Alberta Conservation Association
2015/16 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

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

Key Findings

- We are trialing eDNA methods with water and sediment samples collected from three ponds at the Carmon Creek project near Peace River. The trials are being conducted at a university molecular laboratory to look for trace amounts of wood frog, boreal chorus frog and western (boreal) toad DNA.
- Samples will also be tested for inhibiting substances that may lead to inaccurate test results. We will also determine if excessive amounts of eDNA from one species affects the detection of rarer species.
- Brandon Booker successfully defended his MSc thesis entitled "Developing and Assessing an Environmental DNA Protocol for Detecting Amphibian Species in Lentic Systems in Alberta, Canada" in January 2016.
- The Shell Carmon Creek work will contribute to the refinement of Booker's eDNA field sampling protocol and laboratory assay, and will allow us to better understand the limitations of the eDNA method for monitoring amphibians in an industrial setting.

Introduction

Living organisms can leave a DNA signature in 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. We are partnering with the University of Alberta to develop and test a novel approach for detecting the presence of amphibians using environmental DNA (eDNA) collected from water and sediment samples. Although eDNA detection is a new survey technique, 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; Hobbs and Goldberg 2015) and sediment (e.g., Willerslev et al. 2003; Turner et al. 2015).

We engaged a graduate student (Brandon Booker) at 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 assessed the effects of sampling method, number of samples collected per site and seasonal timing of sampling on the detectability of low- and high-abundance amphibian species per site. To support the study, we provided water samples from the Edmonton area in 2012 and from the Shell Carmon Creek project near Peace River in 2014 and 2015. The Shell Carmon Creek work will contribute to the refinement of Booker's eDNA field sampling protocol and laboratory assay, and will allow us to better understand the limitations of the eDNA method for monitoring amphibians.

Methods

We visited all waterbodies within the greenfield areas at the Shell Carmon Creek project between July 7 and 9, 2015; all of these waterbodies were constructed borrow pits within 100 m of a road. We assessed these waterbodies for the presence of amphibians by conducting visual encounter surveys that consisted of walking along the edge of the waterbody and watching carefully for the movement of amphibians underfoot or in shallow water. Before accessing the Shell Carmon Creek project site, we disinfected our rubber boots and other field equipment (i.e., footwear and dip nets) with a 20% bleach-to-water solution to prevent the spread of amphibian diseases.

Using field protocols developed by Brandon Booker as part of his thesis work, we collected two concurrent replicate water and sediment samples per sampling station at each waterbody surveyed. For the water sampling technique, we collected 10 separate 15 mL water samples by dipping 50 mL centrifuge tubes into the waterbody and pouring off all but 15 mL of the water within the tube. Samples were preserved with absolute ethanol and sodium acetate and stored at -20°C until processed. For the sediment sampling technique, we filled 2 mL microcentrifuge tubes with surficial material from the top 2 cm of the sediment profile. Sediment samples were placed in a cooler with ice before being stored at -20ºC until processed.

All samples were submitted to the molecular laboratory in the Department of Biological Sciences at the University of Alberta to be analyzed by a geneticist.

Results

In January 2016, Booker successfully defended his thesis: "Developing and Assessing an Environmental DNA Protocol for Detecting Amphibian Species in Lentic Systems in Alberta, Canada." This work has helped clarify approaches for both field sampling and laboratory work, although we are currently exploring further opportunities for refinement.

During our survey of waterbodies at the Shell Carmon Creek project in July, we observed amphibians at all three waterbodies. We confirmed western (boreal) toad and wood frog breeding

at all three waterbodies sampled. Boreal chorus frogs were detected at two waterbodies, including evidence of breeding at one waterbody. Amphibian breeding was confirmed by the presence of tadpoles or young-of-the-year (YOY). Wood frog sub-adults were detected at two sites and boreal chorus frog sub-adults at one site (Table 1).

Table 1. Amphibian observations at ponds surveyed within greenfield areas at the Shell Carmon Creek project in 2015.

