AN ENVIRONMENTAL DNA APPROACH TO SAMPLING FOR THE PRESENCE AND ABUNDANCE OF THE ENDANGERED SPOTTED TURTLE (*Clemmys guttata*)

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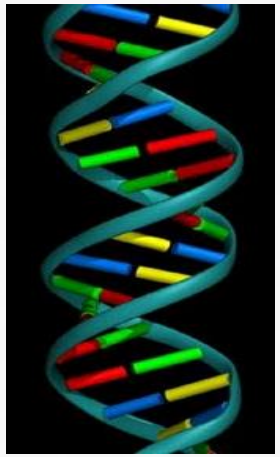

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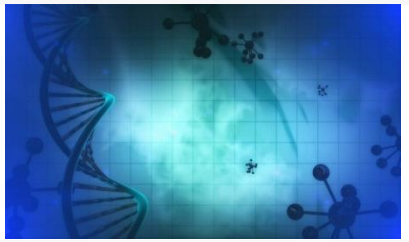
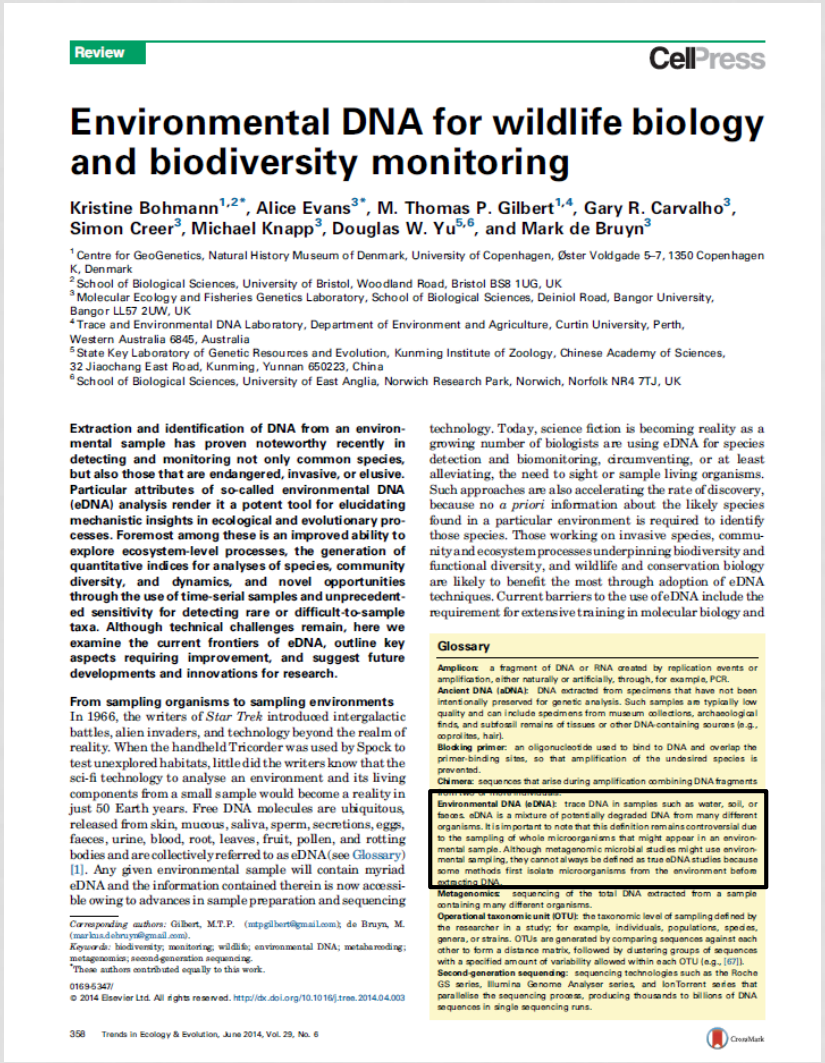
WHAT IS eDNA?





Review

CellPress

Environmental DNA for wildlife biology and biodiversity monitoring

Kristine Bohmann[1,2*], Alice Evans[3*], M. Thomas P. Gilbert[1,4], Gary R. Carvalho[3], Simon Creer[3], Michael Knapp[3], Douglas W. Yu[5,6], and Mark de Bruyn[3]

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Extraction and identification of DNA from an environmental sample has proven noteworthy recently in detecting and monitoring not only common species, but also those that are endangered, invasive, or elusive. Particular attributes of so-called environmental DNA (eDNA) analysis render it a potent tool for elucidating mechanistic insights in ecological and evolutionary processes. Foremost among these is an improved ability to explore ecosystem-level processes, the generation of quantitative indices for analyses of species, community diversity, and dynamics, and novel opportunities through the use of time-serial samples and unprecedented sensitivity for detecting rare or difficult-to-sample taxa. Although technical challenges remain, here we examine the current frontiers of eDNA, outline key aspects requiring improvement, and suggest future developments and innovations for research.

From sampling organisms to sampling environments

In 1966, the writers of *Star Trek* introduced intergalactic battles, alien invaders, and technology beyond the realm of reality. When the handheld Tricorder was used by Spock to test unexplored habitats, little did the writers know that the sci-fi technology to analyse an environment and its living components from a small sample would become a reality in just 50 Earth years. Free DNA molecules are ubiquitous, released from skin, mucous, saliva, sperm, secretions, eggs, faeces, urine, blood, root, leaves, fruit, pollen, and rotting bodies and are collectively referred to as eDNA (see Glossary) [1]. Any given environmental sample will contain myriad eDNA and the information contained therein is now accessible owing to advances in sample preparation and sequencing technology. Today, science fiction is becoming reality as a growing number of biologists are using eDNA for species detection and biomonitoring, circumventing, or at least alleviating, the need to sight or sample living organisms. Such approaches are also accelerating the rate of discovery, because no *a priori* information about the likely species found in a particular environment is required to identify those species. Those working on invasive species, community and ecosystem processes underpinning biodiversity and functional diversity, and wildlife and conservation biology are likely to benefit the most through adoption of eDNA techniques. Current barriers to the use of eDNA include the requirement for extensive training in molecular biology and

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Keywords: biodiversity; monitoring; wildlife; environmental DNA; metabarcoding; metagenomics; second-generation sequencing.
[*]These authors contributed equally to this work.

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Glossary

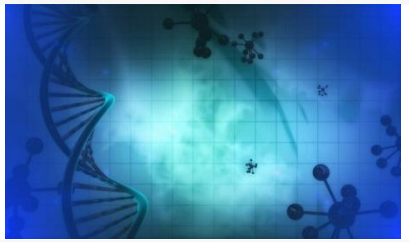
Amplicon: a fragment of DNA or RNA created by replication events or amplification, either naturally or artificially, through, for example, PCR.
Ancient DNA (aDNA): DNA extracted from specimens that have not been intentionally preserved for genetic analysis. Such samples are typically low quality and can include specimens from museum collections, archaeological finds, and subfossil remains of tissues or other DNA-containing sources (e.g., coprolites, hair).
Blocking primer: an oligonucleotide used to bind to DNA and overlap the primer-binding sites, so that amplification of the undesired species is prevented.
Chimera: sequences that arise during amplification combining DNA fragments from two or more individuals.
Environmental DNA (eDNA): trace DNA in samples such as water, soil, or faeces. eDNA is a mixture of potentially degraded DNA from many different organisms. It is important to note that this definition remains controversial due to the sampling of whole microorganisms that might appear in an environmental sample. Although metagenomic microbial studies might use environmental sampling, they cannot always be defined as true eDNA studies because some methods first isolate microorganisms from the environment before extracting DNA.
Metagenomics: sequencing of the total DNA extracted from a sample containing many different organisms.
Operational taxonomic unit (OTU): the taxonomic level of sampling defined by the researcher in a study; for example, individuals, populations, species, genera, or strains. OTUs are generated by comparing sequences against each other to form a distance matrix, followed by clustering groups of sequences with a specified amount of variability allowed within each OTU (e.g., [67]).
Second-generation sequencing: sequencing technologies such as the Roche GS series, Illumina Genome Analyser series, and IonTorrent series that parallelise the sequencing process, producing thousands to billions of DNA sequences in single sequencing runs.

