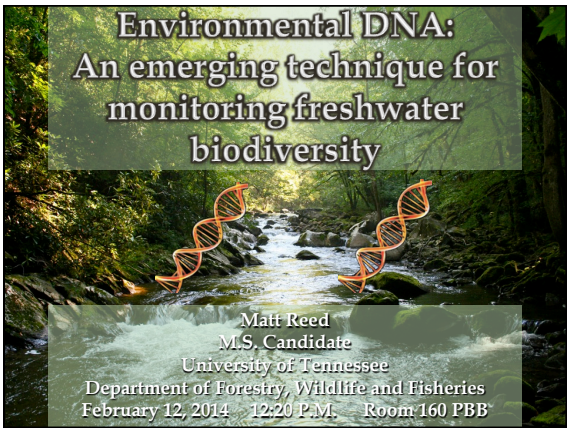

Outline

- Introduction & Background
- Field Applications
- Problems and Limitations
- Case Studies
- Future Research Directions
- Conclusions

Introduction

- **Precise data for species distribution is crucial for efficient conservation of aquatic biodiversity**
- Many aquatic species are notoriously hard to detect
- Recent research has enabled aquatic species detection through analysis of DNA taken from water samples

(Dejean et al. 2011, Ficetola et al. 2008, Goldberg et al. 2011, Hebert et al. 2003, Harvey et al. 2009, Jerde et al. 2011, Margurran 2004, Mehta et al. 2007, Pilloid et al. 2013, Smith 2006, Waits and Paetkau 2005)

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Hebert's Proposal

Taxonomic
is colla

Few taxonomists
> 0.01% of
mated 10-15
species

Most efficient way to sustain species identification
=
utilizing DNA sequences as "taxon barcodes"

Persistence in Aquatic Systems

biology letters

Species detection using environmental DNA from water samples

Gentile Francesco Ficetola, Claude Miaud, François Pompanon and Pierre Taberlet

Biol. Lett. 2008 **4**, doi: 10.1098/rsbl.2008.0118, published 23 August 2008

incubated in filtered lake water with EDTA

- DNA of 400 bp may persist in lake environments for ~1 week at 18°C (64.4°F)

(Matsui et al. 2001)

What is eDNA?

- eDNA = cell-bound or dissolved DNA that persists in the environment
  - Shed cells, excretions (feces), decaying tissues, urine
- Every species has a unique DNA sequence
- Degrades naturally

(Dejean et al. 2011, Ficetola et al. 2008, Goldberg et al. 2011, Waits and Paetkau 2005)

Methodology

- Collecting water at sample sites
- Filter water to concentrate DNA
- Quantitative Polymerase Chain Reaction (qPCR)
- Screen PCR results for sequence of target species

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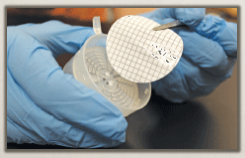


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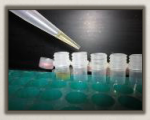


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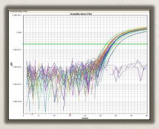
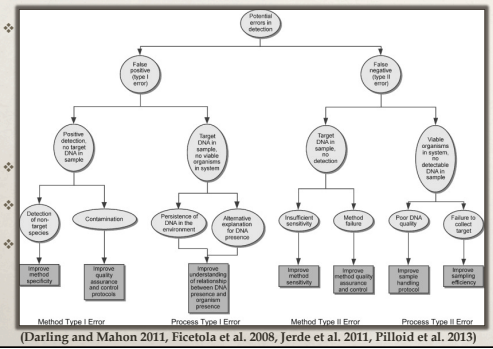


Proposed advantages

- Non-invasive alternative
- "Sight-unseen" detection
- Detection sensitivity
- Cost-effective, reduced physical labor
- Invasive and/or cryptic species

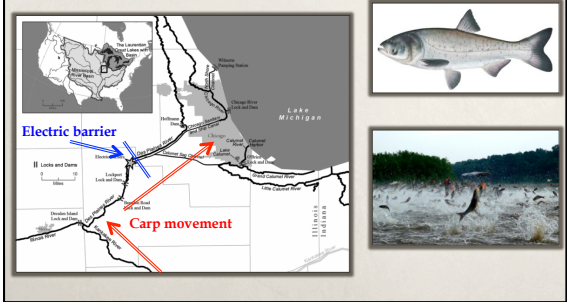
(Dejean et al. 2011, Ficetola et al. 2008, Goldberg et al. 2011, Jerde et al. 2011, Pilloid et al. 2013, Takahara et al. 2012, Thomsen et al. 2012)