Waterbody	Date visited dd/mm/yy	Life stage of species observed		
		WETO	WOFR	BCFR
288418B	09/07/15	Tadpole	YOY	–
018518A	09/07/15	Tadpole	YOY/Sub-adult	Sub-adult
108518B	08/07/15	Tadpole/YOY	YOY/Sub-adult	YOY

WETO = western (boreal) toad; WOFR = wood frog; BCFR = boreal chorus frog; YOY= young-of-the-year

At each sampling station, we collected a concurrent replicate water, sediment and respective control sample. In total, we collected 60 water samples and 60 sediment samples from the three ponds surveyed, as well as 12 water and sediment control samples (Table 2).

Table 2. Number of samples and controls taken for each volume of water and sediment sampled.

Waterbody	Date visited dd/mm/yy	Number of samples and controls taken for each volume of water and sediment sampled				
		Water (10 mL)		Sediment (2 mL)		Control
		Set 1	Set 2	Set 1	Set 2	
288418B	08/07/15	10	10	10	10	4
018518A	09/07/15	10	10	10	10	4
108518B	09/07/15	10	10	10	10	4
Total		30	30	30	30	12

We successfully detected DNA in both water and sediment samples. Overall detection rates were highest in sediment samples compared with water samples. We detected the DNA of all three target species in only one waterbody using the water sample collection method. Sediment samples showed positive results for all three species at two of three waterbodies. PCR inhibitors (substances that affect the genetic analysis) affected eDNA detection rates in 60% of the water samples collected (18/30), resulting in an inability to detect the target DNA if present (false negatives) (Table 3).

Table 3. eDNA detection rates for each target amphibian species from 10 water and 10 sediment samples collected from each waterbody.

Species	eDNA detection rate					
	Water			Sediment		
	288418B	018518A	108518B	288418B	018518A	108518B
WETO	0/10	1/10	2/10	8/10	4/10	3/10
WOFR	0/10	0/10	4/10	8/10	7/10	4/10
BCFR	0/10	0/10	3/10	0/10	2/10	1/10

WETO = western (boreal) toad; WOFR = wood frog; BCFR = boreal chorus frog

Conclusions

Monitoring the presence of amphibians using eDNA allows for detection of amphibians by simply taking a water or sediment sample and having it analyzed in a genetics laboratory. While there are some details to be resolved with collection and assay protocols, 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 samples at any time of day or night, minimal time spent at a location, and the flexibly to engage non-specialists. The Carmon Creek water and sediment samples will contribute to the refinement of Booker's eDNA field sampling protocol and laboratory assay, and will allow us to better understand the limitations of the eDNA method.

Communications

Conference poster presentations

- Joint Meeting: Idaho Chapter of The Wildlife Society, Washington Chapter of The Wildlife Society, Society Northwestern Vertebrate Biology, and Northwest Partners in Amphibian and Reptile Conservation, Coeur d'Alene, Idaho, February 22–26, 2016.

Publications

- Kendell, K. 2015. Amphibian monitoring using environmental DNA: Shell Canada Ltd. Carmon Creek Project: 2015 Field Report. Unpublished manuscript, ACA, Sherwood Park, Alberta, 8 pp.

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.

Hobbs, J., and C.S. Goldberg. 2015. Pacific water shrew and coastal giant salamander inventory and method assessment environmental DNA (eDNA) study. Prepared for BC Ministry of Forests, Lands and Natural Resources Operations. Surrey, British Columbia. 29 pp.

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.

Turner, C.R., K.L. Uy, and R.C. Everhart. 2015. Fish environmental DNA is more concentrated in aquatic sediments than surface water. Biological Conservation 183: 93–102.

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

Alberta Conservation Association biologist Amanda Rezansoff conducts an amphibian survey at an eDNA sampling waterbody at the Shell Carmon Creek project near Peace River, Alberta. Photo: Kris Kendell

Alberta Conservation Association biologist Kris Kendell collects a 15 mL water sample from a waterbody at the Shell Carmon Creek project near Peace River, Alberta. Photo: Amanda Rezansoff

Alberta Conservation Association biologist Kris Kendell collects a 2 mL sediment sample from a waterbody at the Shell Carmon Creek project near Peace River, Alberta. Photo: Amanda Rezansoff

Boreal chorus frog at the Shell Carmon Creek project near Peace River, Alberta. Photo: Kris Kendell