NORTHERN ILLINOIS UNIVERSITY

Using Environmental DNA to Detect the Presence of Blanding's Turtle (*Emydoidea blandingii*)

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By

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Capstone Approval Page

Capstone Title (print or type)

Using Environmental DNA to Detect the Presence of Blanding's Turtle (*Emydoidea blandingii*)

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HONORS THESIS ABSTRACT

Guidelines

Blanding's turtle (*Emydoidea blandingii*) has been designated as threatened or endangered throughout a large portion of its range largely due to destruction and degradation of wetland and terrestrial habitat. More precise knowledge of locations where Blanding's turtles are found would greatly aid efforts to protect this species. Unfortunately, Blanding's turtles can be difficult to observe or trap, making detection difficult. Instead, environmental DNA (eDNA) collected from an aquatic habitat may be utilized as a cost and time-effective alternative to determine whether *E. blandingii* have recently occupied a given locale. For this study, we designed species-specific primers to amplify a 219 bp segment of the cytochrome-b gene. Following water filtration and eDNA extraction from Millipore filters, the extracts were amplified using the specifically designed primers. The PCR products were then run on a 1% agarose gel to infer the presence or absence of *E. blandingii* based on corresponding gel results. Our preliminary results have demonstrated that the primers are species-specific to *E. blandingii* and will amplify *E. blandingii* DNA from a 2-3 L water sample from water used to house this species in captivity. Future research will focus on obtaining positive results from field sites where *E. blandingii* is known to occur and will test the utility of this method at historic *E. blandingii* sites as well as sites where the current presence/absence status of *E. blandingii* is unknown. Furthermore, we plan to include a relative cost analysis, with respect to both time and money, comparing traditional survey methods to eDNA methods.

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ABSTRACT (100-200 WORDS):

Blanding's turtle (*Emydoidea blandingii*) has been designated as threatened or endangered throughout a large portion of its range largely due to destruction and degradation of wetland and terrestrial habitat. More precise knowledge of locations where Blanding's turtles are found would greatly aid efforts to protect this species. Unfortunately, Blanding's turtles can be difficult to observe or trap, making detection difficult. Instead, environmental DNA (eDNA) collected from an aquatic habitat may be utilized as a cost and time-effective alternative to determine whether *E. blandingii* have recently occupied a given locale. For this study, we designed species-specific primers to amplify a 219 bp segment of the cytochrome-b gene. Following water filtration and eDNA extraction from Millipore filters, the extracts were amplified using the specifically designed primers. The PCR products were then run on a 1% agarose gel to infer the presence or absence of *E. blandingii* based on corresponding gel results. Our preliminary results have demonstrated that the primers are species-specific to *E. blandingii* and will amplify *E. blandingii* DNA from a 2-3 L water sample from water used to house this species in captivity. Future research will focus on obtaining positive results from field sites where *E. blandingii* is known to occur and will test the utility of this method at historic *E. blandingii* sites as well as sites where the current presence/absence status of *E. blandingii* is unknown. Furthermore, we plan to include a relative cost analysis, with respect to both time and money, comparing traditional survey methods to eDNA methods.

Using Environmental DNA to Detect the Presence of Blanding's Turtle (*Emydoidea blandingii*)

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Northern Illinois University

3 May 2013

Introduction

The project, "Using Environmental DNA to Detect the Presence of Blanding's Turtle," aimed to develop eDNA methods to be used as a conservation tool to monitor this state-endangered species. Blanding's Turtle (*Emydoidea blandingii*) has been designated as threatened or endangered throughout a large portion of its range largely due to destruction and degradation of wetland and terrestrial habitat (Congdon et al. 2008). More precise knowledge of locations where Blanding's Turtles are found would greatly aid efforts to protect this species. Unfortunately, Blanding's Turtles can be difficult to observe or trap, making detection difficult. Instead, environmental DNA (eDNA) collected from an aquatic habitat may be utilized as a cost- and time-effective alternative to determine whether Blanding's Turtles have recently occupied a given locale (Ficetola et al. 2008; Dejean et al. 2011; Jerde et al. 2011).

