring / bioassessment
- when conventional surveys:
  - target individual species or species groups
  - are biased toward individual species or groups of species
  - require many types of surveys to detect multiple species

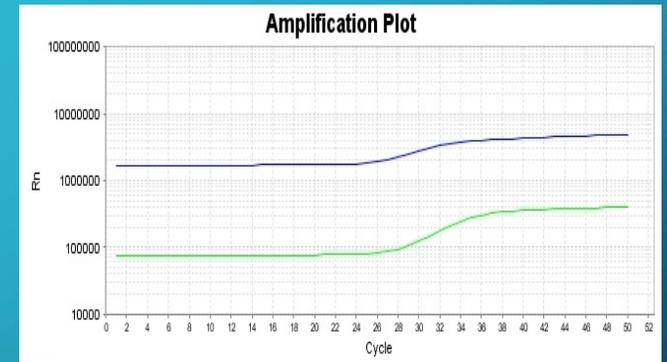
# Deciding when to use eDNA methods



# Selecting an eDNA approach

## 1. Target species

- One or a few species at a time
- Species-specific primers and probes



## 2. Metabarcoding

- Many species at a time
- Generic primers



## Selecting an eDNA approach



Target species eDNA?

OR

eDNA metabarcoding?

*Management concern is targeted toward one or several species*

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- Threatened, Endangered, or at-risk species
- Target invasive species

*Management goal is biodiversity monitoring*

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- Clean Water Act - 303(d)
- Long list of target species

# Developing an eDNA sampling design



Design a pilot sampling strategy that considers:

- Seasonal timing
- Spatial sampling design
- Number of samples
- Sample volume
- Filter type
- Preservation method
- Environmental covariates



# Developing an eDNA sampling design

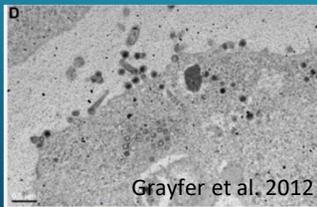


Conduct a pilot study:

1. Implement pilot sampling protocol
2. Record environmental and sampling covariates when collecting eDNA water samples
3. Analyze covariates to identify important factors for species detection
4. Adapt sampling strategy to improve detection