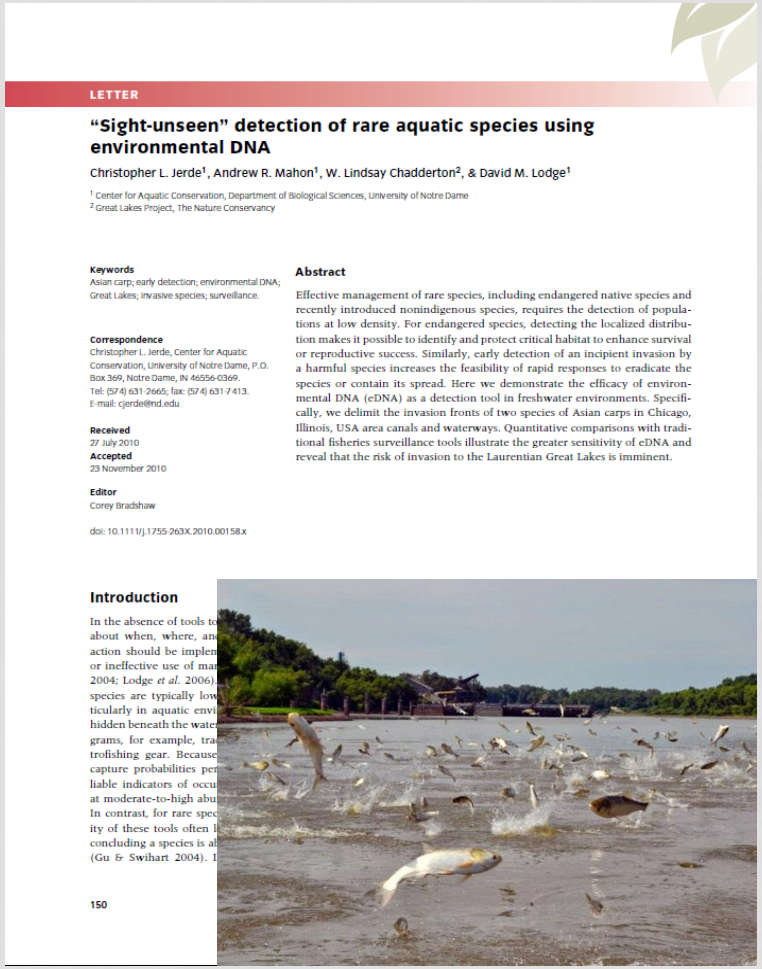
CrossMark

- Environmental DNA is genetic material (short DNA fragments; [ca. 80 – 250 bp; shed from skin, urine, feces, mucus, or dead cells] in nonliving components of the environment (e.g. soil, sediment, or water).

- A mixture of potentially degraded DNA from many different organisms.

THE PROMISE OF eDNA





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]

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Keywords
Asian carp; early detection; environmental DNA; Great Lakes; invasive species; surveillance.

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Abstract

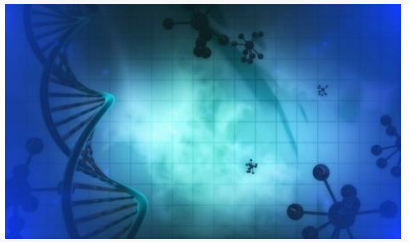
Effective management of rare species, including endangered native species and recently introduced nonindigenous species, requires the detection of populations at low density. For endangered species, detecting the localized distribution makes it possible to identify and protect critical habitat to enhance survival or reproductive success. Similarly, early detection of an incipient invasion by a harmful species increases the feasibility of rapid responses to eradicate the species or contain its spread. Here we demonstrate the efficacy of environmental DNA (eDNA) as a detection tool in freshwater environments. Specifically, we delimit the invasion fronts of two species of Asian carps in Chicago, Illinois, USA area canals and waterways. Quantitative comparisons with traditional fisheries surveillance tools illustrate the greater sensitivity of eDNA and reveal that the risk of invasion to the Laurentian Great Lakes is imminent.

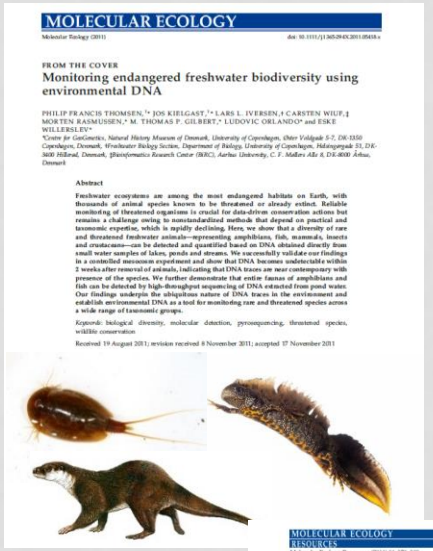
Introduction

In the absence of tools to
about when, where, an
action should be implem
or ineffective use of ma
2004; Lodge *et al.* 2006).
species are typically low
ticularly in aquatic envi
hidden beneath the wate
grams, for example, tra
trofishing gear. Because
capture probabilities per
liable indicators of occu
at moderate-to-high abu
In contrast, for rare spec
ity of these tools often l
concluding a species is a
(Gu & Swihart 2004). I

- eDNA detection of invasive carp in the Illinois River
- A "**revolutionary**", "**silver-bullet**" approach to sampling cryptic, rare, or difficult to detect species
- **Effective Selectivity**
- **Improved Sensitivity**
- **Reduced effort and costs**

THE PROMISE OF eDNA




MOLECULAR ECOLOGY

FROM THE COVER

Monitoring endangered freshwater biodiversity using environmental DNA

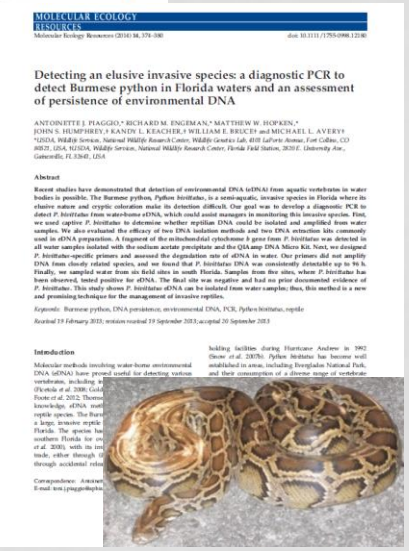
PHILIP FRANCIS THOMSEN,*† JOS KIELGAST,*† LARS L. IVERSEN,‡ CARSTEN WIUF,§ MORTEN RASMUSSEN,* M. THOMAS P. GILBERT,* LUDOVIC ORLANDO* and ESKE WILLERSLEV*

Abstract

Freshwater ecosystems are among the most endangered habitats on Earth, with thousands of animal species known to be threatened or already extinct. Reliable monitoring of threatened organisms is crucial for data-driven conservation actions but remains a challenge owing to nonstandardized methods that depend on practical and taxonomic expertise, which is rapidly declining. Here, we show that a diversity of rare and threatened freshwater animals—representing amphibians, fish, mammals, insects and crustaceans—can be detected and quantified based on DNA obtained directly from small water samples of lakes, ponds and streams. We successfully validate our findings in a controlled mesocosm experiment and show that DNA becomes undetectable within 2 weeks after removal of animals, indicating that DNA traces are near contemporary with presence of the species. We further demonstrate that entire faunas of amphibians and fish can be detected by high-throughput sequencing of DNA extracted from pond water. Our findings underpin the ubiquitous nature of DNA traces in the environment and establish environmental DNA as a tool for monitoring rare and threatened species across a wide range of taxonomic groups.

