Alberta Conservation Association

2018/19 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 employed a new sampling method to improve our detection of amphibian eDNA that may have a patchy distribution within ponds.
- We detected amphibian eDNA for species that were not detected during conventional survey methods at 40% of ponds sampled.
- We compared two approaches for collecting eDNA and the filtration technique performed better than the sediment technique for detecting the presence of amphibians in ponds.
- The filtration technique was as good as conventional surveys for detecting the target amphibians in all ponds.

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; Booker 2016) and pond sediments (Willerslev et al. 2003; Turner et al. 2015; Booker 2016) for numerous 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 aquatic 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 for the detection of amphibians using eDNA (grab water, filter, and aquatic sediment). We submitted the samples to Washington State University (WSU), where they were analyzed in 2017 for the presence of genetic material of three species of amphibian: boreal toads (*Anaxyrus boreas*), wood frogs (*Lithobates sylvaticus*), and boreal chorus frogs (*Pseudacris maculata*). Laboratory results suggested that the sampling methods employed in 2016 has shortcomings for detecting amphibians that occur at low densities, or where eDNA from tadpoles with schooling behaviour is not well mixed into the aquatic system.

In 2018, we adjusted our sampling approach so that eDNA in ponds from species with low density or tadpoles with schooling behaviour would not be missed as easily during sampling. Our new approach helped ensure more complete coverage of sampling ponds so that target species were more fully and uniformly represented in the set of samples collected.

Methods

In late June 2018, we surveyed five ponds near Edmonton for amphibians using traditional methods (ACA and Alberta Sustainable Resource Development 2010). We also collected eDNA samples using two approaches (i.e., water filter vs aquatic sediment) following protocols used by Goldberg and Strickler (2015) and Booker (2016), with slight modification to improve spatial sampling at each pond. Sample collection occurred late-June and early-July 2018.

We identified ten equally spaced sampling stations around the perimeter of each of the five ponds. From each station, we collected three 100 ml pond-water samples and placed them into three separate 1 L containers. At each subsequent sampling station, a 100 ml sample was added into its respective 1 L container, creating three mixed 1 L samples. From each of the three mixed 1 L samples, we subsampled and filtered 250 ml water, in triplicate. Three small grab samples of pond-bottom sediment were also collected at each of the 1ten stations and placed into three separate small plastic bags. Each subsequent grab bag sample was added into its respective plastic bag creating three bags of mixed pond sediment. From each of the three bags of mixed pond sediment, we subsampled 2 ml of sediment, in triplicate. We prepared field controls for each sampling technique, for each pond. The controls were subjected to all aspects of sample collection, field processing, preservation, transportation, and laboratory handling, and analyzed as environmental samples. The sediment samples were stored at WSU in a -80°C freezer and the prepared filters were stored at room temperature inside a cabinet, until extraction.

We extracted eDNA from the filters and aquatic sediment samples using established protocols used by Goldberg et al. (2011) and Booker (2016), respectively, for boreal toads, wood frogs, and boreal chorus frogs. Each species was analyzed separately, in triplicate (three repeat analyses of the same filter and sediment 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 three species across the five ponds. Wood frog was the most commonly encountered amphibians at ponds surveyed (Table 1).

Table 1. Amphibian species detected at study ponds surveyed in June and July 2018.

Pond	Date surveyed	Field species detected				
		WOFR	BCFR	BOTO	CATO	TISA
315420-B	21/06/18	●	-	-	-	-
	27/06/18	●	-	-	-	-
045923-S	21/06/18	●	-	●	-	-
	28/06/18	●	-	●	-	-
315822	21/06/18	●	●	-	-	-
	29/06/18	●	●	-	-	-
025923	21/06/18	●	●	●	-	-
	29/06/18	●	●	-	-	-
315420-A	21/06/18	●	-	-	-	-
	04/07/18	●	-	-	-	-

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

In total, we processed (on-site) 50 filters and 50 aquatic sediment samples from five ponds: nine of each from each pond, plus a field negative of each sample type from each pond (Table 2).