# Pilot study example: Tiger salamander/ATV system



Grayfer et al. 2012

iridovirus



# Sonora tiger salamander - Year 1

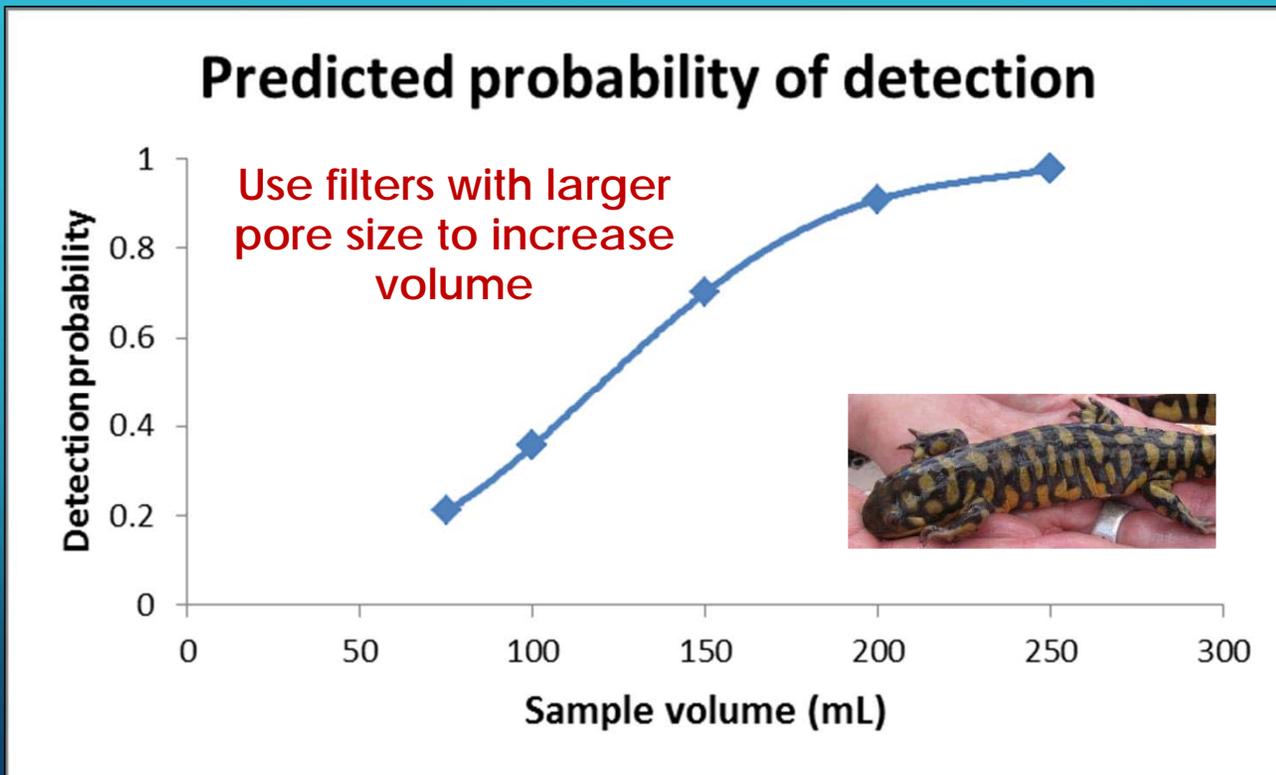


eDNA Detection

Field Detection

	Y	N
Y	8	1
N	3	11

# Implementing adaptive sampling



# Sonora tiger salamander - Year 2

- Switched from 0.45 CN to 6  $\mu\text{m}$  cellulose filter



eDNA Detection

Field Detection

	Yes	No
Yes	10	3
No	0	6

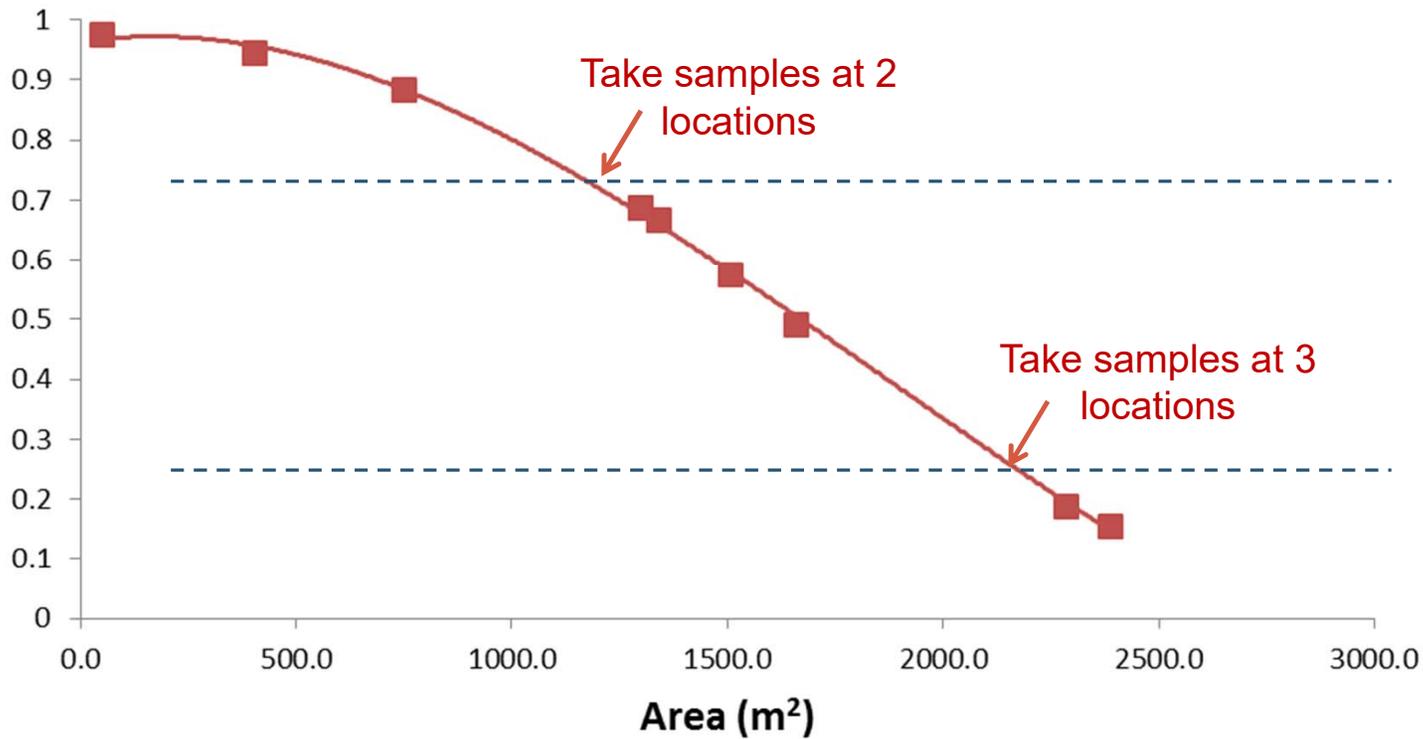