Keywords: biological diversity, molecular detection, pyrosequencing, threatened species, wildlife conservation

Received 19 August 2011; revision received 8 November 2011; accepted 17 November 2011


MOLECULAR ECOLOGY RESOURCES

Detecting an elusive invasive species: a diagnostic PCR to detect Burmese python in Florida waters and an assessment of persistence of environmental DNA

ANTOINETTE J. PIAGGIO,* RICHARD M. ENGEMAN,* MATTHEW W. HOPKEN,* JOHN S. HUMPHREY,† KANDY L. KEACHER,† WILLIAM E. BRUCE† and MICHAEL L. AVERY†

Abstract

Recent studies have demonstrated that detection of environmental DNA (eDNA) from aquatic vertebrates in water bodies is possible. The Burmese python, *Python bivittatus*, is a semi-aquatic, invasive species in Florida where its elusive nature and cryptic coloration make its detection difficult. Our goal was to develop a diagnostic PCR to detect *P. bivittatus* from water-borne eDNA, which could assist managers in monitoring this invasive species. First, we used captive *P. bivittatus* to determine whether reptilian DNA could be isolated and amplified from water samples. We also evaluated the efficacy of two DNA isolation methods and two DNA extraction kits commonly used in eDNA preparations. A fragment of the mitochondrial cytochrome *b* gene from *P. bivittatus* was detected in all water samples isolated with the sodium acetate precipitate and the QIAamp DNA Micro Kit. Next, we designed *P. bivittatus*-specific primers and assessed the degradation rate of eDNA in water. Our primers did not amplify DNA from closely related species, and we found that *P. bivittatus* DNA was consistently detectable up to 96 h. Finally, we sampled water from six field sites in south Florida. Samples from five sites, where *P. bivittatus* has been observed, tested positive for eDNA. The final site was negative and had no prior documented evidence of *P. bivittatus*. This study shows *P. bivittatus* eDNA can be isolated from water samples; thus, this method is a new and promising technique for the management of invasive reptiles.

Keywords: Burmese python, DNA persistence, environmental DNA, PCR, *Python bivittatus*, reptile

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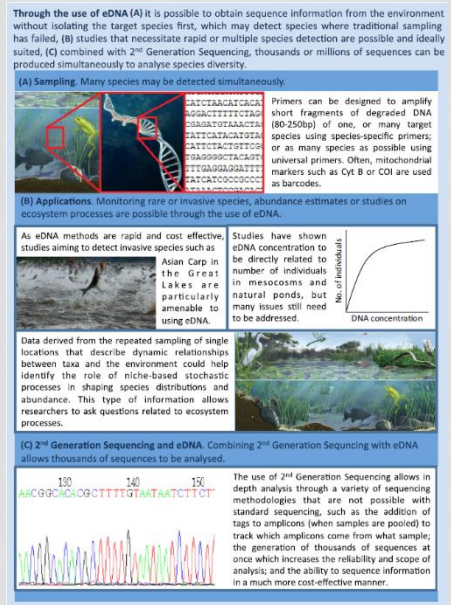
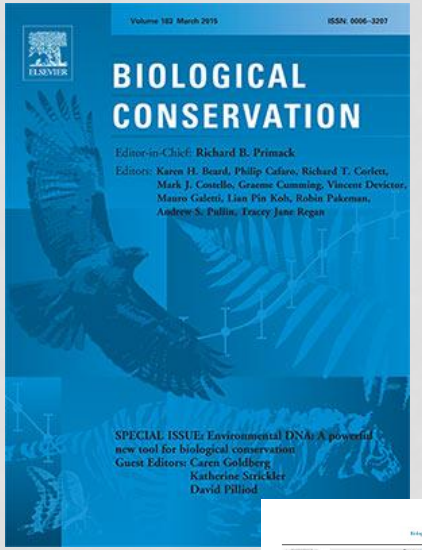
Introduction

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BIOLOGICAL CONSERVATION

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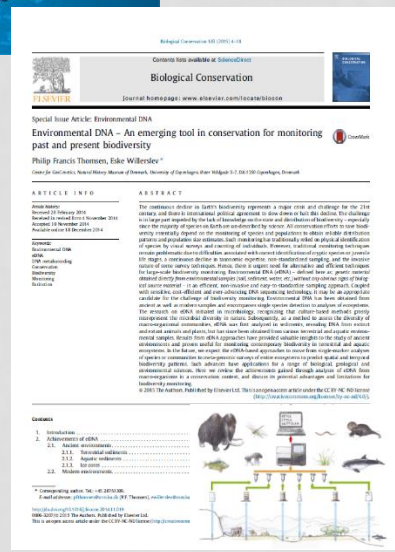
SPECIAL ISSUE: Environmental DNA: A powerful new tool for biological conservation
Guest Editors: Caren Goldberg, Katherine Strickler, David Pilliod


Biological Conservation

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Special Issue Article: Environmental DNA

Environmental DNA – An emerging tool in conservation for monitoring past and present biodiversity

Philip Francis Thomsen, Eske Willerslev

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ABSTRACT

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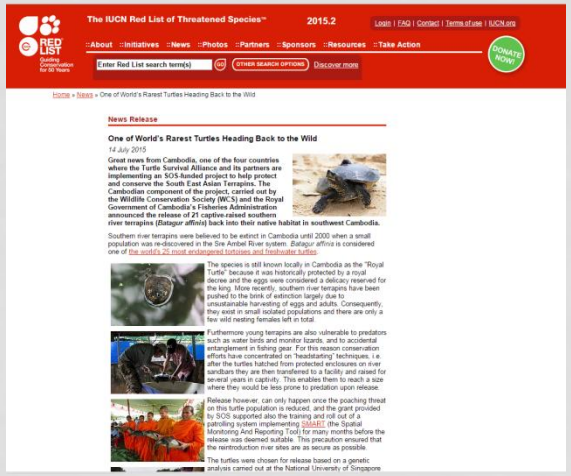
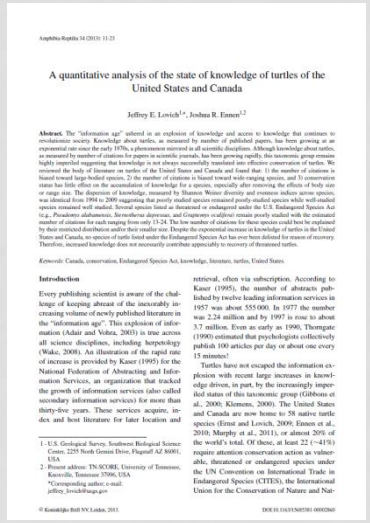
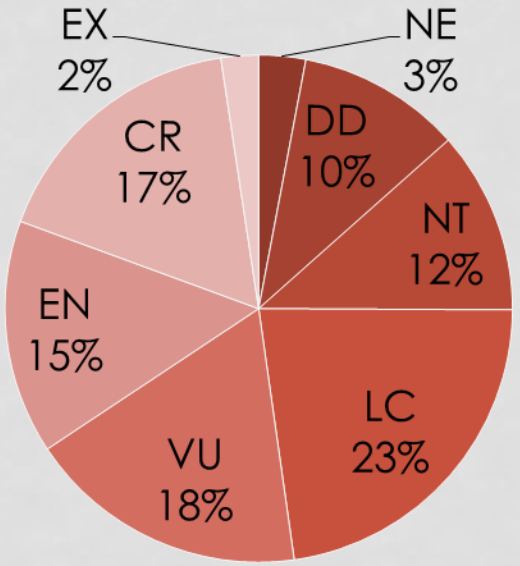
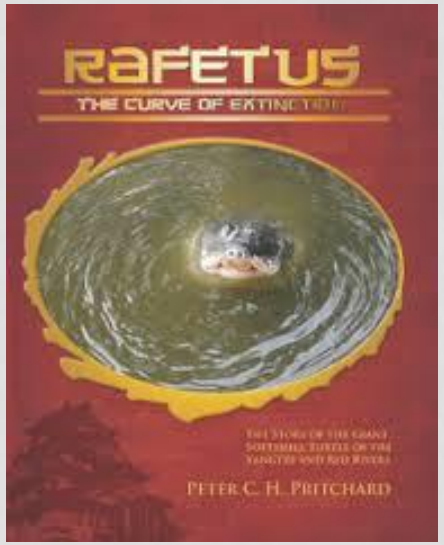


- ≅ 60% of turtles are threatened or extinct
- We know little about most of them!
- ≅ 13% are DD or NE – we know little about all of them!