Blanding's Turtle (*Emydoidea blandingii*) is a semi-aquatic species inhabiting shallow freshwater environments and nearby uplands across North America (Fig. 1). Currently, this species is listed as threatened or endangered in several states and Canadian provinces, including Illinois (Rubin et al. 2004). Blanding's Turtle, once common in the northern 2/3 of Illinois, has seen a decline due to incidences of road mortality, private collecting, and widespread destruction or degradation of its preferred aquatic and terrestrial habitats (Congdon et al. 2008).

Effective monitoring of a threatened species is crucial to any conservation strategy. Traditionally, monitoring of Blanding's Turtle populations has been achieved through the use of aquatic hoop nets, self-standing traps, radio telemetry, and manual searches for basking turtles (Rubin et al. 2004). However, the low density of Blanding's Turtle populations, and the tendency of this species to burrow into freshwater banks

make them particularly difficult to locate (Deiean et al. 2010). Consequently, traditional detection methods require substantial field work, and the required equipment can be costly. A relatively new procedure using environmental DNA, or eDNA, to detect the presence of Blanding's Turtles in aquatic environments, may have the potential to reduce both the amount of time and money required when used instead of more traditional methods.

Methods

The design of this study was based on primary literature sources that had utilized eDNA methods for ecological applications (Ficetola et al. 2008; Dejean et al. 2011; Goldberg et al. 2011; Jerde et al. 2011). The methods of this study involved four main components: primer design and optimization, sample collection, eDNA isolation, and polymerase chain reaction (PCR) amplification.

The first step in this project was primer design. Primers are single-stranded DNA fragments (approximately 20 nucleotides in length) that are complementary to the nucleotide sequences that flank the sequence of interest. Their complementarity allows them to bind to the regions on either side of the target DNA and signal which portion of the DNA to amplify. This allows the PCR reaction to amplify, or copy, a specific portion of the genome. Primer design involved aligning the available *E. blandingii* sequences for the mitochondrial gene, cytochrome b (cyt b), from Genbank (http://www.ncbi.nlm.nih.gov/genbank/) and locating regions that were conserved among *E. blandingii*. To ensure that primers did not also amplify other local turtle species, cyt b sequences were also included for the following species: Spotted Turtle, Ornate Box Turtle, Map Turtle, False Map Turtle, Red-eared Slider, Softshell Turtle, Alligator Snapping

Turtle, Common Musk Turtle, Bog Turtle, Wood Turtle, Common Snapping Turtle, Painted Turtle, and Eastern Box Turtle, which are likely to occur in the same habitat as *E. blandingii* (Fig. 2). Thus, to design primers that would selectively amplify only Blanding's Turtle DNA the primer sequences had to be conserved among Blanding's Turtles but variable among other species. In this way, I was able to identify four possible pairs of primers for this project. Each of the pairs produced PCR fragments of various lengths. However, after some initial trials, I found that a primer pair that amplified a 219 bp region of the cyt b gene worked best. Once primers were selected that would amplify a short region of the cyt b gene, the primers were optimized, largely by varying the annealing temperature, the temperature at which primers were allowed to anneal to the target DNA. Although, the primers were designed to amplify only Blanding's Turtle DNA, mispairing of primers may still occur when PCR conditions are not stringent enough. To test for the optimal PCR conditions, I included blood extractions of *E. blandingii* and five other local turtle species in the PCR with the goal being to amplify only *E. blandingii* DNA. I found that increasing the annealing temperature eliminated the problem of multi-species amplification by decreasing the likelihood that the primers would mispair during the annealing process (Fig. 3).

