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1. Applicant's Information

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2. Type of Project: Environmental Research

3. Project Name and Description: *Can Populations of Blanding's Turtle in Northeastern New York be Detected and Monitored using Environmental DNA? – A Validation Study*

Project Description: Blanding's turtle, *Emydoidea blandingii*, is a semi-aquatic freshwater turtle of the northern United States and southern Canada that is considered imperiled across most of its range. The species is currently listed as Threatened in New York State and the New York Comprehensive Wildlife Conservation Strategy (CWCS) identifies the Blanding's turtle as a Species of Greatest Conservation Need, with a decreasing population in the St. Lawrence Valley. Threats to Blanding's turtle populations are intimately linked to their intrinsic life history traits such as long lifespans, delayed maturity, specific temperature requirements, and long nesting migrations. Degradation of wetlands is responsible for the largest loss of Blanding's turtle populations. Blanding's turtles preferentially reside in permanent, shallow fresh water wetlands and lake edges, but extensively use terrestrial landscapes and small ephemeral wetlands during seasonal movements. A decrease in wetland quality, elimination of ephemeral wetlands, and the encroachment of land development have all contributed to Blanding's turtle habitat degradation. Road mortality, nest predation, and climate change are other important external threats to Blanding's populations.

Obviously a first step to conserving Blanding's turtle is to know where it occurs – what permanent and ephemeral wetlands are used by this species, and whether there are seasonal patterns of usage. To evaluate the effectiveness of wetland habitat restoration or other forms of conservation management (e.g. under-road passage structures), it is also essential to be able to document that turtles actually occur in the mitigated wetlands. For Blanding's turtle, this is a challenge. Blanding's turtle occurs at low densities and is cryptic. Traditional hoop-trapping, the current survey method for this species, is labor-intensive and has a low success rate; given that the capture rate in wetlands where the species is known to occur is on the order of 1% of trap-nights in the St. Lawrence Valley, it requires extraordinary effort and cost to conclude with confidence that the species doesn't occur in a wetland.

Within the last two years, however, a new survey method has been developed that can - if field validated – provide accurate indication of presence or absence of Blanding's turtle and other turtle species in a wetland, at a fraction of the cost of a traditional survey (see *Davy CM, Kidd AG, Wilson CC (2015) Development and Validation of Environmental DNA (eDNA) Markers for Detection of Freshwater Turtles. PLoS ONE 10(7): e0130965. doi:10.1371/journal.pone.0130965* The use of environmental DNA (eDNA) is emerging as valuable survey technique for the detection of rare, cryptic, or invasive aquatic species. Species naturally release small amounts of DNA into their environment (within feces, saliva, dead skin cells, etc.), which can be detected and amplified from water samples with species specific PCR primers (Davey et al. 2015). eDNA is degraded by both biotic and abiotic factors, such as UV exposure and microbial activity, and has been shown to be detectable for less than 30 days (Dejean et al. 2011). By using eDNA, species at low densities can be quickly detected without capture or visual identification; detection rates of rare species by conventional survey methods, such as trapping or visual surveys, can produce varying and low detection probabilities. Detection of Blanding's turtles in New York is typically very low and ranges substantially between locations, which increases the possibility of false negative occupancy results. The sensitivity of eDNA survey techniques and the short time span of detectability provide an opportunity to assess the current known range of Blanding's turtle in the St. Lawrence River ecosystem and determine if the species is actually present in locations that were previously listed as unoccupied according to negative trapping results.

No-one has yet conducted a full field validation study of eDNA surveys for Blanding's turtle. The objective of our proposed study is to determine whether eDNA can be used in the St. Lawrence Valley as a less expensive and more accurate survey method for determining wetland occupancy (including seasonality of occupancy) of Blanding's Turtle. We will do eDNA surveys for Blanding's turtles in a set of wetlands where the species is known to occur and a set of wetlands that we know with certainty that the species does not occur, and use these data to calculate the probability of detection and the probability of a false positive. We will also survey a set of wetlands that appear suitable for the species, but have in the past been surveyed conventionally with no detections. We will develop a protocol for eDNA surveys for Blanding's turtle (and other turtle species), with the goal of seeking future funding to do comprehensive surveys on spatial and temporal patterns of wetland occupancy in the St. Lawrence Valley, should the method prove valid and an improvement over the traditional trapping survey method.

