Monitoring amphibian populations using environmental DNA

KATHERINE STRICKLER AND CAREN GOLDBERG

Monitoring amphibian and reptile populations using environmental DNA (Legacy Projects 12-616, 14-616)

Environmental DNA as a tool for inventory and monitoring of aquatic invertebrates (ESTCP Project RC-201204)

Katherine Strickler
Washington State University

Caren Goldberg
Washington State University

Alex Fremier
Washington State University

Outline

Overview of eDNA technology

eDNA methods

eDNA surveys on DoD installations

Implementing eDNA in aquatic monitoring

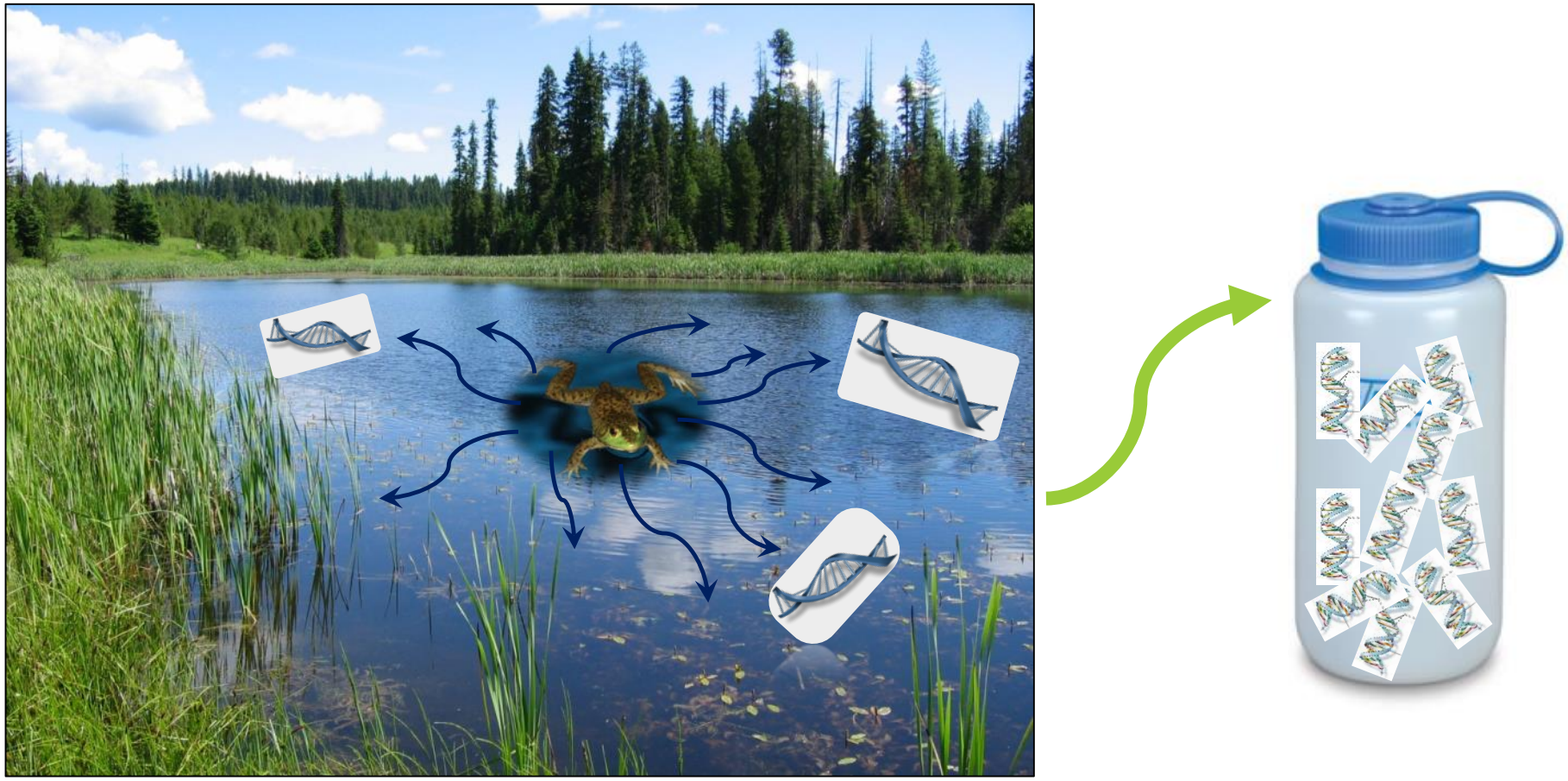
eDNA online resource center

DNA in the environment

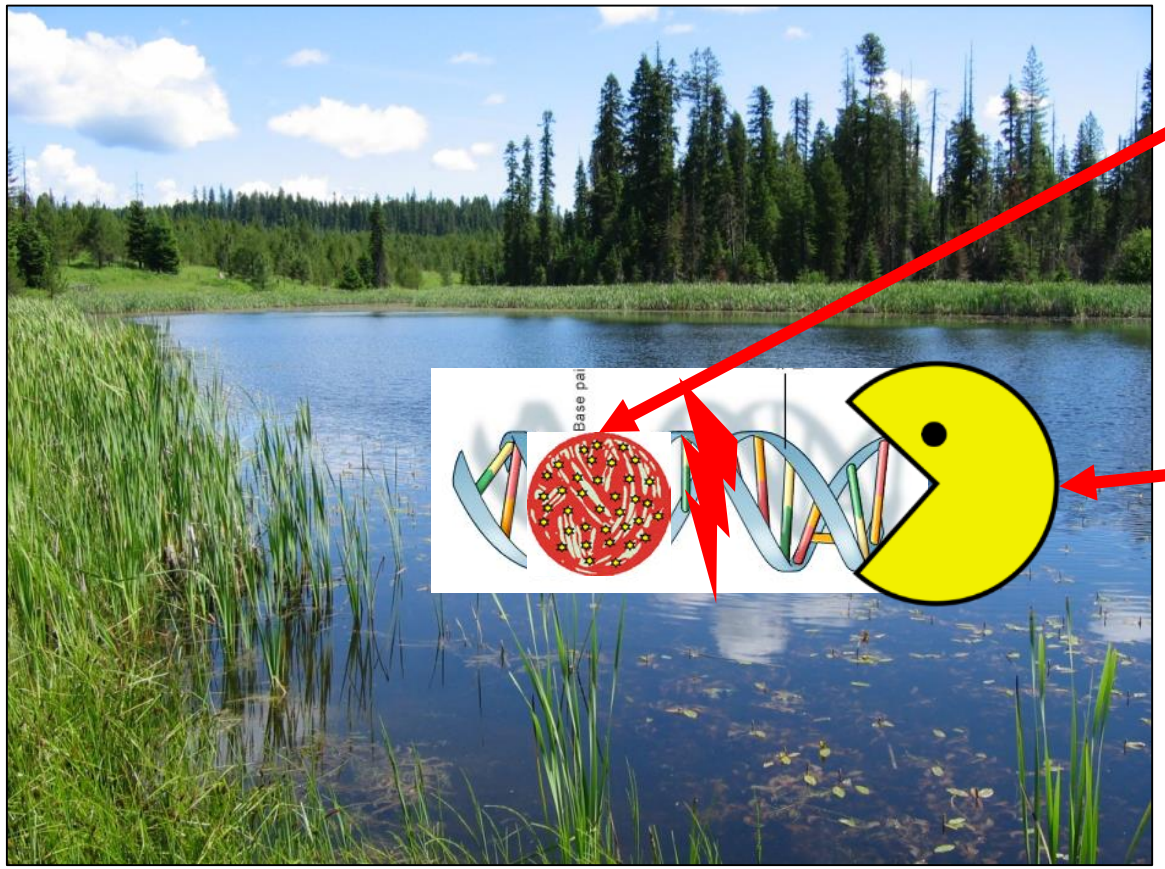
Bioforsk Svanhovd photo

NPS Photo/Cookie Ballou

DNA in the aquatic environment



DNA in the aquatic environment



DNA of ~100 bp can persist

2 – 3 weeks

Pioneering eDNA papers

biology letters

Biol. Lett.
doi:10.1098/rsbl.2008.0118
Published online

Population genetics

Species detection using environmental DNA from water samples

Gentile Francesco Ficetola[1,2,*], Claude Miaud[2], François Pompanon[1] and Pierre Taberlet[1]

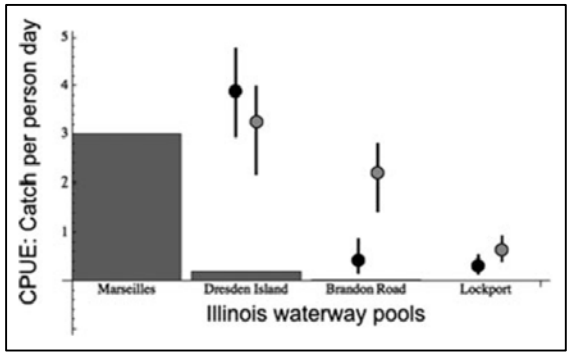
Table 1. Rate of bullfrog detection in water samples.

pond	bullfrog presence and relative abundance	water samples positives at least once	positive PCRs
1	yes-low	2/3	2/9
2	yes-low	3/3	6/9
3	yes-low	2/3	2/9
4	yes-high	3/3	8/9
5	yes-high	3/3	6/9
6	yes-high	3/3	8/10
7	no	0/3	0/9
8	no	0/3	0/9
9	no	0/3	0/15

LETTER