The final optimized PCR protocol was as follows: 1 U taq polymerase, 1X PCR buffer, 2 mM (MgCl2), 0.2 mM dNTPs, 0.25 μM species-specific primers[1], 2 μL DNA template, and ddH2O for a total volume of 20 μL. The PCR program consisted of an initial incubation of 5 min. at 94°C, followed by 35 cycles of denaturation at 94°C for 30s, annealing at 63°C for 30s, extension at 72°C for 30s, and a final extension for 7 min. at 72°C. Following PCR

[1] The primers used in this experiment were (CCACATTGGGCGGGGACTTTAC) and

amplification, the presence or absence of Blanding's Turtles was inferred from presence or absence of a 219-bp band on a 1% agarose gel.

As a positive control, samples were first collected from habitats that are known Blanding's Turtle sites (Boone Co., IL; Grundy Co., IL). At each site, a series of 2-3 L water samples were collected in autoclaved plastic Nalgene bottles. Shortly thereafter, the water samples were filtered through 0.45 μm nitrocellulose Millipore filters using a peristaltic pump. Filters were stored in 95% ethanol at –20°C until eDNA extraction. After air-drying the filters overnight, eDNA was isolated using the prescribed isolation protocols of the Qiagen Puregene® Core Kit A.

Results

With the USOAR grant, I was able to demonstrate the feasibility of this method by amplifying Blanding's Turtle eDNA from water in which captive turtles were housed, while still controlling for amplification of other species (Fig. 1). Five out of the six samples that were tested produced positive results with bands of the appropriate size. The amplified DNA was later confirmed to be Blanding's Turtle DNA through sequencing and verification using the Basic Local Alignment Search Tool (BLAST). Furthermore, none of the negative controls, which included DNA samples from five other locally occurring species, amplified during the PCR, indicating that the designed primers will selectively amplify Blanding's Turtle DNA.

I was also able to extract eDNA from water samples collected from known Blanding's Turtle habitats in Boone and Grundy County, Illinois. Efforts to amplify Blanding's Turtle DNA from these extracts are ongoing.

Further Research

After demonstrating the feasibility of this molecular tool, I am now testing the method with water samples collected from field sites where Blanding's Turtles are known to occur. Following successful amplification of Blanding's Turtle eDNA from these sites, other sites where Blanding's Turtles have been present historically and sites where the current status of Blanding's Turtles is unknown will be tested. Samples collected from the NIU lagoon, where Blanding's Turtles are thought to be absent, will be used as a negative control. Just as before, the DNA will be amplified using species-specific primers, which amplify a short segment of the cytochrome b gene. As a final analysis of the relative advantages of this method compared to more traditional survey methods, I will compare the relative costs, with respect to both time and money, between traditional survey methods and eDNA methods.

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References

Congdon, J.D., T.D. Graham, T. B. Herman, J.W. Lang, M.J. Pappas, and B.J. Brecke. (2008). *Emydoidea blandingii* (Holbrook 1838)-Blanding's Turtle. Conservation Biology of Freshwater Turtles and Tortoises: A Compilation Project of the IUCN/SSC Tortoise and Freshwater Turtle Specialist Group Chelonian Research Monographs No.5: 015.1-015.12.

Dejean, T., A. Valentini, A. Duparc, S. Pellier-Cuit, F. Pompanon, P. Taberlet, and C. Miaud. (2011). Persistence of environmental DNA in freshwater ecosystems. *PLoS ONE* 6(8): e23398.

Ficetola, G. F., C. Miaud, F. Pompanon, and P. Taberlet. (2008). Species detection using environmental DNA from water samples. *Biology Letters* 4: 423-425.

Goldberg C. S., D. S. Pilliod, R. S. Arkle, and L. P. Waits. (2011). Molecular detection of vertebrates in stream water: a demonstration using rocky mountain tailed frogs and Idaho giant salamanders. *PLoS ONE* 6(7): e22746.

Jerde, C. L., A. R. Mahon, W. L. Chadderton, and D.M. Lodge. (2011). "Sight-unseen" detection of rare aquatic species using environmental DNA. *Conservation Letters* 4: 150-157.

Olsen, Z. H., J. T. Briggler, and R. N. Williams. (2012). An eDNA approach to detect eastern hellbenders (Cryptobranchus a. alleganiensis) using samples of water. *Wildlife Research* 39: 629-636.

