Alberta Conservation Association

2019/20 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 – Dr. Caren Goldberg

Key Findings

- In 2018, we employed a new sampling method to improve the detection of amphibian eDNA that may have a patchy distribution within ponds.
- We are in the process of publishing a comparison of water filtration and sediment collection approaches for detecting the presence of amphibian eDNA in ponds, in collaboration with Washington State University.
- We delivered a learn-at-lunch presentation to Shell Canada Energy to share our research findings.

Abstract

Environmental DNA, or eDNA, refers to the DNA that organisms leave behind or shed as they pass through the environment. DNA technology has evolved to allow researchers to detect DNA signatures from material such as mucus, feces, urine, or sloughed skin that is naturally contained within pond water and aquatic sediment. We have worked towards a reliable method of detecting amphibians using eDNA. The first phase of this work involved a MSc project developing an approach for detecting three amphibians in water and aquatic sediment samples. The second phase involved a partnership with Washington State University to further refine and evaluate water and aquatic sediment sampling methods. In 2018, we adjusted our eDNA sampling methods to ensure more complete coverage of study ponds so that target species were more fully represented in the set of samples collected. This new strategy improved 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. The water filtration technique was as good as field surveys for confirming the presence of boreal toads, wood frogs, and boreal chorus frogs at the ponds sampled; whereas detection was lower using aquatic sediment. These results indicate eDNA sampling can be an effective alternative to more traditional amphibian monitoring methods. The next step is to write up what we have learned and make the results available to our partners and conservation community through a peer-reviewed publication.

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 (U of A) 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.

To create a scientific record of our methodologies and facilitate their reference, we are in the process writing up and publishing the comparison of sampling water and sediment for the evaluation of amphibian eDNA in lentic waterbodies.

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 10 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 ten 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) 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. 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.

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. By following robust laboratory assay design, we estimate the probability of false-positive events to be low. Overall, filters had higher proportions of samples (analyzed separately, in triplicate) testing positive for species detected in field surveys compared to sediment.

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. In 2020/21 we will continue to write up what we have learned and make the results available to our partners and conservation community.

Literature Cited

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Photos

Traditional amphibian surveys (left) are highly influenced by temperature and time of day. Alternatively, eDNA sampling (right) can improve detectability of target species at different times of the day or under certain weather conditions when amphibians are normally inactive. Photos: Amanda Rezansoff.