"Sight-unseen" detection of rare aquatic species using environmental DNA

Christopher L. Jerde[1], Andrew R. Mahon[1], W. Lindsay Chadderton[2], & David M. Lodge[1]



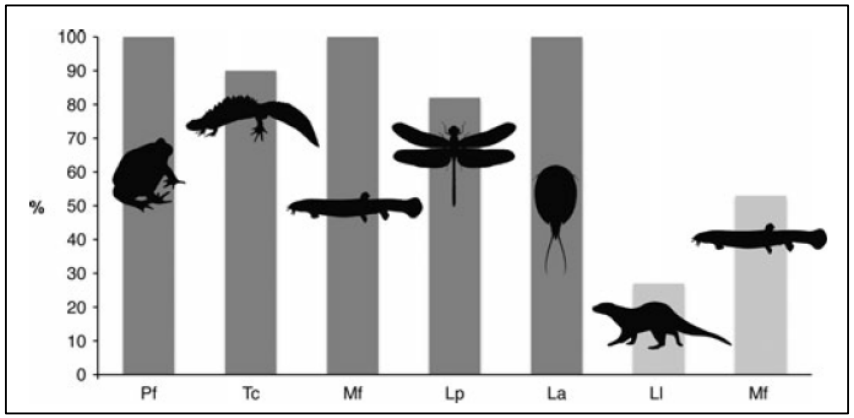
MOLECULAR ECOLOGY

Molecular Ecology (2011) doi: 10.1111/j.1365-294X.2011.05418.x

FROM THE COVER

Monitoring endangered freshwater biodiversity using environmental DNA

PHILIP FRANCIS THOMSEN,[1]* JOS KIELGAST,[1]* LARS L. IVERSEN,† CARSTEN WIUF,‡ MORTEN RASMUSSEN,* M. THOMAS P. GILBERT,* LUDOVIC ORLANDO* and ESKE WILLERSLEV*



OPEN ACCESS Freely available online PLoS one

Molecular Detection of Vertebrates in Stream Water: A Demonstration Using Rocky Mountain Tailed Frogs and Idaho Giant Salamanders

Caren S. Goldberg[1]*, David S. Pilliod[2], Robert S. Arkle[2], Lisette P. Waits[1]

Table 1. Sampling sites, dates of sampling, PCR success for each species, and densities of Idaho giant salamanders (*Dicamptodon aterrimus;* DIAT) and Rocky Mountain tailed frogs (*Ascaphus montanus;* ASMO) where stream filter samples were taken, estimated using field methods in summer 2010.

Site	Latitude	Longitude	Date sampled	DIAT per m^2	DIAT PCR success (%)	ASMO per m^2	ASMO PCR success (%)
Phase 1							
Nasty Creek	44.877	−115.696	25Sept10	0.032	100	0.228	100
Phase 2							
Camp Creek	44.890	−115.706	27Mar11	0.036	100	0.097	16.7
Deadman Creek	44.966	−115.663	27Mar11	0.011	100	0.149	0
Goat Creek	44.759	−115.684	27Mar11	0.029	100	0.052	33.3
Nasty Creek	44.877	−115.696	03Apr11	0.032	100	0.228	33.3
Reegan Creek	44.949	−115.587	27Mar11	0.011	100	0.337	16.7