THE VALUE OF eDNA TO FRESHWATER TURTLE CONSERVATION



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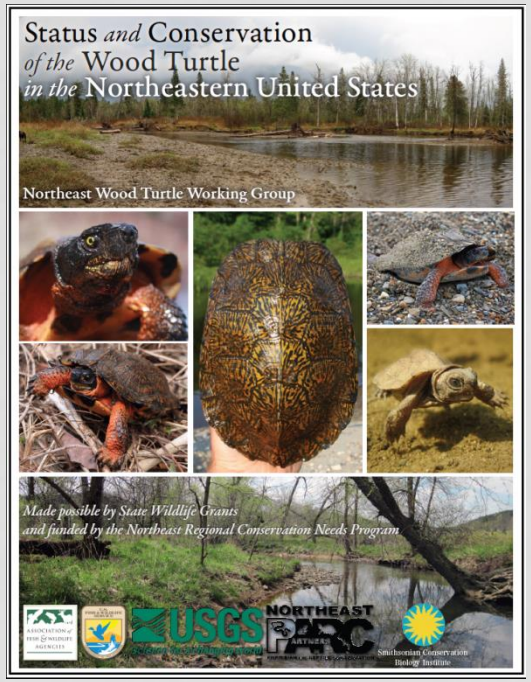

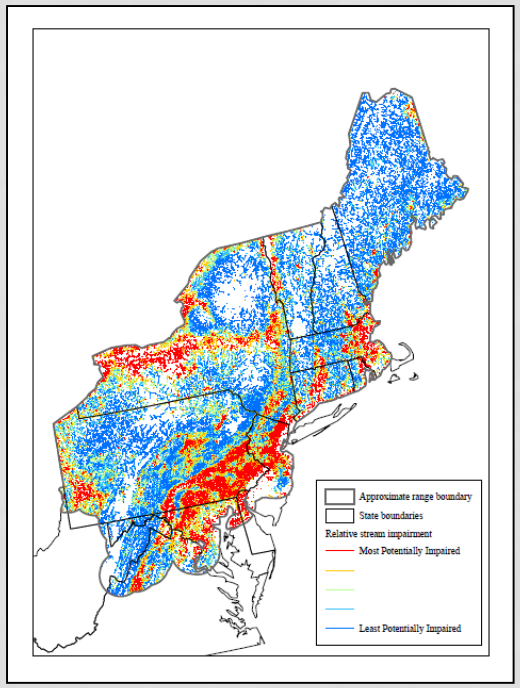

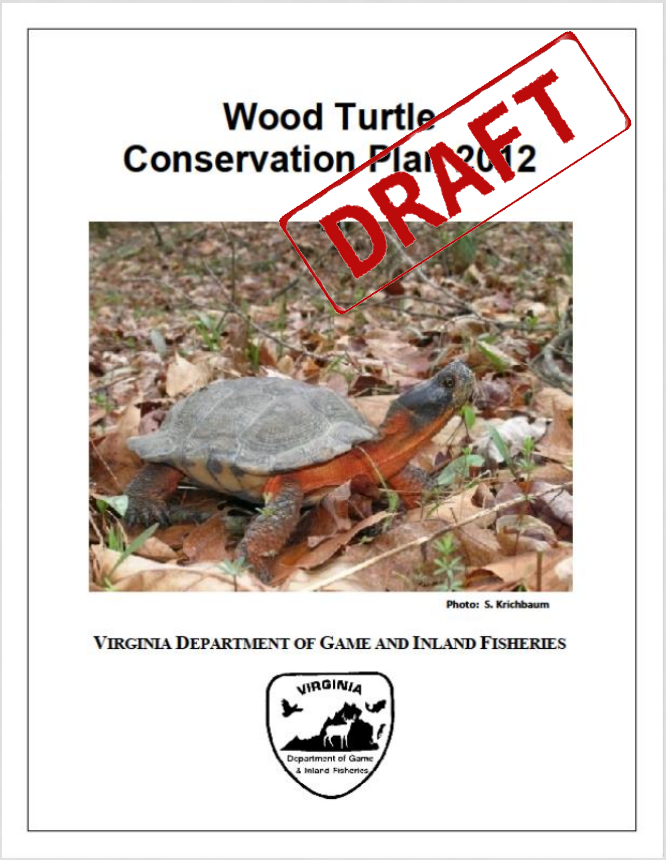

THE VALUE OF eDNA TO WOOD TURTLE CONSERVATION

PLOS ONE

RESEARCH ARTICLE

Concurrent visual encounter sampling validates eDNA selectivity and sensitivity for the endangered wood turtle (*Glyptemys insculpta*)

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OPEN ACCESS

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Data Availability Statement: All relevant data are within the manuscript and its Supporting Information files. We realize that the expectation is to have a study site description and attending figure(s) included in the manuscript. However, because the wood turtle is an IUCN endangered species and listed accordingly in most states and provinces within its range, we do not believe it is ethical to publish place names or geographic locations for this species. We are supported in this position by the state herpetologist for Virginia, JD Kleopfer, and can provide testimonial if needed.