(per sample detection probability = 0.77)

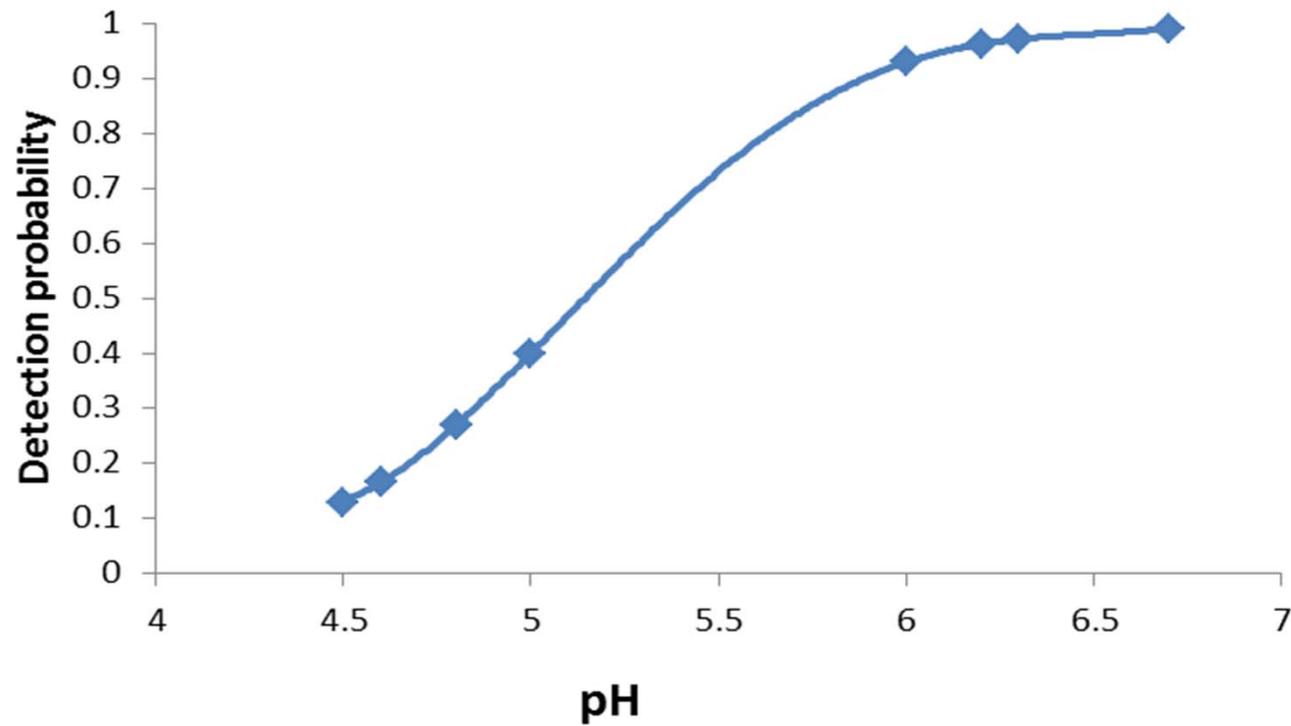
# Chiricahua leopard frog



**Predicted probability of detection per sample**



# Reticulated flatwoods salamander



# Integrating eDNA and field methods



# eDNA field sampling protocols - published

## Published sampling protocols

WSU: [https://labs.wsu.edu/edna/edna\\_protocols/](https://labs.wsu.edu/edna/edna_protocols/)