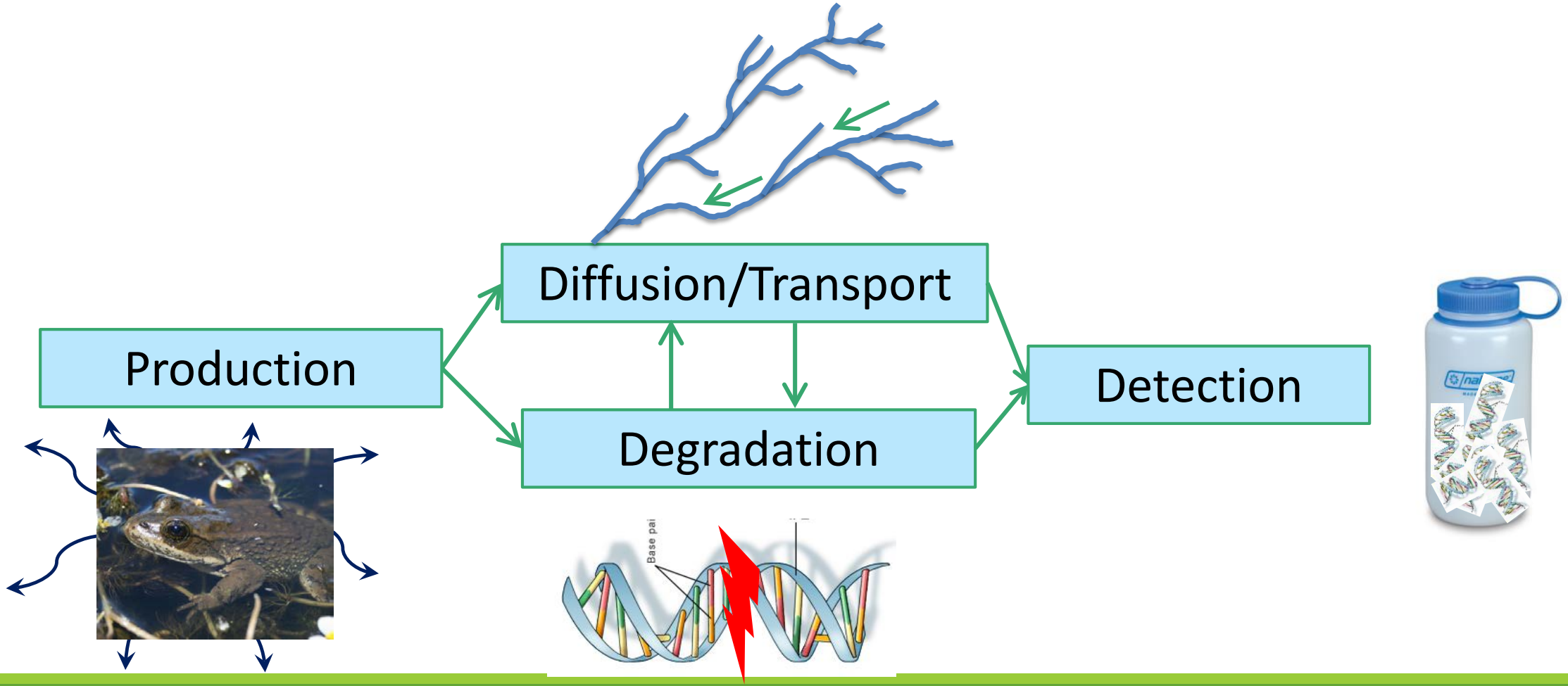
eDNA in practice

- Salamanders
- Frogs
- Snakes
- Marine mammals
- Freshwater fish
- Marine fish
- Freshwater turtles
- Sea turtles
- Freshwater insects
- Crustaceans
- Mollusks
- Nematodes
- Aquatic plants
- Bd
- Ranavirus
- …

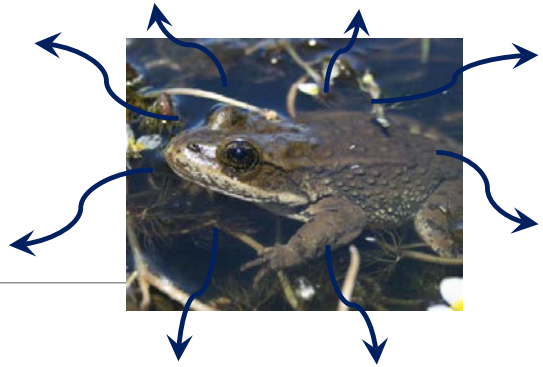
Advantages of eDNA

- Non-destructive
- Highly sensitive – higher detection probabilities
- Multi-species detections (including pathogens)
- Reduced need for taxon-specific field training
- Reduced permitting requirements

Processes affecting eDNA detection



eDNA Production



Species

Life stage

Disease status

Reproductive status

Diet

Season

Habitat structure

Density

and more…

eDNA Removal

Diffusion

Wind

Radiation

Stratification and Turnover

Transport

Discharge

Mixing

Transient storage

eDNA Removal

Degradation

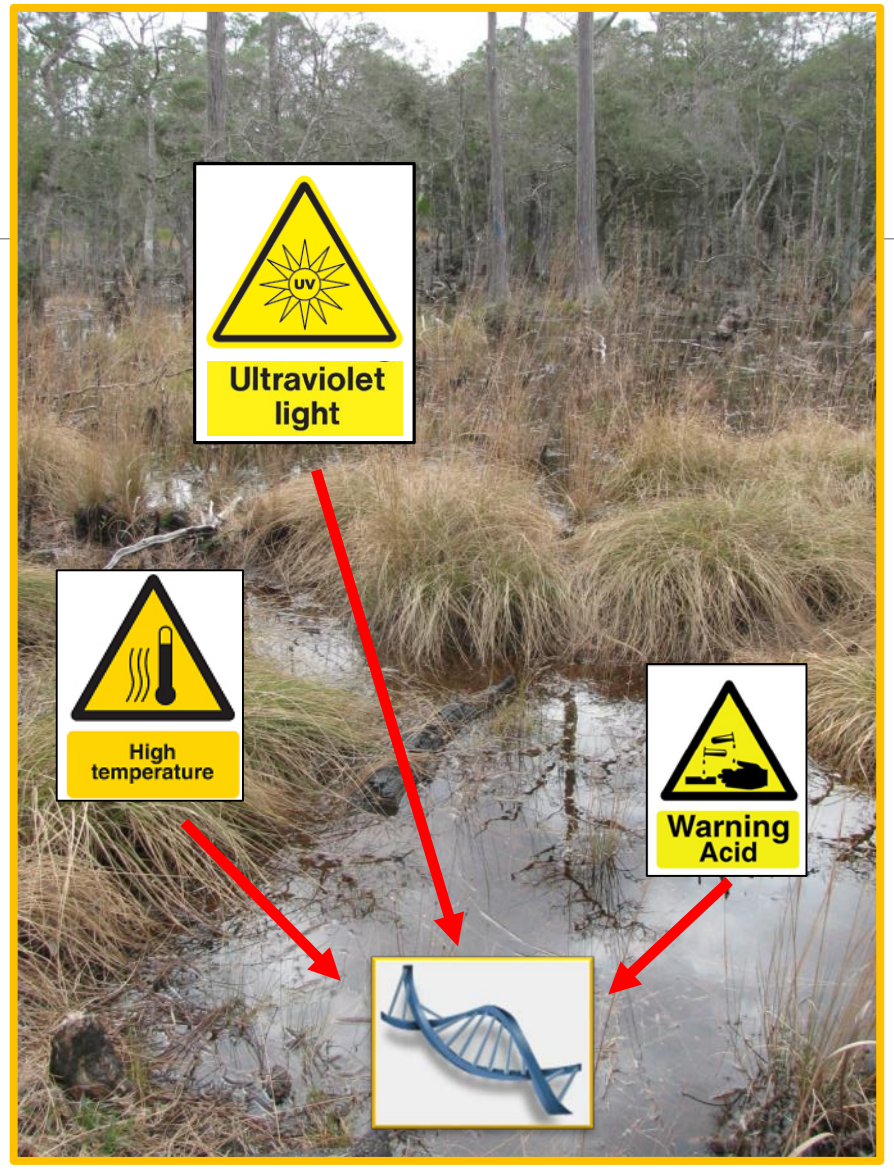
pH

UV

Temperature

Settling

Adsorption to particles



eDNA detection

DNA barcoding: All individuals within a species share particular sequences

Thamnophis eques (mtDNA):