Abstract

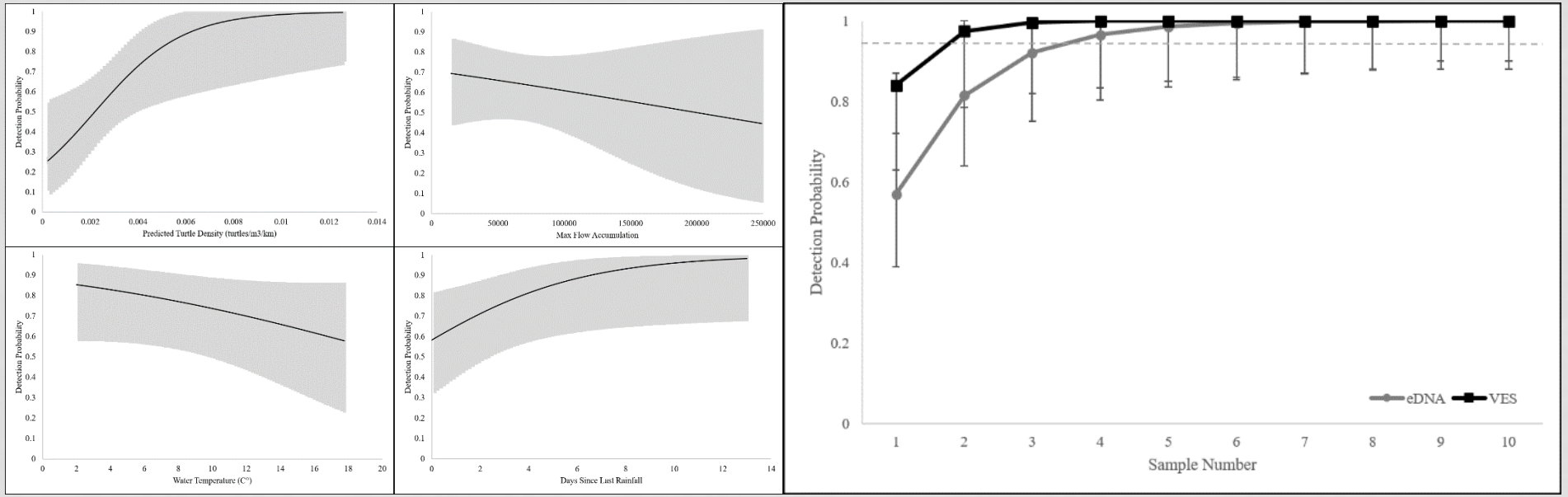
Environmental DNA (eDNA) has been used to record the presence of many different organisms in several different aquatic and terrestrial environments. Although eDNA has been demonstrated as a useful tool for the detection of invasive and/or cryptic and declining species, this approach is subject to the same considerations that limit the interpretation of results from traditional survey techniques (e.g. imperfect detection). The wood turtle is a cryptic semi-aquatic species that is declining across its range and, like so many chelonian species, is in-need of a rapid and effective method for monitoring distribution and abundance. To meet this need, we used an eDNA approach to sample for wood turtle presence in northern Virginia streams. At the same time, we used repeat visual encounter surveys in an occupancy-modelling framework to validate our eDNA results and reveal the relationship of detection and occupancy for both methods. We sampled 37 stream reaches of varying size within and beyond the known distribution of the wood turtle across northern Virginia. Wood turtle occupancy probability was 0.54 (0.31, 0.76) and while detection probability for wood turtle occupancy was high (0.88; 0.58, 0.98), our detection of turtle abundance was markedly lower (0.28; 0.21, 0.37). We detected eDNA at 76% of sites confirmed occupied by VES and at an additional three sites where turtles were not detected but were known to occur. Environmental DNA occupancy probability was 0.55 (0.29, 0.78); directly comparable to the VES occupancy estimate. Higher probabilities of detecting wood turtle eDNA were associated with higher turtle densities, an increasing number of days since the last rainfall, lower water temperatures, and lower relative discharges. Our results suggest that eDNA technology holds promise for sampling aquatic chelonians in some systems, even when discharge is high and biomass is relatively low, when the approach is validated and sampling error is quantified.

THE VALUE OF eDNA TO WOOD TURTLE CONSERVATION

	Turtle Occupancy	eDNA Occupancy
Naïve Occupancy	0.46 (38, 54)	0.43 (36, 51)
Detection Probability	0.88 (58, 98)	0.55 (38, 71)
Occupancy Probability	0.54 (31, 76)	0.55 (29, 78)

Direct comparison of VES & eDNA survey results confirms eDNA is nearly as good as VES for determining occupancy!

THE VALUE OF eDNA TO WOOD TURTLE CONSERVATION

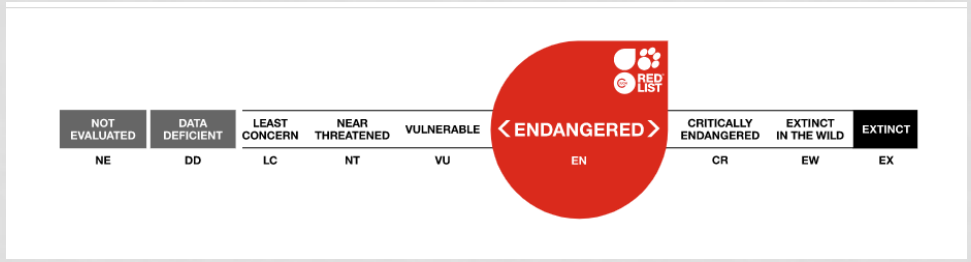


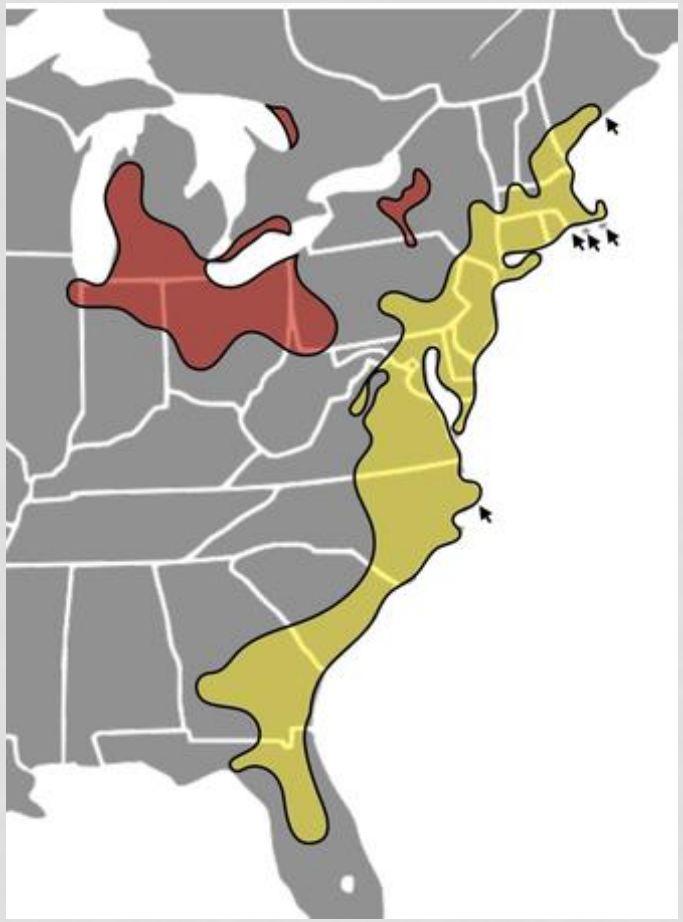
Where and when you survey matters

eDNA is ~2-7X cheaper than conventional surveys

- $51 per sample v. $362 survey
- $308 per site v. $733 per site

THE VALUE OF eDNA TO SPOTTED TURTLE CONSERVATION





STATUS ASSESSMENT AND CONSERVATION PLAN FOR THE SPOTTED TURTLE IN THE EASTERN U.S.

STATUS ASSESSMENT AND **CONSERVATION PLAN** FOR THE **SPOTTED TURTLE** IN THE EASTERN UNITED STATES

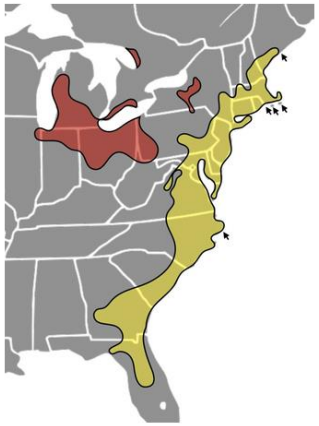
Photos (above, below): Jonathan Mays

Project Summary

Spotted Turtles (*Clemmys guttata*) have declined across their range and are of conservation concern throughout the United States and Canada. They are identified as Species of Greatest Conservation Need (SGCN) in all 21 states in which they occur, considered Endangered by the International Union for Conservation of Nature (IUCN), and have been petitioned for federal listing under the Endangers Species Act. The goal of this project is to quantify the Spotted Turtle status and distribution from Maine to Florida (yellow region in figure)–as well as the effects of climate change and habitat fragmentation on the species–in order to prioritize both habitat conservation and management. As part of this project, we will conduct standardized population assessments at multiple spatial scales, with centralized data analysis, to (1) establish population baselines, (2) inform a comprehensive adaptive management strategy, and (3) identify priority habitat and population management actions at the regional, state, and local levels.