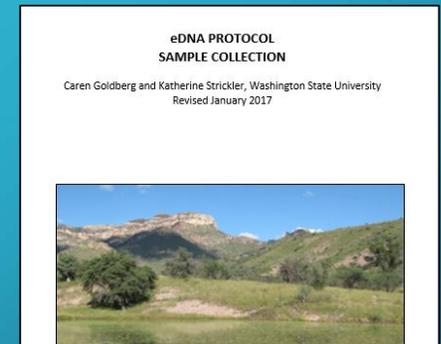
USGS: <https://pubs.usgs.gov/tm/02/a13/tm2a13.pdf>

USFS: [http://www.fs.fed.us/rm/pubs/rmrs\\_gtr355.pdf](http://www.fs.fed.us/rm/pubs/rmrs_gtr355.pdf)

## ▶ Every sampling situation is unique

- Protocols may need small tweaks to work in your system

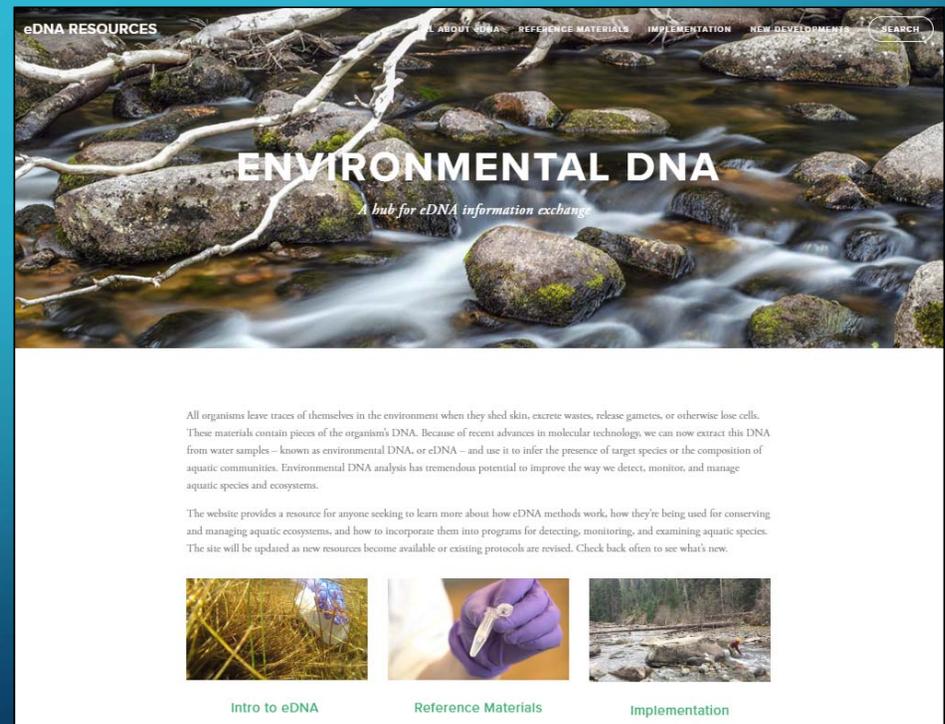
*(but be careful!)*



# eDNA online resource center

- Knowledge base
  - Intro to eDNA
  - Project profiles
  - Lessons learned
  - Links to research and commercial labs
- Guidance
  - Protocols
  - Technical details
- Community hub
  - Information exchange
  - Emerging technology

[www.ednareources.com](http://www.ednareources.com)



eDNA RESOURCES

ABOUT eDNA REFERENCE MATERIALS IMPLEMENTATION NEW DEVELOPMENTS SEARCH

## ENVIRONMENTAL DNA

*A hub for eDNA information exchange*

All organisms leave traces of themselves in the environment when they shed skin, excrete wastes, release gametes, or otherwise lose cells. These materials contain pieces of the organism's DNA. Because of recent advances in molecular technology, we can now extract this DNA from water samples – known as environmental DNA, or eDNA – and use it to infer the presence of target species or the composition of aquatic communities. Environmental DNA analysis has tremendous potential to improve the way we detect, monitor, and manage aquatic species and ecosystems.

The website provides a resource for anyone seeking to learn more about how eDNA methods work, how they're being used for conserving and managing aquatic ecosystems, and how to incorporate them into programs for detecting, monitoring, and examining aquatic species. The site will be updated as new resources become available or existing protocols are revised. Check back often to see what's new.

[Intro to eDNA](#) [Reference Materials](#) [Implementation](#)

# Thank You