…GAAAGGCCCTAACCT**G**GTAGGACCAATA…

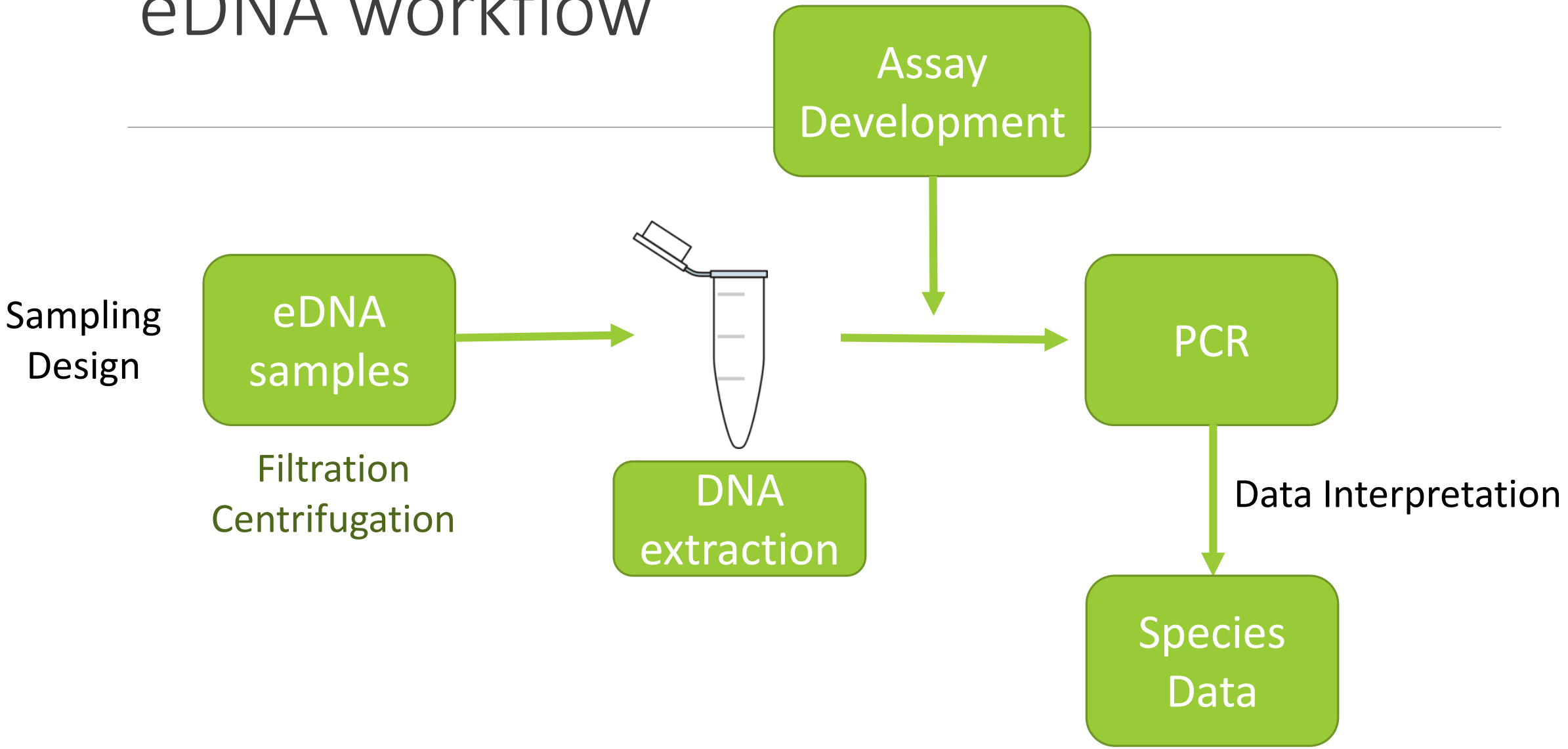
Thamnophis cyrtopsis (mtDNA):

…GAAAGGCCC**C**AACCT**A**GTAGGACCAATA…

Wood et al. 2011

www.barcodeoflife.org

eDNA workflow



Methodological Approaches

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

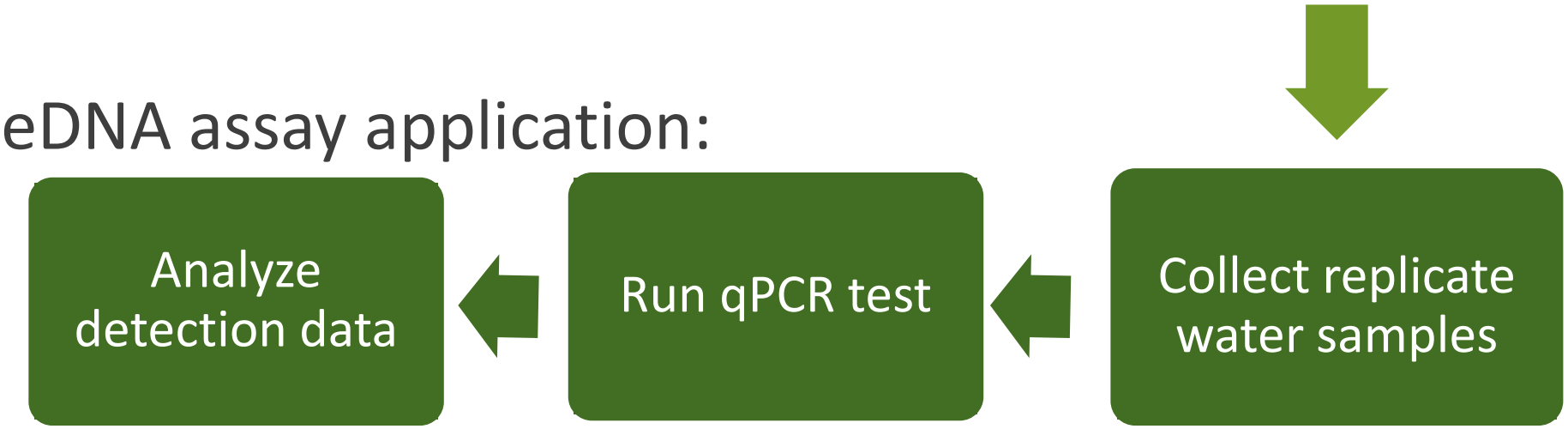
Methods: Target Species Approach

- Useful when management is focused on a single species
- High specificity and sensitivity

Species-specific eDNA detection

eDNA assay development:

eDNA assay application:



eDNA Inference for target species

eDNA *can* tell us:

- Recent target species presence
- Amount of eDNA in a sample
  - *Correlated at some scale with population density*
- Pathogen presence
- Presence of potential hybridizing non-native species

eDNA *can't* tell us:

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

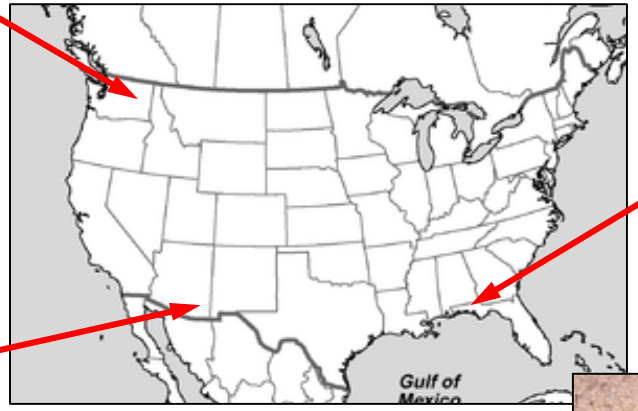
U.S. Air Force Photo/Heide Couch

DoD eDNA demonstration sites

FWS photo/Eric Engbretson

Yakima Training Center, WA

FWS photo/Dan Cox



USGS photo

Eglin AFB, FL

FWS photo/John Jensen

Fort Huachuca, AZ

DoD eDNA demonstration sites

Fort Huachuca (AZ)