Our Goal:

To maintain populations of Spotted Turtles at or above their current levels throughout the participating states, and to achieve zero net loss of suitable habitat at high priority sites by (1) identifying and enhancing priority seasonal wetland and terrestrial habitats in the eastern United States, and (2) applying conservation principles and practices to increase population size and support healthy metapopulations of Spotted Turtles and associated SGCN.



Documents and Forms

MONITORING PROTOCOL

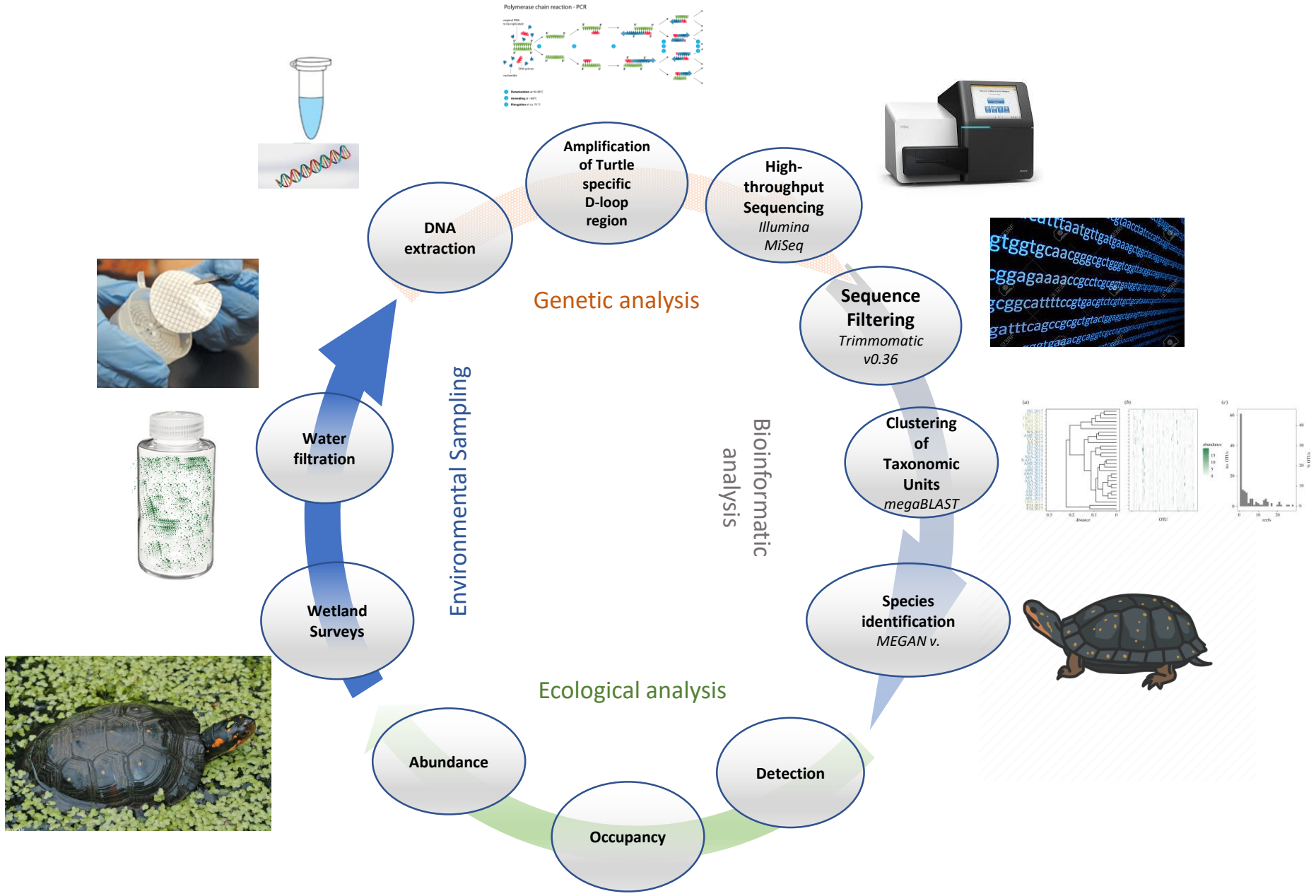
FIELD FORMS

DATA ENTRY TEMPLATE

FIELD FORM INSTRUCTIONS

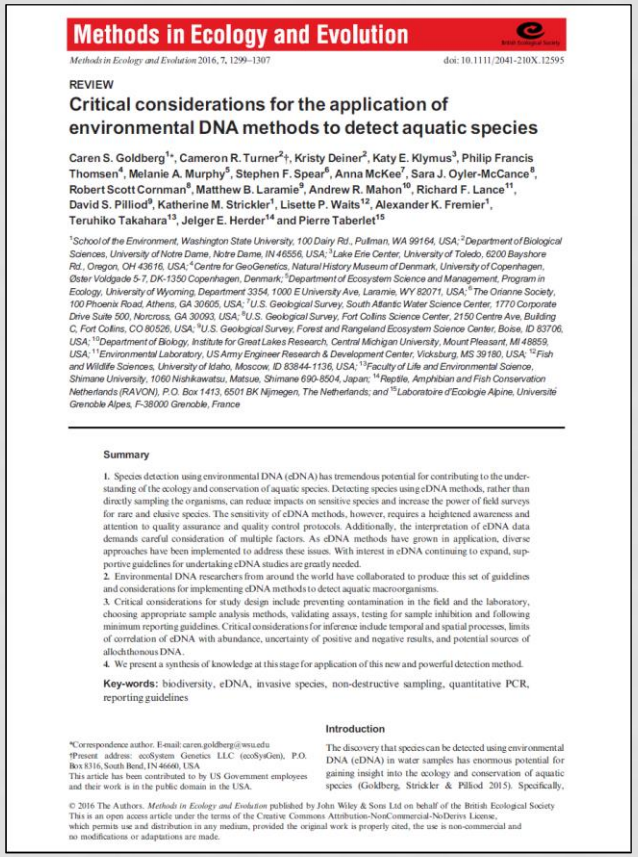
Data Entry

SUBMIT DATA HERE


Polymerase chain reaction - PCR
DNA extraction
Amplification of Turtle specific D-loop region
High-throughput Sequencing
Illumina MiSeq
Sequence Filtering
Trimmomatic v0.36
Clustering of Taxonomic Units
megaBLAST
Species identification
MEGAN v.
Genetic analysis
Bioinformatic analysis
Water filtration
Wetland Surveys
Environmental Sampling
Ecological analysis
Abundance
Occupancy
Detection

THE LIMITATIONS OF eDNA

- Sources of error – method and process error
- Factors affecting eDNA half-life
- eDNA generation - ecology and physiology of the source material (biomass, type of organism, etc.)
- eDNA degradation – UV, pH, temperature, DNAases (microbial activity)
- eDNA dilution – system volume and/or discharge, distance from source, settling/trapped in sediments
- PCR inhibitors – tannins, etc.



Methods in Ecology and Evolution

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REVIEW

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

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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

Introduction

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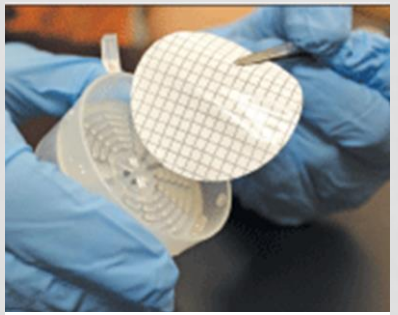
This article has been contributed to by US Government employees and their work is in the public domain in the USA.

