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
DNA in the aquatic environment
DNA in the aquatic environment

DNA of ~100 bp can persist 2 – 3 weeks
Species detection using environmental DNA from water samples

Gentile Francesco Ficetola¹,²,*, Claude Miaud², François Pompanon¹ and Pierre Taberlet¹

Table 1. Rate of bullfrog detection in water samples.

<table>
<thead>
<tr>
<th>pond</th>
<th>bullfrog presence and relative abundance</th>
<th>water samples positives at least once</th>
<th>positive PCRs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>yes-low</td>
<td>2/3</td>
<td>2/9</td>
</tr>
<tr>
<td>2</td>
<td>yes-low</td>
<td>3/3</td>
<td>6/9</td>
</tr>
<tr>
<td>3</td>
<td>yes-low</td>
<td>2/3</td>
<td>2/9</td>
</tr>
<tr>
<td>4</td>
<td>yes-high</td>
<td>3/3</td>
<td>8/9</td>
</tr>
<tr>
<td>5</td>
<td>yes-high</td>
<td>3/3</td>
<td>6/9</td>
</tr>
<tr>
<td>6</td>
<td>yes-high</td>
<td>3/3</td>
<td>8/10</td>
</tr>
<tr>
<td>7</td>
<td>no</td>
<td>0/3</td>
<td>0/9</td>
</tr>
<tr>
<td>8</td>
<td>no</td>
<td>0/3</td>
<td>0/9</td>
</tr>
<tr>
<td>9</td>
<td>no</td>
<td>0/3</td>
<td>0/15</td>
</tr>
</tbody>
</table>
**LETTER**

“Sight-unseen” detection of rare aquatic species using environmental DNA
Christopher L. Jerde¹, Andrew R. Mahon¹, W. Lindsay Chadderton², & David M. Lodge³

**MOLECULAR ECOLOGY**

Monitoring endangered freshwater biodiversity using environmental DNA
PHILIP FRANCIS THOMSEN,¹ JOS KIELGAST,¹ LARS J. IVERSEN, CARSTEN WIUF, MORTEN RASMUSSEN, M. THOMAS P. GILBERT, LUDOVIC ORLANDO and ESKE WILLERSLEV

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

<table>
<thead>
<tr>
<th>Site</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Date sampled</th>
<th>DIAT per m²</th>
<th>DIAT PCR success (%)</th>
<th>ASMIO per m²</th>
<th>ASMIO PCR success (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Party Creek</td>
<td>44.877</td>
<td>-115.600</td>
<td>25 Sep 2010</td>
<td>0.002</td>
<td>100</td>
<td>0.220</td>
<td>100</td>
</tr>
<tr>
<td>Camp Creek</td>
<td>44.890</td>
<td>-115.706</td>
<td>27 Mar 2011</td>
<td>0.006</td>
<td>100</td>
<td>0.037</td>
<td>16.7</td>
</tr>
<tr>
<td>Shoshone Creek</td>
<td>44.966</td>
<td>-115.661</td>
<td>08 Mar 2011</td>
<td>0.017</td>
<td>100</td>
<td>0.140</td>
<td>0</td>
</tr>
<tr>
<td>Goat Creek</td>
<td>44.759</td>
<td>-115.684</td>
<td>27 Mar 2011</td>
<td>0.009</td>
<td>100</td>
<td>0.052</td>
<td>33.3</td>
</tr>
<tr>
<td>Natty Creek</td>
<td>44.877</td>
<td>-115.696</td>
<td>03 Apr 2011</td>
<td>0.002</td>
<td>100</td>
<td>0.220</td>
<td>33.3</td>
</tr>
<tr>
<td>Nezper Creek</td>
<td>44.944</td>
<td>-115.687</td>
<td>27 Mar 2011</td>
<td>0.001</td>
<td>100</td>
<td>0.337</td>
<td>16.7</td>
</tr>
</tbody>
</table>
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

Diffusion/Transport

Production

Degradation

Detection
<table>
<thead>
<tr>
<th>eDNA Production</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
</tr>
<tr>
<td>Life stage</td>
</tr>
<tr>
<td>Disease status</td>
</tr>
<tr>
<td>Reproductive status</td>
</tr>
<tr>
<td>Diet</td>
</tr>
<tr>
<td>Season</td>
</tr>
<tr>
<td>Habitat structure</td>
</tr>
<tr>
<td>Density</td>
</tr>
<tr>
<td>and more...</td>
</tr>
</tbody>
</table>
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):
...GAAAGGCCCTAACCTG\textbf{G}T\textbf{G}T\textbf{G}AGGACCAATA...