- Arizona treefrog
- Northern Mexican gartersnake
- Chiricahua leopard frog
- American bullfrogs
- Sonora tiger salamander
- Barred tiger salamander
- Ranavirus
- Bd

AZGFD photo

AZGFD photo

DoD eDNA demonstration sites

Eglin Air Force Base (FL)

- Reticulated flatwoods salamander
- Ornate chorus frog

USGS photo

FWS photo/John Jensen

Yakima Training Center (WA)

- Bull trout, spring and fall Chinook, brook trout

FWS photo/Dan Brewer

FWS photo/Dan Cox

FWS photo/Eric Engbretson

Developing species-specific guidance

- Collect 4 replicate water filter samples in coordination with field surveys
- Compare detection probabilities of eDNA vs. field surveys
- Identify environmental covariates that influence detection probabilities

Developing species-specific guidance

Water sampling

- 250 mL - 1 L
- 0.45 – 6 µm cellulose filter
- Preserved in ethanol or dried

Developing species-specific guidance

Measuring environmental covariates

- UV exposure
- Conductivity
- Water temperature
- pH
- Sample volume
- Size of water body

Use occupancy modeling to evaluate effects of covariates on detection probabilities

Fort Huachuca, AZ

Forests and grasslands

Year-round tanks

Summer monsoon pools

- Diffusion: Low
- Degradation: Moderate
  - High temperatures
  - High UV
  - Basic (high pH)

Sonora tiger salamander detection

- Federally endangered subspecies
- Breeds in wetlands

Sonora tiger salamander detection - 2013

4 replicates

≤ 250 mL each

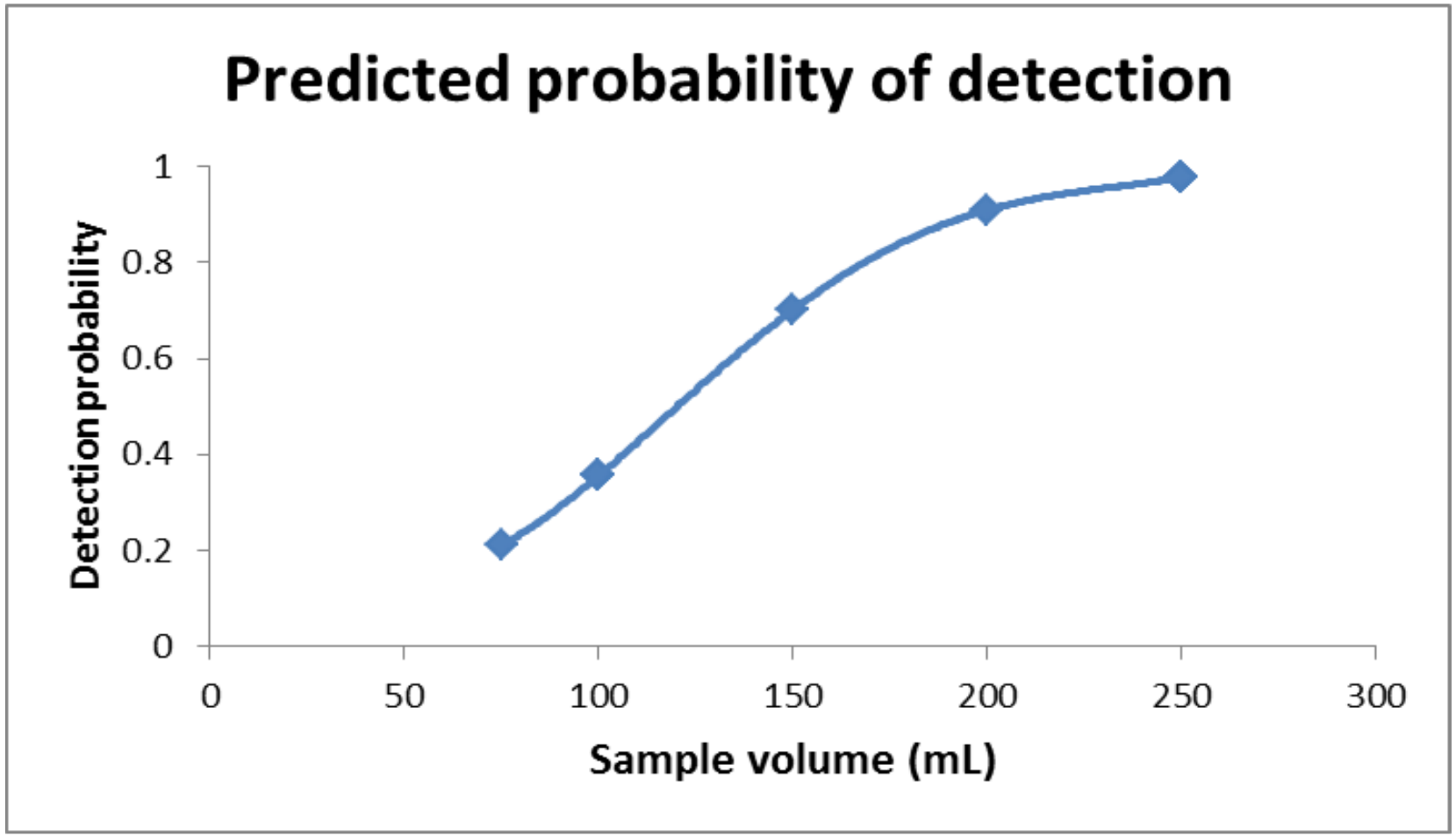
0.45 µm cellulose nitrate filter

Sonora tiger salamander detection - 2013

eDNA Detection \ Field Detection	Yes	No
Yes	8	1
No	3	11

(per sample detection probability = 0.73)

Sonora tiger salamander detection - 2013

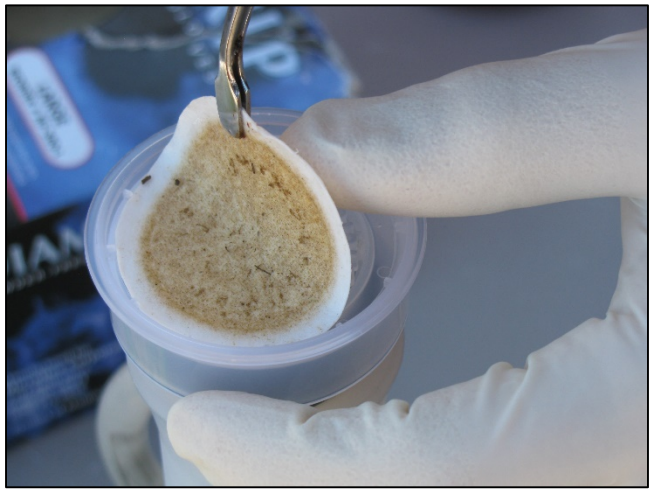


Sonora tiger salamander detection - 2014

4 replicates

250 mL each

6 µm cellulose filter



Sonora tiger salamander detection

2013

Field Detection

eDNA Detection

	Yes	No
Yes	8	1
No	3	11

2014

Field Detection

eDNA Detection

	Yes	No
Yes	10	3
No	0	6

(per sample detection probability = 0.77)