The discovery that species can be detected using environmental DNA (eDNA) in water samples has enormous potential for gaining insight into the ecology and conservation of aquatic species (Goldberg, Strickler & Pilliod 2015). Specifically,

SAMPLING SPOTTED TURTLE eDNA



A HIERCHICAL APPROACH TO SAMPLING SPOTTED TURTLES AND THEIR eDNA

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Site occupancy models in the analysis of environmental DNA presence/absence surveys: a case study of an emerging amphibian pathogen

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Summary

1. The use of environmental DNA (eDNA) to detect species in aquatic environments such as ponds and streams is a powerful new technique with many benefits. However, species detection in eDNA-based surveys is likely to be imperfect, which can lead to underestimation of the distribution of a species.
2. Site occupancy models account for imperfect detection and can be used to estimate the proportion of sites where a species occurs from presence/absence survey data, making them ideal for the analysis of eDNA-based surveys. Imperfect detection can result from failure to detect the species during field work (e.g. by water samples) or during laboratory analysis (e.g. by PCR).
3. To demonstrate the utility of site occupancy models for eDNA surveys, we reanalysed a data set estimating the occurrence of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* using eDNA. Our reanalysis showed that the previous estimation of species occurrence was low by 5–10%. Detection probability was best explained by an index of the number of hosts (frogs) in ponds.
4. Per-visit availability probability in water samples was estimated at 0·45 (95% CRI 0·32, 0·58) and per-PCR detection probability at 0·85 (95% CRI 0·74, 0·94), and six water samples from a pond were necessary for a cumulative detection probability > 95%. A simulation study showed that when using site occupancy analysis, researchers need many fewer samples to reliably estimate presence and absence of species than without use of site occupancy modelling.
5. Our analyses demonstrate the benefits of site occupancy models as a simple and powerful tool to estimate detection and site occupancy (species prevalence) probabilities despite imperfect detection. As species detection from eDNA becomes more common, adoption of appropriate statistical methods, such as site occupancy models, will become crucial to ensure that reliable inferences are made from eDNA-based surveys.

Key-words: environmental DNA, survey, monitoring, detection probability, site occupancy model

Introduction

Population surveys have one undesirable yet unavoidable feature: it is unlikely that all individuals, populations or species are ever detected (Yoccoz, Nichols & Boulinier 2001; Pollock *et al.* 2002; Kéry & Schmidt 2008). This imperfect detection can bias analyses of survey data and potentially lead to poor management decisions (Yoccoz, Nichols & Boulinier 2001). It is therefore important to account for imperfect detection in the analysis of survey data whenever possible (Yoccoz, Nichols & Boulinier 2001; Pollock *et al.* 2002; Kéry & Schmidt 2008).

Conceptually, the effects of imperfect detection can be described with a simple equation:

$$E(C) = N * p$$

where $E(C)$ is the expected value of a count of individuals, populations or species, N is the number of individuals, populations or species actually present and p is the detection probability (Yoccoz, Nichols & Boulinier 2001; Pollock *et al.* 2002; Kéry & Schmidt 2008). Estimates of p can be used in the analysis of survey data to adjust counts for imperfect detection to obtain a more accurate estimate of N (Pollock *et al.* 2002; MacKenzie *et al.* 2006; Royle & Dorazio 2008). Apart from leading to biased assessments

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RESOURCE ARTICLE

WILEY MOLECULAR ECOLOGY RESOURCES

EDNAOCCUPANCY: An R package for multiscale occupancy modelling of environmental DNA data

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Abstract

In this article, we describe EDNAOCCUPANCY, an R package for fitting Bayesian, multiscale occupancy models. These models are appropriate for occupancy surveys that include three nested levels of sampling: primary sample units within a study area, secondary sample units collected from each primary unit and replicates of each secondary sample unit. This design is commonly used in occupancy surveys of environmental DNA (eDNA). EDNAOCCUPANCY allows users to specify and fit multiscale occupancy models with or without covariates, to estimate posterior summaries of occurrence and detection probabilities, and to compare different models using Bayesian model-selection criteria. We illustrate these features by analysing two published data sets: eDNA surveys of a fungal pathogen of amphibians and eDNA surveys of an endangered fish species.

KEYWORDS

Bayesian, environmental DNA, occupancy survey, species distribution model

1 | INTRODUCTION

Presence–absence surveys are often used to estimate the spatial distribution of a species. During these surveys, individuals that are present can be missed owing to errors in detection by observers. These errors are especially common in surveys of animals that are either rare or elusive (e.g., species with cryptic behaviours or coloration). To account for the possibility of such errors, ecologists often use repeated surveys of each sample location and analyse the resulting data (detections and nondetections) using occupancy models (Guillera-Arroita, 2017; Ilanayan, Tingley, Furnas, & Beissinger, 2014; MacKenzie et al., 2006; Royle & Dorazio, 2008). These models allow the occurrence of a species to be estimated accurately while accounting for false-negative errors in detection.

For species that are extremely difficult to detect using conventional (visual or aural) methods, observers often resort to detection of signs of species presence (e.g., animal tracks, scat or fur) that may be observed more reliably. One such sign is environmental DNA (eDNA), which includes short fragments of DNA shed or left behind by individuals in water or soil (Darling & Mahon, 2011; Ficetola, Miaud, Pompanon, & Taberlet, 2008). The sources of eDNA can vary but may include skin cells, mucus, eggs, urine or faeces. Surveys of eDNA are often easier and less expensive than those based on conventional methods of sampling (Rees, Maddison, Middleditch, Patmore, & Gough, 2014; Thomsen & Willerslev, 2015).

Surveys of eDNA are designed to accommodate spatial or temporal heterogeneity in the occurrence of eDNA at a sampling location. This is necessary because even if a location is occupied by a species, not all samples taken from that location will necessarily contain eDNA of the species. Samples collected at each location are usually intended to serve as spatial or temporal replicates of that location. Therefore, the presence of eDNA in these samples can depend on many factors, including the source locations of eDNA, the degradation or transport of eDNA from these source locations and the size of the sample (Darling & Mahon, 2011; Dejean et al., 2011; Pilliod, Goldberg, Arkle, & Waits, 2014). The presence of eDNA in a sample is usually assessed by amplifying the eDNA in each of several subsamples using polymerase chain reaction (PCR) chemistry. The eDNA in each subsample (or PCR replicate) is amplified independently and used to assess whether eDNA is detected or not detected in the subsample (Hunter et al., 2017). Therefore, all eDNA surveys include at least three nested levels of sampling:

