Alberta Conservation Association
2017/18 Project Summary Report

Project Name: Amphibian Monitoring Using Environmental DNA

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Partnerships

Alberta Environment and Parks
Shell Canada Energy
Washington State University – Caren Goldberg

Key Findings

- We tested different techniques involving eDNA sampling and revealed that some methods may be less suitable for detecting certain amphibian species that occur in either lower densities or have tadpoles with schooling behaviour.
- We collaborated with Washington State University to further develop field sampling protocols to improve aquatic sediment sampling.

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. 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. 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 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 (WSU), where they were analyzed in 2017 for the presence of genetic material of three species of amphibian: wood frog, boreal chorus frog, and boreal toad.

Laboratory results revealed that some sampling techniques may be less suitable for detecting certain amphibian species that occur in either lower densities or have tadpoles with schooling behaviour so that their eDNA is not as well mixed into the aquatic system as other amphibians. These findings provided further insight into optimal eDNA sampling methods for Alberta's amphibian species.

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 millilitre centrifuge tube and a filtered sample using a cellulose nitrate filter. For the sediment sampling technique, we filled 2 millilitre microcentrifuge tubes with surficial material from the pond floor. Three identical field samples were taken using each technique at one to three collection stations depending on the size of the pond. 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 analyzed as environmental samples. Water grab and sediment samples were stored at WSU in a -80°C freezer until processed. However, a freezer failure occurred and the samples sat at room temperature for several days before being re-frozen. Filter samples were stored at room temperature inside a cabinet until extraction. In summer 2017, each species was analysed separately by WSU in triplicate (three repeat analysis of the same filter, sediment, and water grab sample).

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).

Table 1. Amphibian species detected at study ponds surveyed in June and July 2016.

Pond	Date surveyed	Field species detected				
		WOFR	**BCFR**	**BOTO**	**CATO**	**TISA**
315822	29/06/16	●	●	x	x	x
	14/07/16	●	●	x	x	x
025923	28/06/16	●	●	x	x	x
	15/07/16	●	●	x	x	x
045923	28/06/16	●	x	●	x	●
	18/07/16	●	●	●	x	x
315420-A	30/06/16	●	●	●	x	x
	19/07/16	●	x	x	x	x
315420-B	30/06/16	●	●	●	x	x
	19/07/16	●	x	●	x	x

Codes: WOFR = wood frog, BCFR = boreal chorus frog, BOTO = boreal toad, CATO = Canadian toad; TISA = tiger salamander
● = Detected
x = Not detected

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			
				Filter	Sediment	Water	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	40-55	2	6	6	6	3
Total			8	24	24	24	15

Laboratory assay results of filter, sediment, and water grab samples revealed that water filter samples had the highest number of total positive tests for species that were present. Where the target species was confirmed present by traditional surveys, 31percent of water filter samples tested positive compared to 16 percent of the water grab samples. Sediment samples had very few positives. Direct water grab sampling was variable in detection as compared to water filter samples and may reflect the patchiness of eDNA in waterbodies and limitations of smaller volumes of water collected. Boreal toads were the most difficult to detect of all the species and were not detected in any sediment or water grab samples suggesting that some sampling techniques may be less suitable for detecting certain amphibian species. At ponds where boreal toads were not detected during field surveys, molecular material for that species were also not detected in the filter, sediment, and water grab samples analyzed for eDNA at the laboratory (Table 3).

Table 3. Laboratory assay results of filter, sediment, and water samples from ponds where target field species were detected. Number of total positive triplicate tests for species that were present.

Pond	Species	Field species detected	Number of positive triplicate tests*		
			Filter	Sediment	Water
315822	**WOFR**	●†	9/9	1/9	9/9
	BCFR	●†	9/9	1/9	6/9
	BOTO	x	-	-	-
025923	**WOFR**	●†	8/9	2/9	0/9
	BCFR	●†	9/9	1/9	8/9
	BOTO	x	-	-	-
045923	**WOFR**	●†	0/27	0/27	0/27
	BCFR	●‡	0/27	0/27	0/27
	BOTO	●†	2/27	0/27	0/27
315420-A	**WOFR**	●†	1/9	0/9	0/9
	BCFR	●†	0/9	0/9	0/9
	BOTO	●†	0/9	0/9	0/9
315420-B	**WOFR**	●†	18/18	2/18	8/18
	BCFR	●‡	0/18	0/18	0/18
	BOTO	●†	5/18	0/18	0/18
Percent of total number of positive tests			61/198 (31%)	7/198 (4%)	31/198 (16%)

Codes: WOFR = wood frog, BCFR = boreal chorus frog, BOTO = boreal toad
● = Detected
x = Not detected
* A single positive test is not generally considered confirmatory evidence of species presence
† Confirmed amphibian breeding by the presence of tadpoles or young-of-the-year
‡ Sub-adults or adults detected only

Conclusions

Monitoring using eDNA allows for the detection of amphibian presence by simply taking a water or aquatic sediment sample and having it analysed in a genetics laboratory. Major benefits of this approach are the ability to collect samples at any time of day or night, minimal time spent at a location, and the flexibly to engage non-specialists. Although eDNA sampling methods for Alberta's amphibian species stand in need of further assessment, collecting a small amount of surficial sediment may be the most economical and straightforward approach when considering citizen science applications and amphibian surveys over large regions. Our results for sediment yielded poor detections; however, our sediment samples were likely degraded from the unforeseen degradation caused by a freezer failure. Given the higher success rate found by Booker (2016) for sediment, we will recast the aquatic sediment sampling technique in 2018/19.

Communications

- St. Albert Fish and Game Association meeting; Kris Kendell; St. Albert, AB; January 9, 2018.
- Edmonton Nature Club; Kris Kendell; Edmonton, AB; February 16, 2018.

Literature Cited

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Willerslev, E., A.J. Hansen, J. Binladen, T.B. Brand, M.T.P Gilbert, B. Shapiro, et al. 2003. Diverse plant and animal genetic records from Holocene and Pleistocene sediments. Science 300:791–795.

Photos

Boreal chorus frog tadpole (left) and tiger salamander larva (right). Photo: Kris Kendell

The eDNA sampling technique can be especially useful when trying to detect species that rely on their camouflage and little movement to elude detection and that occur in low densities within the survey area. Photo: Kris Kendell

More elaborate water sampling techniques for eDNA involve greater volumes of water and a flow-through filter system. Photo: Kris Kendell