Problems & Limitations



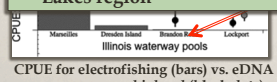
(Darling and Mahon 2011, Ficetola et al. 2008, Jerde et al. 2011, Pilloid et al. 2013)

Asian carp in Lake Michigan
(Jerde et al. 2011)



Asian carp in Lake Michigan
(Jerde et al. 2011)

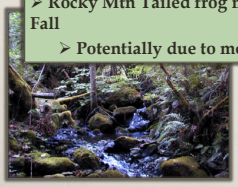
- Collected 1,000 2L surface samples
- Developed species-specific test for bighead & silver carp
- No false positives in rivers w/o Asian carp & when tested w/ other species
- eDNA surveillance = best approach for the vast scale of monitoring efforts needed in the Great Lakes region



CPUE for electrofishing (bars) vs. eDNA sampling for silver (gray dots) and bighead (black dots) carp in 2009-2010.

Headwater amphibians, Idaho
(Goldberg et al. 2011)


- No false positives
- 4 co-occurring amphibians species
- Rocky Mtn Tailed frog more difficult to detect in Spring than Fall
  - Potentially due to metamorphosis timing?

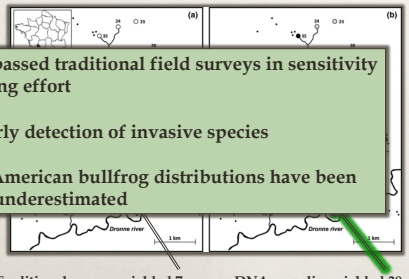


- Species-specific PCR test
- Peristaltic pump used for water samples
- PCR tests and sequencing

American bullfrogs in France
(DeJean et al. 2012)


- Compared detection sensitivity of traditional field methods with new eDNA techniques
- 48 sites surveyed using both methods

American bullfrogs in France
(DeJean et al. 2012)



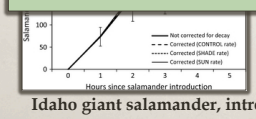
- eDNA surpassed traditional field surveys in sensitivity & sampling effort
- Enables early detection of invasive species
- Suggests American bullfrog distributions have been strongly underestimated

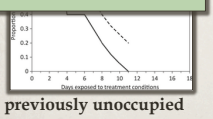
Traditional surveys yielded 7 bullfrog detections

eDNA sampling yielded 38 positive bullfrog detections

Detection factors
(Pilloid et al. 2014)

- Temperature and light conditions play key role in rapid DNA degradation
- eDNA persisted for roughly 8-18 days (longer in shaded treatment)
- Detectability decreased significantly ~50 m downstream





Idaho giant salamander, introduced to previously unoccupied streams

Future Research Directions

- Influences on eDNA detection:
  - Field methods
  - Lab protocols
  - Environmental conditions
- Factors influencing:
  - Lower limits of detection
  - Residence time of eDNA in varying aquatic environments
- Correlation between DNA concentration & species density

(Darling and Mohan 2011, Pilloid et al. 2013, Thomsen et al. 2012)

Conclusions

- Vast potential for monitoring aquatic systems
- Proven efficiency in multi-species detection
- Effective detection of cryptic species
- Early detection & monitoring of invasive species
- Cost effective / reduced physical labor
- Many uncertainties remain!

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Photos

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- Jason Jones, NV Department of Wildlife
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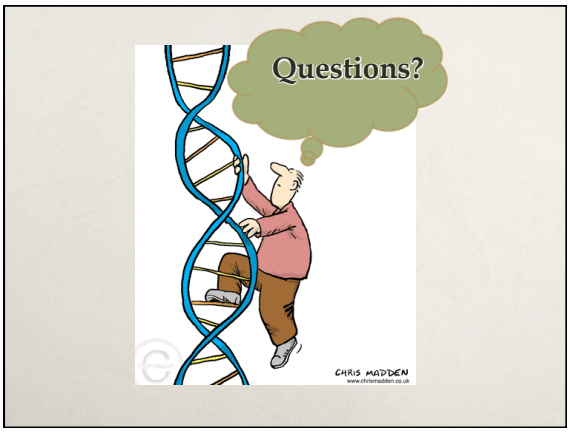
- Paul Hebert, Biodiversity Institute of Ontario
- U.S. Fish and Wildlife Service, Midwest Region
- U.S. Forest Service, Pacific Southwest Research Station
- U.S. Army Corps of Engineers


Questions?
CHRIS MADDEN