Table 2. Number of filter, aquatic sediment and control samples taken for each eDNA sampling technique at ponds visited in July 2018.

Pond	Date	Filter[†]					Sediment[‡]				
		250 mL			Control	Total	2 mL			Control	Total
		A	B	C			A	B	C		
315420-B	27/06/18	3	3	3	1	10	3	3	3	1	10
045923-S	28/06/18	3	3	3	1	10	3	3	3	1	10
315822	29/06/18	3	3	3	1	10	3	3	3	1	10
025923	29/06/18	3	3	3	1	10	3	3	3	1	10

315420-A	04/07/18	3	3	3	1	10	3	3	3	1	10
Total						50	**Total**				50

†10 stations, 100 mL water collected per station, mixed into three 1L containers (A, B, C); 250 mL subsampled in triplicate

‡10 stations, grab sample of aquatic sediment collected per station, mixed into three plastic bags (A, B, C); 2 mL subsampled in triplicate

All field and laboratory controls tested negative, meaning there was no cross contamination among samples within a pond and among ponds, or among extracts. All species detected using traditional field surveys were also detected using the filter technique, at all five ponds. In comparison, detection was lower using the sediment sampling technique which failed to detect boreal chorus frogs and boreal toads at two of five ponds where they were known to be present based on field surveys.

At two ponds, eDNA sampling preformed better than conventional amphibian monitoring methods. At the first pond (315420-B), boreal toads were detected in filters but not seen during field surveys. At the second pond (045923-S), boreal chorus frogs were detected in both filters and sediment, where they were not seen during field surveys. By following robust laboratory assay design, we estimate the probability of false-positive events to be low. Overall, filters (55%) had higher proportions of samples (analyzed separately, in triplicate) testing positive for species detected in field surveys compared to sediment (39%). The wood frog had the highest number of total positive tests (100% filters, 87% sediment) for species confirmed present during field surveys (Table 3).

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

Pond	**Species**	**Detected Field Survey**	**Number of positive triplicate tests**	
			Filter	**Sediment**
315420-B	WOFR	●	9/9	9/9
	BCFR	-	0/9	0/9
	BOTO	-	1/9	0/9

	WOFR	●	9/9	3/9
045923-S	BCFR	-	4/9	3/9
	BOTO	●	5/9	0/9
	WOFR	●	9/9	9/9
315822	BCFR	●	9/9	0/9
	BOTO	-	0/9	0/9
	WOFR	●	9/9	9/9
025923	BCFR	●	9/9	9/9
	BOTO	●	1/9	1/9
	WOFR	●	9/9	9/9
315420-A	BCFR	-	0/9	0/9
	BOTO	-	0/9	0/9
Total number of triplicate positive tests (%)			74/135 55%	52/135 39%

Codes: BOTO = boreal toad, WOFR = wood frog, BCFR = boreal chorus frog; (●) = Detected, (-) = Not detected

Conclusions

We have demonstrated that eDNA can be used to detect amphibian presence in both filtered pond water and surficial pond-bottom sediment samples. Our results suggest that these molecular techniques can perform just as well as traditional amphibian monitoring methods. By adjusting our eDNA sampling strategy, we were able to improve our ability to detect certain species that occur in either lower densities or have tadpoles with schooling behaviour that may result in patchy distribution of their eDNA in a pond. We found that filtration had higher levels of detection than sediment collected at the same time. Nonetheless, sediment collection may warrant further investigating because of its sampling simplicity when compared with the filter sampling method.

Communications

- Alberta Amphibian and Reptile Specialist Group, A comparison of two eDNA sampling strategies. Red Deer, AB. March 12, 2019.

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Photos

ACA biologist, Amanda Rezansoff, processes water and aquatic sediment collected from an eDNA sampling pond near Edmonton. Photo: Kris Kendell

A wood frog tadpole is captured for identification during an amphibian survey at an eDNA sampling pond. Photo: Kris Kendell