Rubin, C. S., Warner, R. E., Ludwig, D. R., &Thiel, R. (2004). Survival and population structure of Blanding's Turtles (*Emydoidea blandingii*) in two suburban Chicago forest preserves. *Natural Areas Journal* 24: 44-48.

Takahara, T., T. Minamoto, and H. Doi. (2013). Using environmental DNA to estimate the distribution of an invasive fish species in ponds. *PLoS ONE* 8(2): e56584.

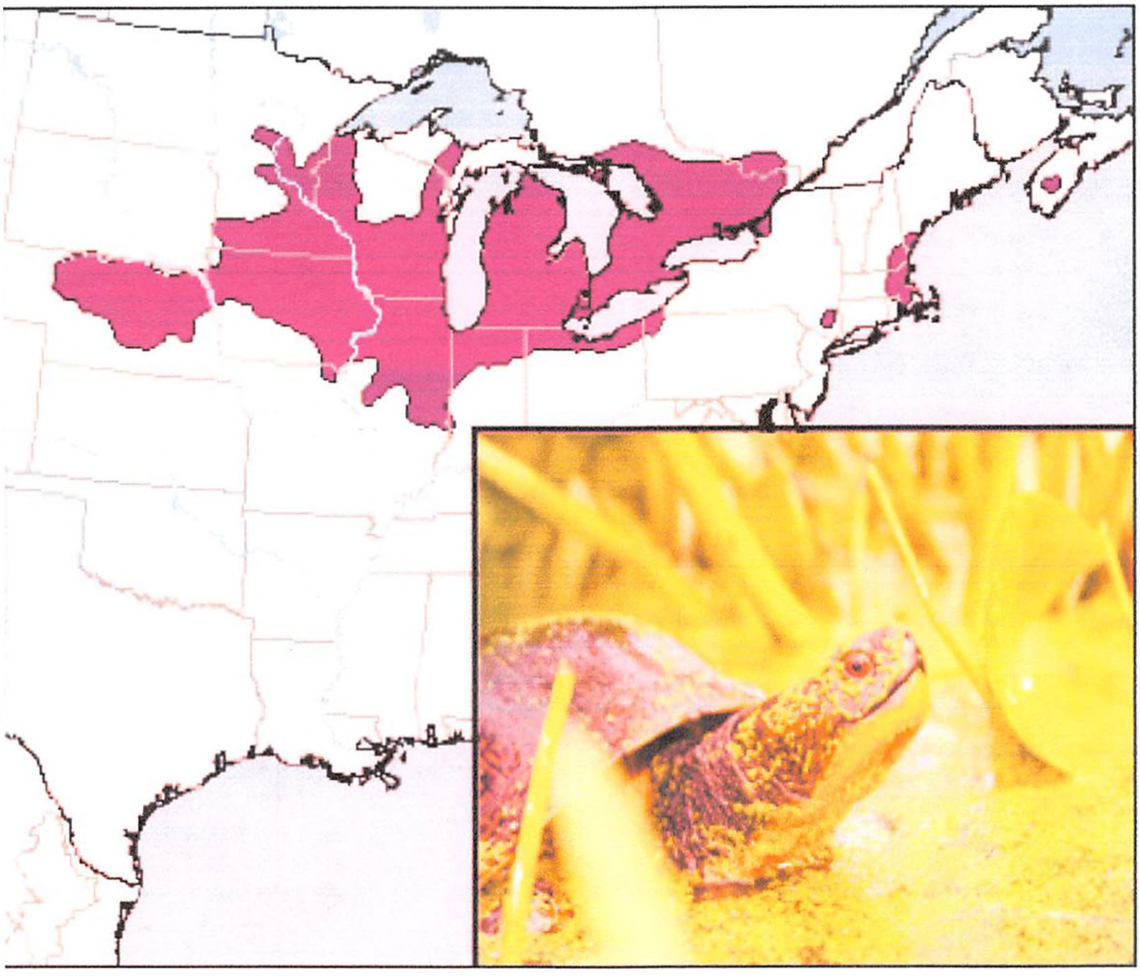

Fig. 1 Historic Range of Blanding's Turtle (Natureserve.org) and photo of Blanding's Turtle courtesy of J. Ross