Blanding's turtle surveys using eDNA potentially have several advantages over conventional surveys: (1) eDNA surveys may be much less costly. We estimate that eDNA surveys are 5-10% the cost of a conventional trapping survey. (2)

eDNA surveys may be more accurate. If Davy et al. (2015) results are valid for this region, then eDNA surveys are likely to have a higher detection rate and a comparable false positive rate to conventional trap surveys. (3) eDNA surveys require much less field technical skills and training, and are safer; no animals are handled, there is no wading through marshes etc. (4) eDNA surveys eliminate stress and risk of injury to the turtles (and non-target species that enter traps), since eDNA collection is non-invasive. From an animal welfare standpoint it is always desirable to minimize risk to animals, and this is especially true for rare and threatened species.

For this validation study, to test the sensitivity of the assay we will collect water samples at 27 locations in St. Lawrence County, NY where, based on Dr. Glenn Johnson's trapping surveys, Blanding's turtles have been detected, including both high and low population density wetlands. We will also test 27 wetlands for which trapping surveys did not detect Blanding's turtles or else have not been trapped. Six wetlands where we are certain that the species does not occur will be surveyed to serve as negative controls. At each location, 30 mL water samples will be collected at twenty locations around the wetland (600 mL total), pooled, and kept on ice until transported to the lab. Each 30 mL subsample will be taken at a location that matches an ideal trapping location as recommended by the Northeast Blanding's Turtle Working Group. Six 15 mL subsamples will be added to 50 mL micro centrifuge tubes (with 33 mL of absolute ethanol and 1.5 ml of sodium acetate) and centrifuged; the material will then be pooled and mailed to the processing lab following the USFWS's Quality Assurance Project Plan (QAPP); we have communicated with three processing labs capable of doing the appropriate analyses (e.g. with set protocols for rare and degraded DNA samples). At the processing lab, samples then will be analyzed with qPCR. Each sample will be run in 8-12 replicates, including positive and negative controls. The high number of replicates is fairly standard among eDNA protocols, especially with low density target species.

By developing and validating a more accurate and less costly method to survey for Blanding's turtle presence, our study is intended to address the information needs of agencies working to restore and manage Blanding's turtle habitat and populations along the St. Lawrence River. Better survey methods will result in better monitoring and adaptive management of the currently ongoing Habitat Improvement Projects (HIPs) initiated under the relicensing of NYPA's hydropower projects. Several of the HIPs are being implemented in Wilson Hill Wildlife Management Area and Coles Creek State Park and focus on improving wildlife habitat along the St. Lawrence River including for the Blanding's turtle. The information from this project can also benefit and compliment other regional conservation and management projects such as the New York State Blanding's' turtle recovery plan, and the Northeast Blanding's turtle conservation plan.

The results will be available in a report produced for the NYSDEC and other stakeholders. The technical findings will also be disseminated via oral presentations to the Massena AOC Remedial Action Committee (RAC), NYSDEC conservation planners and managers, the international Blanding's turtle recovery team, and personnel involved with implementing the HIPs. Results will also be presented at the St. Lawrence River and Great Lakes conferences, and via peer-reviewed papers in appropriate disciplinary journals (e.g. Herpetological Conservation & Biology). The long-term goal is to use this validation study is to justify funding for a detailed comprehensive study of Blanding's turtle distribution and seasonality in the St. Lawrence Valley.