Chiricahua leopard frog detection

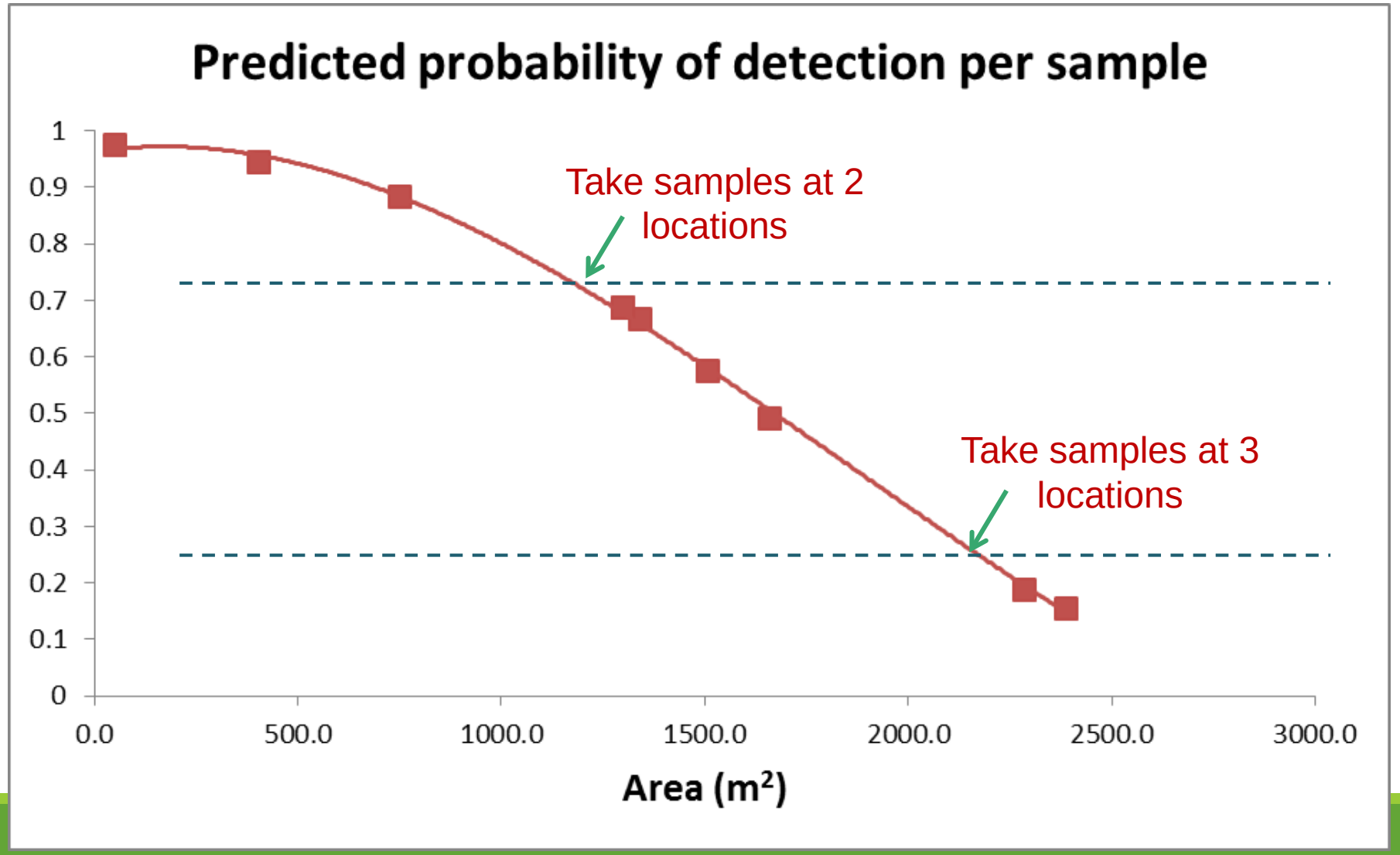
- Federally threatened
- Year-round breeder
- Permanent wetlands

Chiricahua leopard frog detection - 2012

eDNA Detection \ Field Detection	Yes	No
Yes	7	2
No	1	10

(per sample = 0.62)

Adaptive sampling design - spatial



Chiricahua leopard frog detection - 2013

2013

Field Detection

eDNA Detection	Yes	No
Yes	7	2
No	1	10

2014

Field Detection

eDNA Detection	Yes	No
Yes	4	2
No	1	10

(per sample = 0.63)