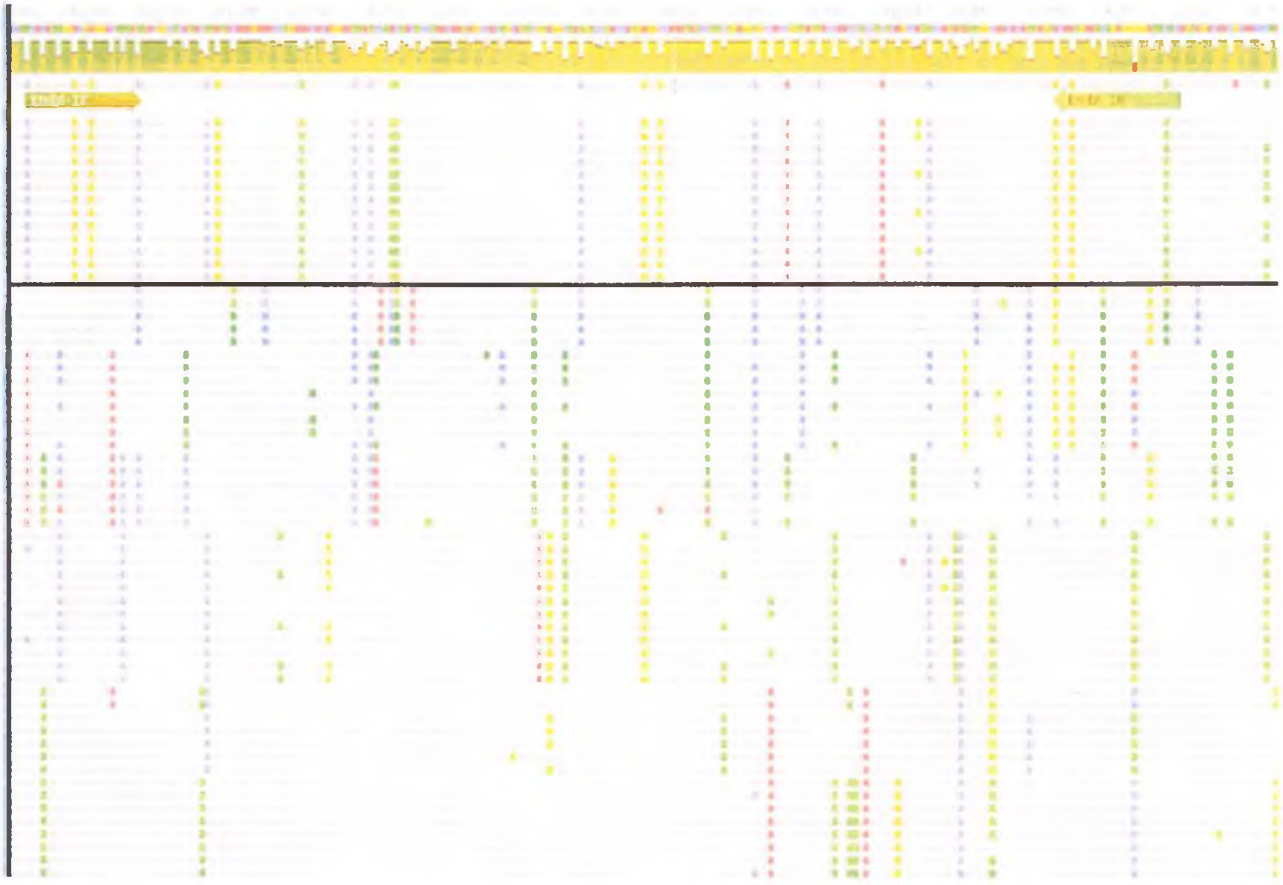

Figure 2. Cytochrome b nucleotide alignment used to design species-specific primers (*Embl-1F* and *Embl-1R*) for Blanding's Turtle. The first 14 lines represent Blanding's Turtle sequences and the remainder are other turtle species. The green arrows indicate primer binding sites.

