

Alberta Conservation Association (ACA)

Date: 2014-2015

Project Name: Amphibian Monitoring Using Environmental DNA

Wildlife Program Manager: Doug Manzer

Project Leader: Kris Kendell

Primary ACA staff on project:

Kris Kendell and Doug Manzer

Partnerships

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

Key Findings

- Using three water sampling techniques, we collected 162 water samples from five ponds at the Shell Carmon Creek project near Peace River, Alberta.
- We have developed and tested environmental DNA extraction protocols for each water sampling method used.
- Early results demonstrate fairly consistent detection of wood frog environmental DNA from water samples, and we are refining techniques to improve consistency with other species.

Introduction

Living organisms can leave a DNA signature from organic matter suspended in water (e.g., mucus, feces, urine, sloughed tissue) that is 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 samples. Although eDNA detection is a new survey technique, a few studies have shown that it is possible to detect aquatic and semi-aquatic organisms through traces of their DNA suspended in water (e.g., Dejean et al. 2012; Thomsen et al. 2012; Goldberg et al. 2011; Jerde et al. 2011; Ficetola et al. 2008).

We engaged a graduate student (Brandon Booker) at 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. Brandon Booker is preparing DNA amplification and extraction protocols to detect all amphibian species found in Alberta.

Methods

In 2014, we explored greenfield areas at the Shell Carmon Creek project to identify the types and distribution of waterbodies known or suspected to contain boreal toads (*Anaxyrus boreas*). We conducted a visual encounter survey for amphibians at each water collection site. Surveys consisted of walking along the edge of the wetland, watching carefully for the movement of amphibians underfoot or in shallow water, and scanning or dip-netting shallow water areas for tadpoles. To prevent the spread of amphibian diseases, rubber boots and other field equipment were disinfected between waterbodies using a 20% bleach-to-water solution.

We collected water samples at 10 locations distributed at equal distances along the edge of each waterbody. We tested three water sampling techniques: 1) one 15 mL water sample at each sample location; 2) one 300 mL water samples at each sample location, passed through filter paper; and 3) ten 50 mL samples of water from each waterbody pooled into a single sample and subsampled six separate 15 mL water samples, and one 300 mL water sample passed through a filter paper. Samples were preserved with absolute ethanol and sodium acetate and stored at -20°C until processed.

Results

We surveyed and sampled nine waterbodies within greenfield areas at the Shell Carmon Creek project between July 22 and 25, 2014. All waterbodies were constructed wetlands (i.e., borrow pits).

We observed amphibians in the field at seven of nine waterbodies surveyed, with confirmed boreal toad and wood frog (*Lithobates sylvaticus*) breeding at five and three sites, respectively. Evidence of breeding was confirmed by presence of tadpoles or young-of-the-year (YOY). Boreal toad and wood frog subadults or adults were detected at one and three sites, respectively (Table 1).

Table 1. Amphibian observations at waterbodies surveyed within greenfield areas at the Shell Carmon Creek project.

Waterbody	Date visited dd/mm/yy	Life stage of species observed	
		Boreal toad	Wood frog
338418B	22/07/14	0	0
288418A	22/07/14	0	0
018518B	23/07/14	0	Subadult
108518B	23/07/14	YOY/tadpole	YOY
108518A	23/07/14	Tadpole	Subadult
058518A	24/07/14	YOY	YOY
338418A	25/07/14	Adult	0
288418B	25/07/14	Tadpole	YOY
018518A	25/07/14	YOY	Subadult

Using three water sampling techniques, we collected a total of 162 water samples from the five confirmed boreal toad breeding sites, as well as one site where an individual adult boreal toad was detected (Table 2).

Table 2. Number of samples taken for each volume of water at each waterbody where boreal toads were detected.

Waterbody	Date visited dd/mm/yy	No. of samples taken for each collection method				
		15 mL	300 mL	50 mL x 10 pooled		Control
				15 mL	300 mL	
338418A	25/07/14	10	10	6	1	4
288418B	25/07/14	10	10	6	1	4
018518A	25/07/14	10	10	6	1	4
058518A	24/07/14	10	10	6	1	4
108518B	23/07/14	10	10	6	1	4
108518A	23/07/14	10	10	6	1	4
Subtotal		60	60	36	6	24

To date, Brandon Booker has processed water samples from three of six waterbodies. At two of the sites, boreal toad was not detected for any eDNA collection technique. At the third site, boreal toad was detected in two of the 15 mL samples but not with the other sampling techniques. He is currently developing approaches for improving detectability with eDNA.

Conclusions

Monitoring using eDNA allows for the detection of amphibian presence by simply taking a water 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, which commonly require highly skilled personnel often working at night in remote areas. This new approach, if successful, will allow us to collect water samples at any time of day or night with minimal time spent at a location; it also provides the flexibility to engage non-specialists such as volunteers.

Communications

Conference poster presentations

- Society for Northwestern Vertebrate Biology, Kris Kendell, February 2015
- Canadian Herpetological Society, Kris Kendell, September 2014

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.
- 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.

Photos



Brandon Booker, MSc. student at the University of Alberta, conducts an amphibian survey at an environmental DNA water sampling site at the Shell Carmon Creek project near Peace River, Alberta. Photo: Kris Kendell



Alberta Conservation Association biologist Kris Kendell collects an environmental DNA water sample at the Shell Carmon Creek project near Peace River, Alberta. Photo: Brian Eaton, AI-TF



Brandon Booker, MSc. student at the University of Alberta, collects an environmental DNA water sample at the Shell Carmon Creek project near Peace River, Alberta. Photo: Kris Kendell



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