*Thamnophis cyrtopsis* (mtDNA):
...GAAAGGCCCCAACCT\textbf{A}T\textbf{A}T\textbf{A}G\textbf{G}T\textbf{G}T\textbf{G}AGGACCAATA...

Wood et al. 2011
eDNA workflow

Sampling Design

eDNA samples

Filtration
Centrifugation

DNA extraction

Assay Development

PCR

Data Interpretation

Species Data
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:
1. Identify target species set
2. Collect DNA sequence data
3. Create and validate qPCR test

eDNA assay application:
1. Collect replicate water samples
2. Run qPCR test
3. Analyze detection data
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
DoD eDNA demonstration sites

- Fort Huachuca, AZ
- Yakima Training Center, WA
- Eglin AFB, FL

Sources:
- USGS photo
- FWS photo/Eric Engbretson
- FWS photo/Dan Cox
- FWS photo/John Jensen
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
DoD eDNA demonstration sites

Eglin Air Force Base (FL)
• Reticulated flatwoods salamander
• Ornate chorus frog

Yakima Training Center (WA)
• Bull trout, spring and fall Chinook, brook trout
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

<table>
<thead>
<tr>
<th>eDNA Detection</th>
<th>Field Detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>Yes 8</td>
</tr>
<tr>
<td>No</td>
<td>3</td>
</tr>
</tbody>
</table>

(per sample detection probability = 0.73)
Sonora tiger salamander detection - 2013

Predicted probability of detection

Detection probability

Sample volume (mL)
Sonora tiger salamander detection - 2014

4 replicates
250 mL each
6 µm cellulose filter
### Sonora tiger salamander detection

#### 2013

<table>
<thead>
<tr>
<th>Field Detection</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>No</td>
<td>3</td>
<td>11</td>
</tr>
</tbody>
</table>

#### 2014

<table>
<thead>
<tr>
<th>Field Detection</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>No</td>
<td>0</td>
<td>6</td>
</tr>
</tbody>
</table>

(per sample detection probability = 0.77)
Chiricahua leopard frog detection

- Federally threatened
- Year-round breeder
- Permanent wetlands
Chiricahua leopard frog detection - 2012

<table>
<thead>
<tr>
<th>eDNA Detection</th>
<th>Field Detection</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>Yes</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>2</td>
</tr>
<tr>
<td>No</td>
<td>Yes</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>10</td>
</tr>
</tbody>
</table>

(per sample = 0.62)
Adaptive sampling design - spatial

Predicted probability of detection per sample

Take samples at 2 locations

Take samples at 3 locations
Chiricahua leopard frog detection - 2013

<table>
<thead>
<tr>
<th></th>
<th>2013</th>
<th>2014</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>eDNA Detection</strong></td>
<td><strong>Field Detection</strong></td>
<td><strong>Field Detection</strong></td>
</tr>
<tr>
<td>Yes</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>No</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

(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
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

<table>
<thead>
<tr>
<th>eDNA Detection</th>
<th>Field detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>Yes 8</td>
</tr>
<tr>
<td>No</td>
<td>2</td>
</tr>
</tbody>
</table>

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 most useful?**

<table>
<thead>
<tr>
<th>Target species are difficult to detect</th>
<th>Conventional survey methods are problematic</th>
</tr>
</thead>
<tbody>
<tr>
<td>– elusive</td>
<td>– low detection rates</td>
</tr>
<tr>
<td>– rare/low density</td>
<td>– expensive</td>
</tr>
<tr>
<td>– difficult to identify</td>
<td>– require extensive training or certification</td>
</tr>
<tr>
<td></td>
<td>– destructive to the species or its habitat</td>
</tr>
</tbody>
</table>
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: Replace with eDNA sampling

No: Do current survey methods have low detection probabilities or require a large investment of time or money?

Yes: Integrate eDNA sampling (e.g., after visual surveys)

No: 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

Ryan Risenmay
Benjamin Shors
eDNA online resource center

Central hub for collaboration and information exchange
eDNA online resource center

- 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/
Thank you

Fort Huachuca
Eglin AFB
Yakima Training Center