Alberta Conservation Association
2013/14 Project Summary Report

Project Name: Amphibian Monitoring Using Environmental DNA

Wildlife Program Manager: Doug Manzer

Project Leader: Kris Kendell

Primary ACA staff on project:

Kris Kendell and Doug Manzer

Partnerships

Natural Sciences and Engineering Research Council of Canada – Industrial Postgraduate Scholarships Program
University of Alberta – Brandon Booker (M.Sc. candidate), David Coltman, Corey Davis and Cynthia Paszkowski

Key Findings

- We obtained tissue for 10 species of amphibians that occur in Alberta and successfully tested lab approaches to identify the unique DNA signatures for each.
- We began developing techniques to detect environmental DNA for individual species from water samples collected from natural ponds as well as from controlled aquariums.
- Early results demonstrate successful detection from water samples, although we are refining techniques to improve consistency.

Introduction

Living organisms can leave a DNA signature from organic matter suspended in water (e.g., mucus, feces, urine, sloughed tissue) that is detectable with genetic analysis. Alberta Conservation Association (ACA) and University of Alberta are partnering to test a novel approach for detecting the presence of amphibians using environmental DNA (eDNA) collected from water samples. Although eDNA detection is a new survey technique, a few studies have shown that it is possible to detect aquatic and semi-aquatic organisms through traces of their DNA suspended in water (e.g., Ficetola et al. 2008; Goldberg et al. 2011; Jerde et al. 2011; Dejean et al. 2012; Thomsen et al. 2012).

We engaged a graduate student (Brandon Booker) from the University of Alberta through a Natural Sciences and Engineering Research Council of Canada Industrial Postgraduate Scholarship to work with us to develop this eDNA survey approach. Our main objective is to develop a reliable approach for detecting the presence of amphibians in natural waterbodies. To develop this approach, we are focusing most of our effort on the ubiquitous wood frog and rarer Canadian toad. However, we will also prepare DNA amplification and extraction protocols to

detect all amphibian species found in Alberta. Other factors being examined include the influence of season on the reliability of eDNA detection and the potential to determine relative abundance of a given species within and among waterbodies.

Methods

We obtained tissue for all 10 amphibian species found in Alberta for refining the lab techniques to detect their genetic signatures. This material was provided by tissue banks (e.g., museums, GenBank) and by local researchers in possession of the required genetic material.

We identified 26 natural ponds and collected four water samples from each using a strict water sample collection and storage protocol to ensure that DNA material within water samples was properly preserved. Water samples were promptly placed into a cooler prior to transferring them to freezer storage. Our protocol also aimed to minimize the potential transfer of waterborne pathogens among ponds. We collected water samples over two seasonal periods to coincide with the individual breeding and metamorphosis periods of Canadian toad and wood frog, respectively. To act as a control, we added Canadian toad tadpoles to an aquarium filled with dechlorinated tap water and collected water samples over a 60-day period.

Results

We tested and refined our lab techniques for identifying the DNA signatures of all 10 amphibian species known to Alberta. We successfully amplified amphibian eDNA in 10 of the 30 water samples using a trial extraction protocol. We are continuing to refine these techniques for greater reliability. Preliminary results suggest eDNA may be detected in water samples stored in freezer conditions for at least one year.

Laboratory tests of the remaining water samples are underway to determine the reliability of amphibian detection in natural ponds over time and in differing volumes of water based on the presence/absence of DNA in the samples. We are also examining the influence of season on the reliability of eDNA detection and our ability to determine relative abundance of a given species within and among waterbodies.

Conclusions

This new technique will allow us to detect amphibian presence by simply taking a water sample and having it analyzed in a genetics laboratory. This technique will be a vast improvement over traditional methods used for surveying amphibians, which commonly require highly skilled personnel often working at night in remote areas. This new approach will allow us to collect water samples at any time of day or night with minimal time spent at a location, as well as the flexibly to engage non-specialists (possibly volunteers) in sampling.

Communications

- ACA email newsletter article: “Seeing Without Eyes: Using eDNA to Track Amphibian Distributions,” November 2013.

- Student oral presentation to Alberta Amphibian Reptile Specialist Group, 17th annual meeting, March 2014.
- Poster presentation to joint meeting devoted to Enhancing Conservation Program Delivery among Agricultural and other Natural Resource Interests, Pasco Washington, February 2014.

Literature Cited

Dejean, T., A. Valentini, C. Miquel, P. Taberlet, E. Bellemain, and C. Miaud. 2012. Improved detection of an alien invasive species through environmental DNA barcoding: the example of the American bullfrog *Lithobates catesbeianus*. Journal of Applied Ecology 49: 953–959.

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

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: 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.

Thomsen, P.F., J. Kielgast, L. Iversen, C. Wiup, M. Rasmussen, T.P. Gilbert, L. Orlando, and E. Willerslev. 2012. Monitoring endangered freshwater biodiversity using environmental DNA. Molecular Ecology 21: 2565–2573.

Photo Captions

Collecting a water sample for eDNA analysis. Collector: Brandon Booker (M.Sc. candidate). Photo: Kyle Welsh
[filename: Photo1_CanadianToadeDNA_2013-14_Kyle Welsh.jpg]

Wood frog (*Lithobates sylvaticus*). Photo: Kris Kendell
[filename: Photo2_CanadianToadeDNA_2013-14_Kris Kendell.jpg]

University of Alberta researchers Sean MacQueen (right) and Arthur Whiting (left) assisting with 2012/13 eDNA water sample collection. Photo: Kris Kendell
[filename: Photo3_CanadianToadeDNA_2013-14_Kris Kendell.jpg]