PI **Tom Langen** has experience with field survey methodology for Blanding's turtle and other species, and has completed a Species Distribution Model for Blanding's turtle in the St. Lawrence Valley. He frequently collaborates with governmental agencies (e.g. NYSDEC, USFWS) and non-governmental organizations (e.g. Nature Conservancy). Co-PI **Glenn Johnson** is on the international Blanding's Turtle Recovery Team and has been highly-involved in conservation and management of the species in the St. Lawrence Valley. Project Participant **Michael Bicknell** will conduct most of the sampling, sample preparation and analyze the final results for his graduate research; he has conducted field surveys for turtles, and has laboratory training in molecular biology, including all of the laboratory techniques and tools needed for this project. Michael is a Ph.D. student in Clarkson University's Interdisciplinary Bioscience and Biotechnology (IB&B) program with a specialization in ecology and evolution.

4. Budget

eDNA extraction and qPCR analysis	$	12,000	*(60 samples at $200 per sample)*
Travel to Collect Samples	$	2000	*(40 rt x 100 miles-rt x $0.5/mile)*
Supplies	$	1,000	*(sampling materials, buffers, molecular grade ethanol, sodium acetate, coolers, ice, shipping materials etc.)*
Matching Funds	$	15,000	*($7800 deferred overhead; $7200 partial summer salary & fringe)*
Total proposal amount	$	30,000	
SLRREF contribution requested	**$**	**15,000**	

5. Schedule: Sample collection will begin mid May 2016 (depending on weather conditions) and continue through July 2016. eDNA sample preparation for analysis will be conducted within 48 hours of sample collection and stored until shipment for qPCR analysis. qPCR analysis will be conducted June-August 2016. Analysis of final results and report writing will be conducted during the fall of 2016 and completed by December 31, 2016.

Results of previous SLRREF proposals to PI Langen.

(1) ***Wildlife Barrier for Reptiles & Amphibians along New York State Highway 68 (SH 68) at Upper and Lower Lakes State Wildlife Management Area (ULL WMA)*** (2008). A 1.2 km turtle wildlife barrier was installed on both sides of SH 68 at ULL WMA, as was an informational kiosk on the project. The project resulted in one publication (*Langen, T.A.. 2011. Design considerations and effectiveness of fencing for turtles: three case studies along northeastern New York state highways. Proceedings of the 2011 International Conference on Ecology and Transportation 545-556. http://www.icoet.net/ICOET_2011/proceedings.asp*) and one book chapter, and presentations to NYS DOT, NYS DEC, and several international and national conferences on wildlife and transportation.

(2) ***Breeding golden-winged warbler use of electrical transmission line corridors in the St. Lawrence Valley of New York*** (2009). Findings are incorporated in the New York Audubon / USDA National Resource Conservation Service private partnership habitat restoration program for Golden-winged Warblers in the St. Lawrence Valley, and in the Golden-winged Warbler Status & Conservation Plan (http://gwwa.org/plan.html). PI Langen is a co-author of a new guide for environmental managers *Best Management Practices for Golden-winged Warbler Habitat on Utility Rights-of-way* that is to be distributed by the Golden-winged Warbler Working Group (a consortium of state and federal agencies and environmental NGOs) under direction of the Cornell Lab of Ornithology.

(3) ***Evaluation of Biodiversity and Water Quality Indicators of Putative Beneficial Use Impairments and Ecosystem Services in Wetlands of the Massena (New York) Area of Concern*** (2012). A final report was submitted to NYPA and NYS DEC. Oral reports were given to the Massena AOC Remedial Action Committee, St. Lawrence River Conference and other venues. A research paper is in press in Journal of Great Lakes Research (*Stryszowska, K.M., M.R. Twiss, and T.A. Langen. 2016. Biodiversity and water quality indicators of Beneficial Use Impairments in wetlands of the Massena (New York) Area of Concern. Journal of Great Lakes Research*).

(4) ***Predictive model for Blanding's turtle distribution in northern New York; a tool for conservation and management*** (2013). A final report was submitted to NYPA and NYS DEC, and the results incorporated into the New York State (draft) Recovery Plan for Blanding's Turtle. A research paper is in press in Journal of Herpetology (*Stryszowska, K.M., G. Johnson, L. Rivera Mendoza, and T.A. Langen. 2016. Species distribution modeling of the threatened Blanding's turtle's (Emydoidea blandingii) range edge as a tool for conservation planning. Journal of Herpetology*).