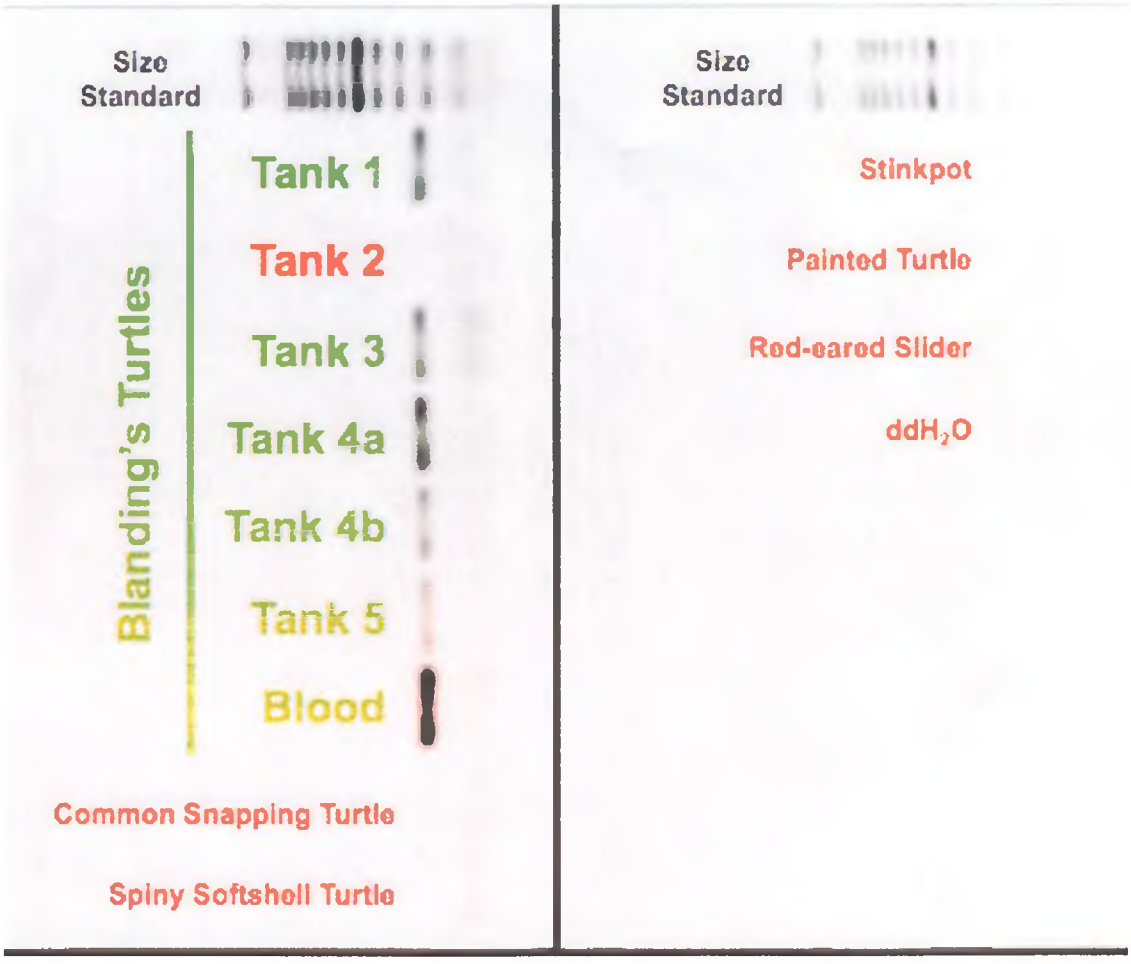

Fig. 3 Agarose gel showing presence of Blanding's Turtle DNA as a 219-bp band (dark bands indicate successful amplification and are also indicated by green text)