1. locations (primary sample units) within a study area,

CONCURRENT TURTLE AND eDNA SAMPLING

Aquatic trap plots

- 4 trap-nights per site
- Collect environmental & weather variables

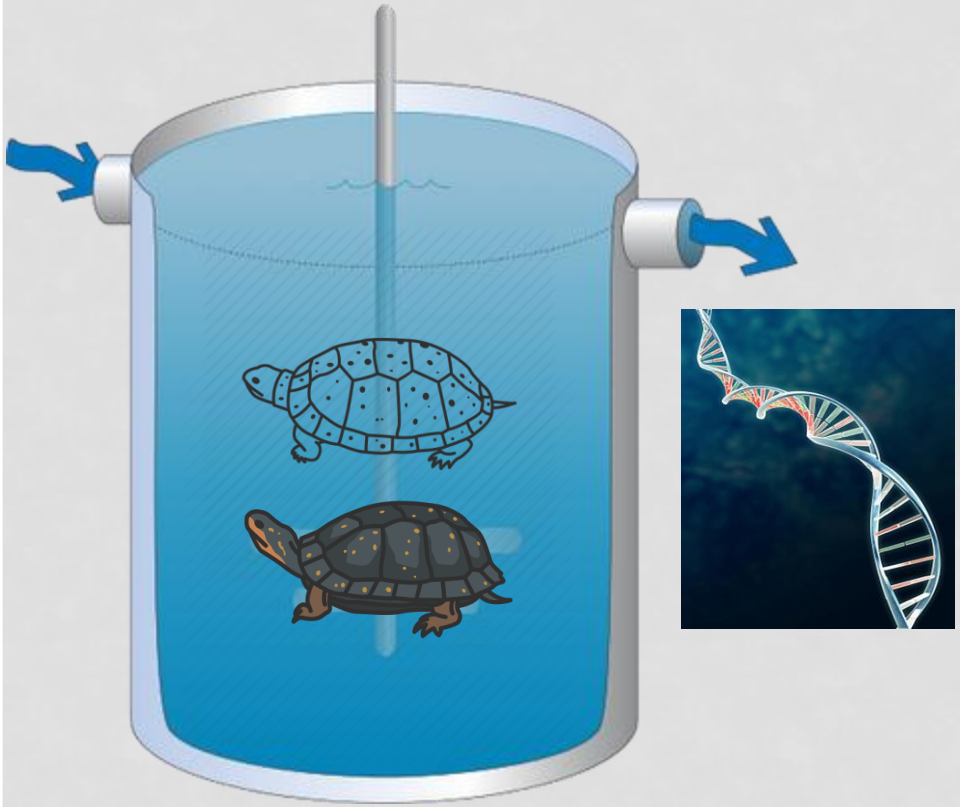
eDNA Sampling

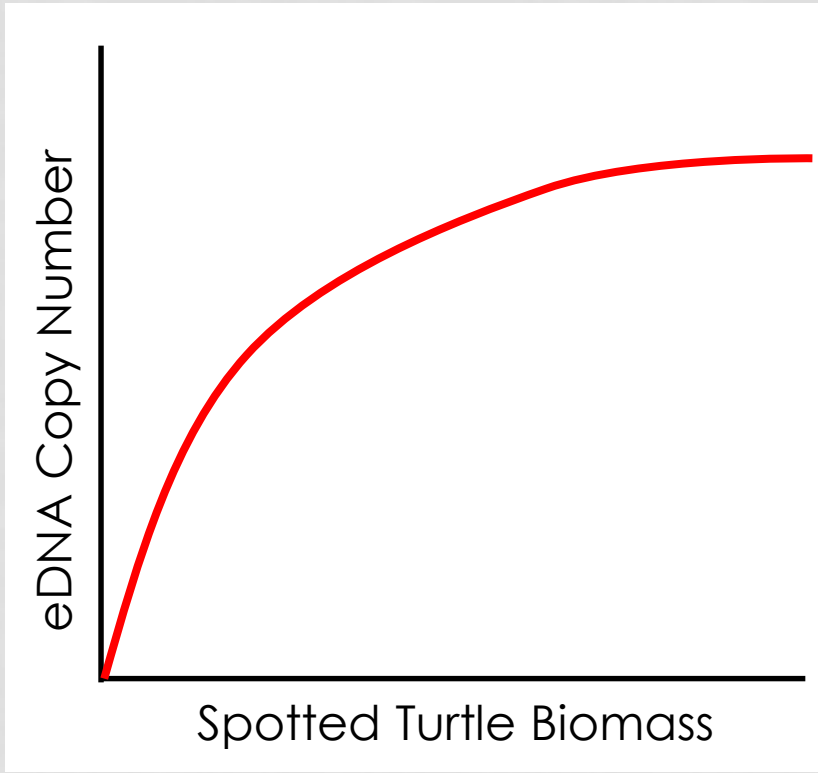
- Filter four 2-liter water samples per site: 3 samples and 1 field blank
- Collect environmental & weather variables

SPOTTED TURTLE AND eDNA DETECTION AND OCCUPANCY

eDNA Occurrence Prob. at a Site	eDNA Conditional Occurrence Prob. in a Water Sample	eDNA Conditional Detection Prob. in a Water Sub-Sample
Water Depth (m)	pH	qPCR reaction
% Woody Cover	Water Temperature (C°)	PCR inhibitors
Water Temperature (C°)	Total Suspended Solids	???

ESTIMATING ABUNDANCE: BUILDING A STANDARD CURVE





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An environmental DNA-based method for monitoring spawning activity: a case study, using the endangered Macquarie perch (*Macquaria australasica*)

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Summary

1. Determining the timing and location of reproductive events is critical for efficient management of species. However, methods currently used for aquatic species are costly, time intensive, biased and often require destructive or injurious sampling. Hence, developing a non-invasive sampling method to accurately determine the timing and location of reproduction for aquatic species would be extremely valuable.
2. We conducted an experimental and field study to determine the influence of spawning, and the mass release of spermatozoa in particular, on environmental DNA (eDNA) concentrations. Using a quantitative PCR approach we monitored changes in nuclear and mitochondrial eDNA concentrations over time.
3. The data from the experimental study and the field survey supported our hypothesis that spawning events are characterized by higher concentrations of nuclear relative to mitochondrial eDNA. Outside of the reproductive period, we find that nuclear and mitochondrial DNA fragments are equally abundant in environmental water samples.
4. We have shown that changes in the relative abundance of nuclear and mitochondrial eDNA can be used to monitor spawning activity of the endangered Macquarie perch. Our method is likely to be transferrable to other aquatic species and can be particularly useful to increase our understanding of the spawning biology of cryptic, rare or threatened species as well as design and evaluate environmental management actions and determine species establishment.

Key-words: aquatic vertebrates, conservation genetics, environmental DNA, reproduction, spermatozoa, wildlife management

Introduction

Monitoring reproduction in aquatic organisms is important for the conservation and management of species and/or populations (Koenig *et al.* 2000; Mozz & Setka 2004; King *et al.* 2010; Di Franco *et al.* 2012; Kearns *et al.* 2012). Individual monitoring methods suffer from biases, do not provide direct evidence for reproduction or are unable to distinguish between reproductive failure and high mortality rates of early life-history stages. DNA-based methods provide promising opportunities to overcome these challenges through the monitoring of environmental DNA (eDNA) signals that are correlated with reproductive activity in aquatic organisms.

Many aquatic organisms reproduce sexually through a process called spawning, i.e. the mass release of reproductive cells (oocytes and spermatozoa) into the water column, allowing external fertilization (Harrison *et al.* 1984; Beebee 1996; Coward *et al.* 2002). Determining the timing and location of spawning events is important to: increase our understanding of the species' biology (Harrison *et al.* 1984; Rose 1993; Grant, Chadwick & Halliday 2009); evaluate the reproductive output of populations (Levitan *et al.* 2014); determine population establishment for both invasive and translocated native species (Pearce 2013); and design and evaluate management actions (Koenig *et al.* 2000; King *et al.* 2010; Kearns *et al.* 2012). For aquatic vertebrates relying on external fertilizations (e.g. most fishes and frogs) monitoring reproductive activity can be achieved by destructive, injurious or non-invasive methods (Table 1)(Lefort *et al.* 2015). The extra mortality rate imposed by destructive sampling methods makes them undesirable for monitoring reproduction in rare and threatened species (Tsukamoto 2006; Wei *et al.* 2009; Engstedt, Engkvist & Larsson 2014). On the other hand, injurious methods (i.e. use of acoustic telemetry) are often unable to deliver direct evidence of spawning and non-invasive methods are sensitive to observer biases and taxonomic misidentification (Caswell *et al.* 2004; Miller *et al.* 2012; Ko *et al.* 2013; Koster *et al.* 2013; Diana, Hanchin & Popoff 2015). Overall, all currently available survey

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NEXT STEPS???

- Use Mesocosm experiments to evaluate same and other factors that influence eDNA occupancy for eDNA concentration(copy number?)
- Develop reference curves for factors that influence eDNA detection of concentration. Model relationships among those factors and turtle abundance.
- Develop field-based assessments of eDNA concentration using turtle sampling network for same and others factors that influence eDNA detection and occupancy
- Develop field-based eDNA concentration model related to primary factors influencing turtle abundance and eDNA detection

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