Eglin Air Force Base, FL

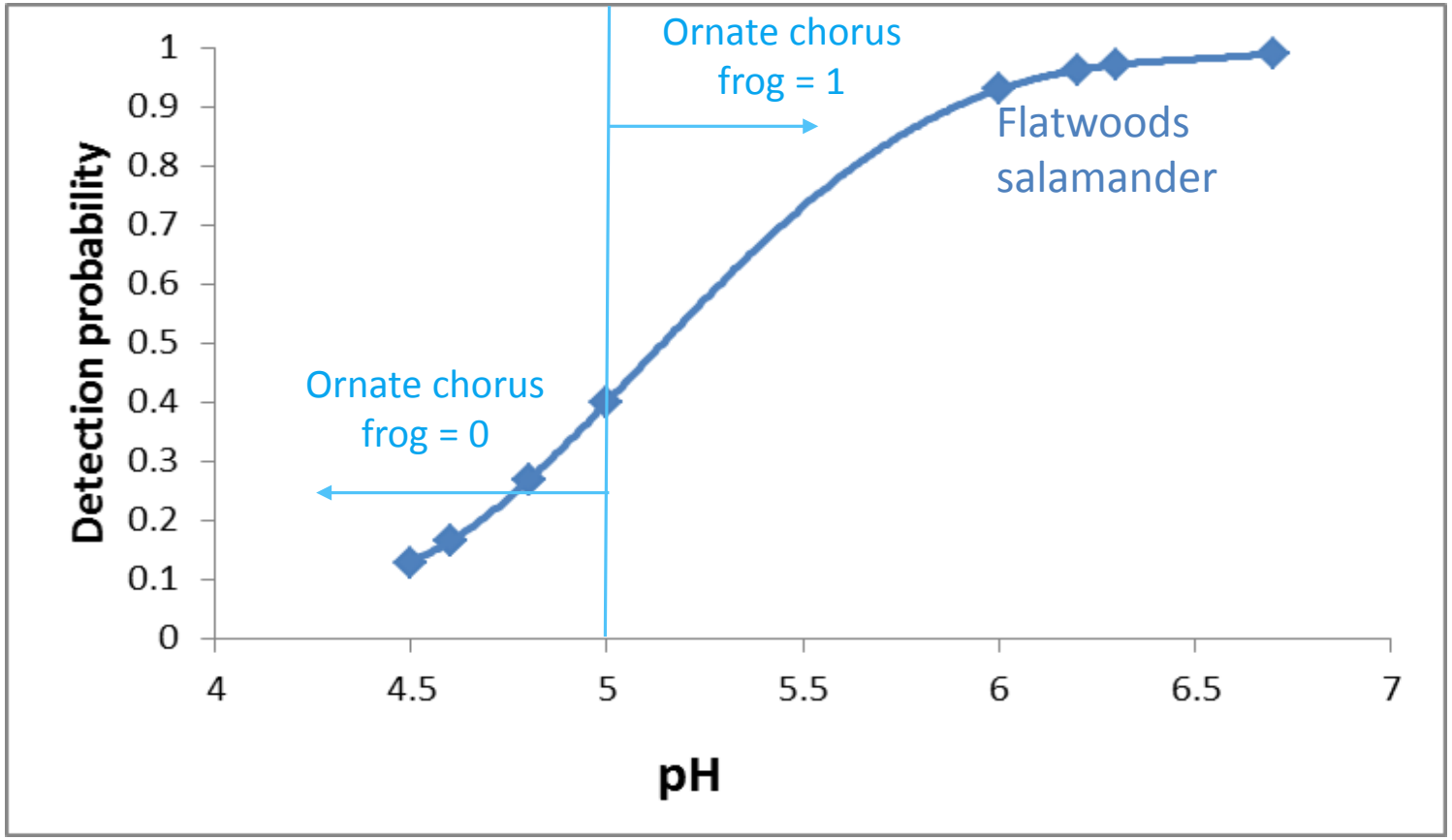
Forested wetlands

- Ephemeral
- Shallow
- Complex

- Diffusion: Very low
- Degradation: Very high
  - High temperature
  - High UV
  - Acidic

Flatwoods salamander and ornate chorus frog detection - 2014

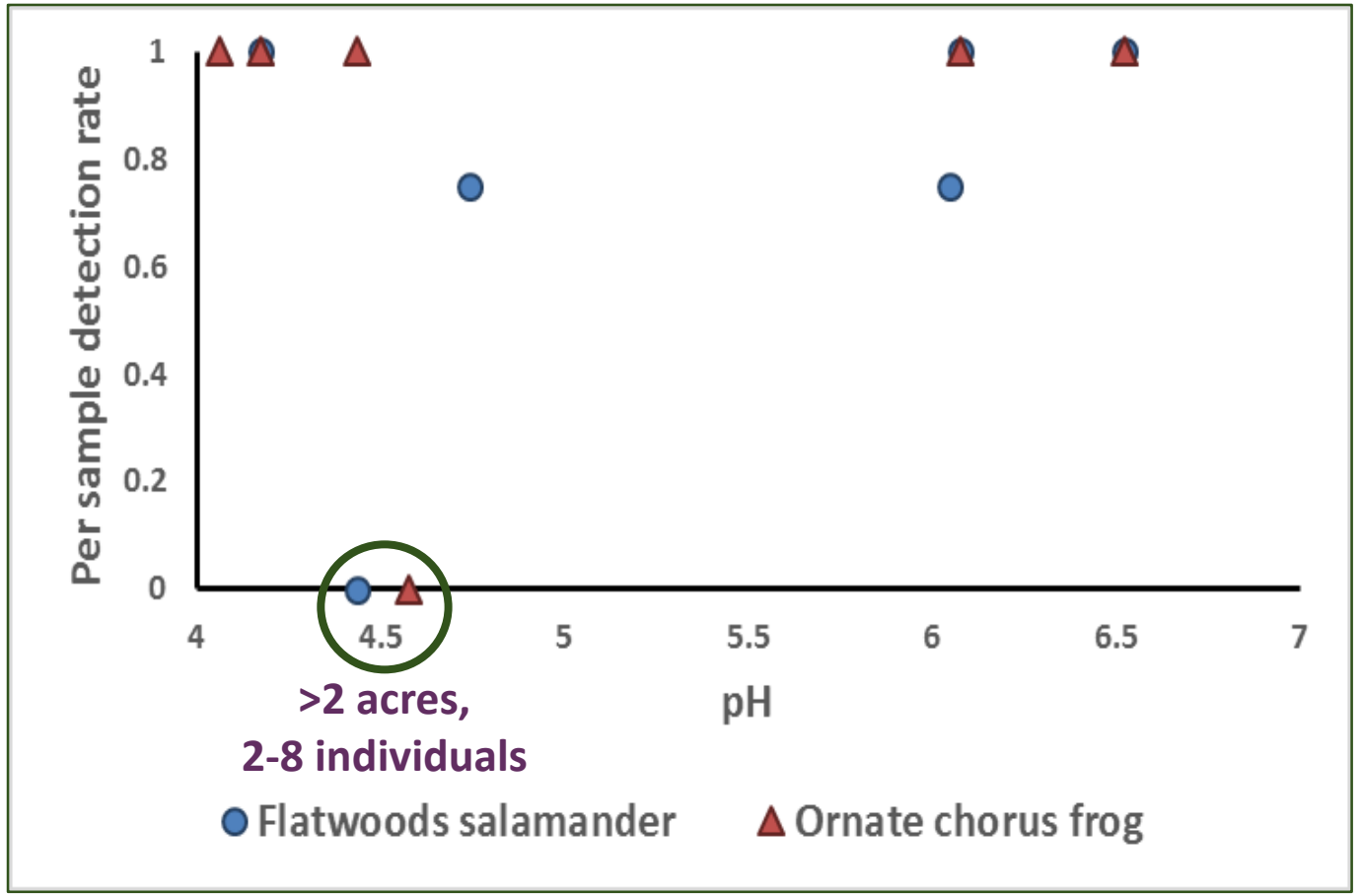
500 mL samples
from 4 locations, mixed



Flatwoods salamander and ornate chorus frog detection - 2015

500 mL samples, mixed

- pH > 5 = sampled at 4 locations
- pH < 5 = sampled at 8 locations



Flatwoods salamander and ornate chorus frog detection - 2015

500 mL samples, mixed

- pH > 5 = sampled at 4 locations
- pH < 5 = sampled at 8 locations

Field detection

eDNA Detection	Yes	No
Yes	8	2
No	2	4

Short surveys (not full protocol)

Large, acidic, low density

Summary

- eDNA methods are very powerful, but imperfect
- Study design needs to be tailored to each system
- A pilot study is necessary to optimize detection probabilities
- Adaptive sampling strategies can increase efficiency and sensitivity

Implementing Environmental DNA in Aquatic Monitoring

Implementing eDNA surveys

1. Critically evaluate eDNA's potential benefits
2. Select appropriate eDNA approach
3. Conduct a pilot survey
4. Implement adaptive sampling protocol
5. Consider how eDNA sampling can complement existing field methods

Step 1: Deciding when to use eDNA

When is eDNA is most useful?

