Alberta Conservation Association
2016/17 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, Doug Manzer and Amanda Rezansoff

Partnerships

Shell Canada Energy
University of Alberta – David Coltman, Corey Davis and Cynthia Paszkowski
Washington State University – Caren Goldberg

Key Findings

- We collaborated with the University of Alberta to develop an approach for detecting three amphibians commonly found in Alberta using eDNA.
- We initiated a partnership with Washington State University to test and refine three sampling protocols to improve amphibian detection using eDNA.
- We tested protocols involving eDNA sampling from water filtered through a cellulose nitrate filter, which will provide new insight into eDNA sampling methods for Alberta's amphibian species.

Introduction

Living organisms can leave a DNA signature from organic matter suspended in the environment from the release and persistence of extracellular matter, such as mucus, feces, urine and sloughed tissue, which becomes detectable with genetic analysis. This environmental DNA, or eDNA, has been successfully detected in water (Ficetola et al. 2008; Goldberg et al. 2011; Jerde et al. 2011; Hobbs and Goldberg 2015) and pond sediments (Willerslev et al. 2003; Turner et al. 2015) for a number of species.

In partnership with the University of Alberta (UofA) and a graduate student, Brandon Booker, we developed a standardized eDNA sampling protocol and assay that allowed us to identify at least three species of amphibians in Alberta by simply taking samples of water and sediment from ponds. To support the study, we provided water and/or sediment samples from the Edmonton area in 2012 and from the Shell Carmon Creek project near Peace River in 2014 and 2015. Brandon Booker published his thesis, "Developing and Assessing an Environmental DNA Protocol for Detecting Amphibian Species in Lentic Systems in Alberta, Canada," in June 2016. Although there are some details to be resolved, his thesis supported the theory that amphibian DNA in the environment can be used as a proxy for directly observing a target species once robust sample collection and assay protocols are established.

In 2016, we also investigated three sample collection techniques to improve the detection of amphibians using eDNA: 1) collecting a simple water grab sample, 2) passing water through a cellulose nitrate filter, and 3) collecting surface material from the top of the substrate on a pond floor. We collected water and sediment samples from five ponds near Edmonton and submitted them to Washington State University, where they are being analyzed to try to detect the presence of up to five species of amphibian: wood frog (*Lithobates sylvaticus*), boreal chorus frog (*Pseudacris maculata*), western toad (*Anaxyrus boreas*), Canadian toad (*Anaxyrus hemiophrys*) and tiger salamander (*Ambystoma mavortium*). Traditional amphibian survey data from these ponds will be used to compare eDNA detection rates in corresponding water, sediment and filter samples. Results of this analysis will provide insight into optimal eDNA sampling methods for Alberta's amphibian species and serves as an important step for validating work completed in partnership with the UofA.

Methods

In June and July 2016, we surveyed five ponds near Edmonton for amphibians using traditional methods. Amphibian surveys consisted of walking along the edge of the waterbody and watching carefully for the movement of amphibians underfoot or in shallow water.

We collected water and sediment samples following protocols developed by Booker (2016) and Goldberg and Strickler (2015). Two methods were used for the water sampling technique: a grab sample using a 50 mL centrifuge tube and a filtered sample using a cellulose nitrate filter. For the sediment sampling technique, we filled 2 mL microcentrifuge tubes with surficial material from the pond floor. Three identical samples were taken using each technique at one to three collection stations depending on the size of the pond. Grab water samples were preserved and along with sediment samples stored at -20°C until processed. Filter samples were dried and kept at room temperature until processed.

We prepared field controls for each sampling technique. The controls were subjected to all aspects of sample collection, field processing, preservation, transportation and laboratory handling, and will be analyzed as environmental samples.

Results

Amphibians were detected at all five ponds using traditional methods. We found at least two species of amphibian at each pond, for a total of four species across the five ponds. Wood frog and boreal chorus frog were the most commonly encountered amphibians at ponds surveyed (Table 1). Amphibian breeding was confirmed at all ponds by the presence of tadpoles or young-of-the-year.

Table 1. Amphibian life-stage observations at ponds surveyed in June and July 2016.

Pond	Date surveyed	Life stage of species observed				
		WOFR	BCFR	WETO	CATO	TISA
315822	29/06/16	L	L	–	–	–
	14/07/16	L, Y	L, Y	–	–	–
025923	28/06/16	L, S/A	L	–	–	–
	15/07/16	Y, S/A	L	–	–	–
045923	28/06/16	S/A	–	L	–	L
	18/07/16	Y, S/A	S/A	Y	–	–
315420-A	30/06/16	L, S/A	L	L, S/A	–	–
	19/07/16	Y, S/A	–	–	–	–
315420-B	30/06/16	L	S/A	L	–	–
	19/07/16	Y, S/A	–	Y	–	–

Codes: WOFR = wood frog, BCFR = boreal chorus frog, WETO = western toad, CATO = Canadian toad; TIGA = tiger salamander, L = larvae, Y = young-of-the-year, S/A = sub-/adult,

On July 14 – 19, we collected eDNA samples from a total of eight sampling stations across the five ponds visited. In total, we collected 87 water, filter, sediment and control samples (Table 2).

Table 2. Number of water, sediment, filter and control samples taken for each eDNA sampling technique at ponds visited in July 2016.

Pond	Date sampled	Pond diameter (m)	No. of sample stations	No. of samples collected using each eDNA collection technique			
				Water	Sediment	Filter	Control
315822	14/07/16	< 40	1	3	3	3	3
025923	15/07/16	< 40	1	3	3	3	3
045923	18/07/16	> 55	3	9	9	9	3
315420-A	19/07/16	< 40	1	3	3	3	3
315420-B	19/07/16	> 40	2	6	6	6	3
	Total		8	24	24	24	15

Conclusions

Monitoring using eDNA allows for the detection of amphibian presence by simply taking a water or sediment sample and having it analyzed in a genetics laboratory. Although there are some details to be resolved with collection and assay protocols for species of amphibians found in Alberta, this technique presents a potential improvement over traditional methods used for surveying amphibians. Major benefits of this new approach are the ability to collect water or sediment samples at any time of day or night, minimal time spent at a location, and the flexibly to engage non-specialists. Samples from 2016/17 are still being analyzed, but we are hopeful that results of this study will provide insight into optimal eDNA sampling methods for Alberta's amphibian species.

Communications

- Joint Meeting: Society for Northwestern Vertebrate Biology, California North Coast Chapter of the Wildlife Society, NW Partners in Amphibian and Reptile Conservation, Arcata, CA, February 28 – March 3, 2017.
- Lunch and Learn Talk: Shell Canada Energy, Calgary AB, March 16, 2017.
- Meeting: 20th Annual Meeting of the Alberta Amphibian and Reptile Specialist Group, Red Deer, AB, March 22, 2017.

Literature Cited

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Photos

Alberta Conservation Association biologist Amanda Rezansoff conducts an amphibian survey at an eDNA study pond near Edmonton. Photo: Kris Kendell

Alberta Conservation Association biologist Kris Kendell filters an eDNA water sample through a cellulose nitrate filter using a disposable filter funnel and a vacuum hand pump. Photo: Amanda Rezansoff

Alberta Conservation Association biologist Peter Aku collects water quality information from an eDNA study pond near Edmonton. Photo: Kris Kendell