Target species are difficult to detect

- elusive
- rare/low density
- difficult to identify

Conventional survey methods are problematic

- low detection rates
- expensive
- require extensive training or certification
- destructive to the species or its habitat

Step 1: Deciding when to use eDNA

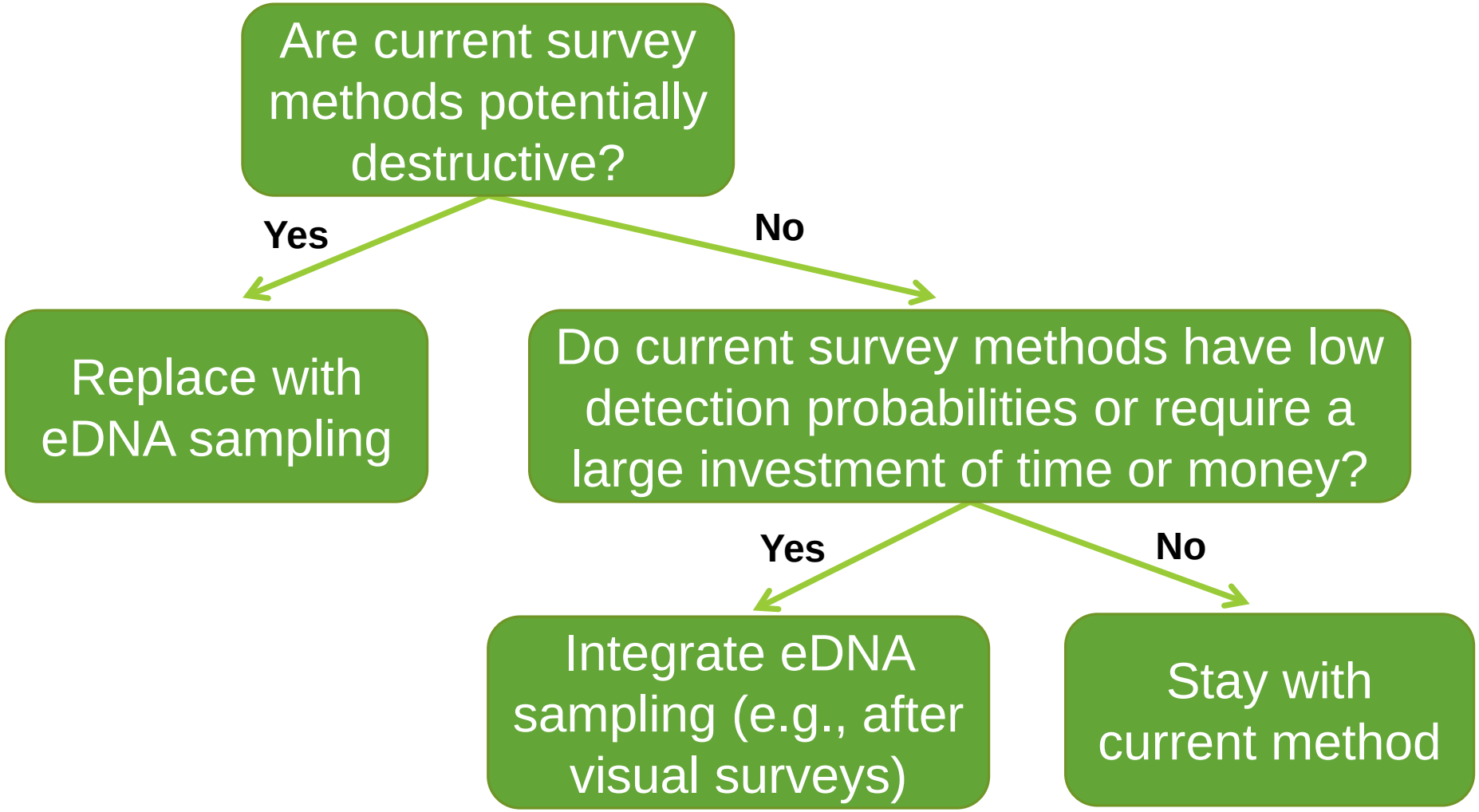
When is eDNA is most useful?

Community-level or system-level information is needed

Biomonitoring (e.g., IBIs)

Conventional surveys are:

- typically targeted toward individual species or species groups
- often biased toward individual species or groups of species
- many types of surveys may be required to detect multiple species


Are current survey methods potentially destructive?
Yes
No
Replace with eDNA sampling
Do current survey methods have low detection probabilities or require a large investment of time or money?
Yes
No
Integrate eDNA sampling (e.g., after visual surveys)
Stay with current method

Step 2: Deciding on eDNA method

Target species approach? OR **eDNA metabarcode approach?**

Management concern is targeted toward one or several species

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

Management goal is biodiversity monitoring

- Clean Water Act - 303(d)
- List of targeted species is long (e.g., vernal pools in CA - 20 listed species)

Step 3: Conduct a pilot survey

Design a pilot protocol that considers:

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

Step 4: Implement adaptive sampling

- Revise sampling strategy to optimize detection probabilities
- Continue to measure environmental and sampling factors
- Periodically re-evaluate sampling strategy

Step 5: Consider how eDNA sampling can complement existing field methods

eDNA online resource center

Image: todaymade.com

THE EDWARD R. Murrow COLLEGE OF COMMUNICATION

Ryan Risenmay

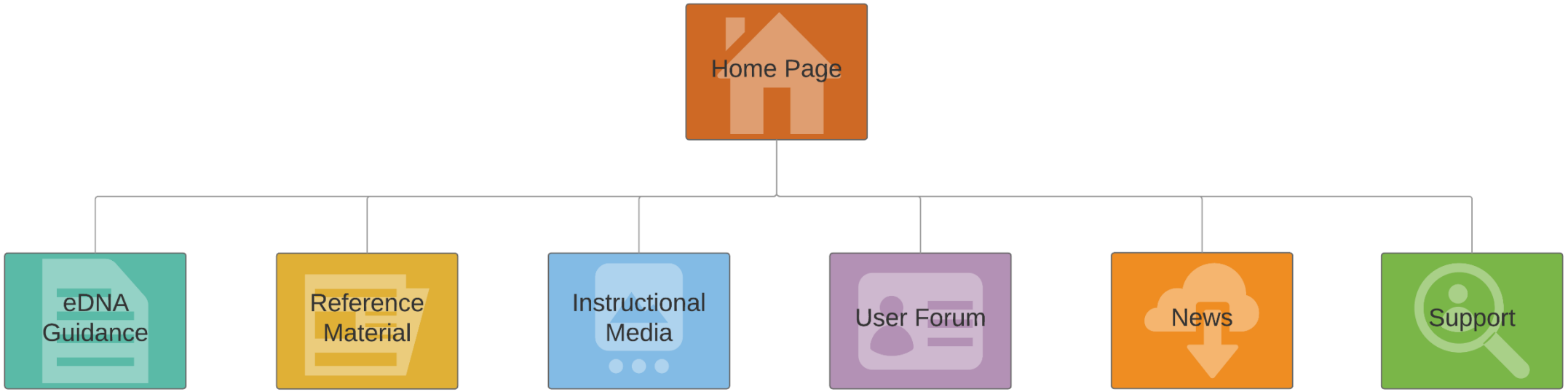
Benjamin Shors

WASHINGTON STATE UNIVERSITY

eDNA online resource center

Central hub for collaboration and information exchange

SIMPLIFIED - NOT FINAL



- Intro to eDNA (if, when, how)
- Field protocols
- Project profiles (examples)
- Lessons learned
- Research (results, relevance)
- Implementation methods
- Lab selection & protocols

- Technical specs & details
- Training materials
- eDNA literature & references
- Materials lists
- Reports (DoD, research, other)
- Sampling examples
- DoD Info Center (projects, species list, requests, recommendations)

- Webinars
- Sample project videos
- Demo videos (eDNA overview, field sampling processes)
- Podcasts/audio
- Workshop videos
- Slideshows

- Open blog
- Advice exchange

- News alerts
- Events
- Social media feeds

- FAQ
- Answers from experts
- Contacts

eDNA online resource center

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

Fort Huachuca
Eglin AFB
Yakima Training Center