Use of Bioacoustic Technology to Evaluate Habitat Use and Road Effects on Two Anuran Amphibians in the Boreal Region of Northeastern Alberta

by

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Habitat loss and fragmentation are thought to be leading causes behind local to global amphibian declines. Recent expansion of the energy sector in the western boreal forest of northeastern Alberta raises concerns for amphibians that occupy this landscape. Methods for monitoring anuran amphibians typically involve call surveys to detect vocalizing males during the breeding seasons. I used passive acoustic monitoring with autonomous recording units (ARUs) to maximize detections of uncommon and common species. ARUs were deployed in the Lower Athabasca Planning Region (LAPR) of northeastern Alberta during the months of April to August (2012 – 2016). ARUs collected acoustic recordings of the soundscape that were processed through human listening of raw recordings and through use of an automated recognizer to identify the unique vocalizations of the Canadian toad (Anaxyrus hemiophrys, Cope 1886) and boreal chorus frog (Pseudacris maculata, Agassiz 1850).

I generated a predictive generalized linear model using logistic regression to identify breeding habitat used by the Canadian toad in northeastern Alberta. I did this using a combination of remote sensing from ARUs and landscape characteristics collected from satellite and LIDAR technology. I found that the presence of this species is associated with fen wetland and upland edge habitat, along with the occurrence of coarser soils that allow toads to dig below the frost line during winter months. A positive relationship between presence and the proximity of roads was seen in the model, indicating that suitable habitat for toads exists near roads. This model had good predictive capacity and accuracy, and would be an effective tool for locating additional breeding locations for this species of concern.

I also used ARUs to understand if boreal chorus frogs show a negative response to roads and associated noise pollution, a major cause of habitat degradation. I used call intensity codes as a measure of the relative abundance of chorus frogs and evaluated if road metrics, calculated with spatial data layers, influenced relative abundance. Boreal chorus frog abundance was shown to be positively influenced by road proximity, suggesting that the species breeds in roadside ditches. I further examined acoustic interference by passing vehicles, by measuring the full chorus amplitude (dB) for boreal chorus frogs before, during and after a vehicle passage. No significant difference in chorus amplitude could be detected, indicating that this species is not affected by traffic noise. It seems this anuran is not affected by roads and human activity on roads in this system.

In conclusion, I provide an argument for the use of passive acoustic monitoring for surveying temperate anuran amphibians. ARU detections greatly increased the sample size of sites and dates surveyed, as well as
expanding the ecological questions that I could address. Future monitoring and conservation plans for anurans in the LAPR are encouraged to use the findings of this research and to continue to use and develop methods for acoustic sampling.
PREFACE

Funding for myself and this project was provided through an industrial postgraduate scholarship through the Natural Sciences and Engineering Council of Canada (NSERC) and Devon Energy Corporation. Field work was funded by the oil sands industry originally through the Ecological Monitoring Committee for the Lower Athabasca (EMCLA). Funding underwent several changes in governance, including the Joint Oil Sands Monitoring (JOSM) initiative from 2013 – 2014, and the Alberta Environmental Monitoring, Evaluation, and Reporting Agency (AEMERA) in 2015. Current funding is received through the Environmental Monitoring and Science Division (ESMD) of Alberta Environment and Parks, a division of the Government of Alberta. Other grants received include the Alberta Biodiversity Monitoring Institute Grant, Alberta Conservation Association Grants in Biodiversity, Circumpolar/Boreal Alberta Research Grants, Northern Scientific Training Program Grants, and University of Alberta Northern Research Awards. Data gathered in collaboration with the University of Alberta – Alberta Biodiversity Monitoring Institute Bioacoustic Unit. I would like to thank Dr. Nicholas Coops and Chris Bater for providing access to the LIDAR data used in my thesis. Thanks also to Ducks Unlimited for providing access to the Enhanced Wetland Classification layer.

This thesis is an original work by Natasha Annich. No part of this thesis has been previously published. Chapters 1 and 4 are written in first person, as the writing is primarily my own. Chapters 2 and 3 were developed with supervisors, Dr. Erin Bayne and Dr. Cindy Paszkowski, and are written in plural to reflect so.
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Thank you to my supervisors, Dr. Erin Bayne and Dr. Cindy Paszkowski, for bringing me into this crazy thing known as graduate school and for helping me navigate my way through this thesis. Erin, thank you for pushing me to really understand my data and providing me with every opportunity to get out into the field. Cindy, thank you for helping me realize my research limitations and broadening my love of herpetology. Thank you to my committee member, Dr. Mark Boyce, and my arms-length examiner, Dr. Heather Proctor. Your thoughts and comments were very much appreciated.

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It may have taken me four years to get here, but it has never been a dull moment. I have had the opportunity to get into the field every year (not always for my own research), and have realized my deep love for the boreal forest. I look forward to spending my summers in the boreal, soaking in the birdsong and the beautiful unique landscapes. There is nothing quite like stepping into nature and feeling like you are the only person to have ever been in this place (until you look to the right and see an empty beer can). I might even miss the mosquitos, blackflies, deer flies, tent caterpillars, and the smell of fresh fen-drenched socks drying on the truck dashboard. So, for all of this, a big thank you to the boreal forest.
Thank you to my fellow Bayne-Boutin and Paszkowski-Tonn graduate students for answering all my statistical questions, reading over my material, and providing feedback along the way. Your company has kept me sane. I could not have done this without you. I also need to thank my field technicians and the graduate students that assisted in data collection for my specific project – you were all ‘fentastic’.

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CHAPTER 1. INTRODUCTION

1.1 GENERAL INTRODUCTION

1.1.1 Boreal Alberta

The boreal forest is one of the largest ecosystems on earth (Foote and Krogman 2006) and Canada’s western boreal forest accounts for 20% of the global boreal ecozone (Bonan and Shugart 1989). The boreal forest is home to a wide variety of species and is used as breeding grounds for many migratory and resident birds and amphibians, including up to 40% of all North American waterfowl (Foote and Krogman 2006). The terrain in Alberta’s western boreal forest is largely undulating and habitats vary throughout the landscape. Natural processes of disturbance, fire and insect outbreaks (Bonan and Shugart 1989), are heavily responsible for the mosaic of habitats present.

Alberta’s boreal forest contains a disproportionate amount of wetland habitat (Foote and Krogman 2006). Boreal wetlands are classified into five groups: bogs, swamps, fens, marshes and shallow water (National Wetlands Working Group 1997). These categories are based on differences in nutrient richness, water depth, water flow and vegetation composition. Fens and bogs are peatlands dominated by black spruce (Picea mariana, (Mill.) Britton, Sterns and Poggenb.) and tamarack (Larix laricina, (Du Roi) K. Koch) (Johnson et al. 1995; Kaheil and Creed 2009). Swamps and marshes are dominated by willow (Salix sp.), birch (Betula sp.), alder (Alnus sp.), sedges (Carex sp. and Eriophorum sp.) and grasses (Poaceae) (Johnson et al. 1995; Kaheil and Creed 2009). Upland habitats in the western boreal forest are dominated by aspen (Populus tremuloides, Michx.), balsam poplar (Populus balsamifera, L.), white spruce (Picea glauca, (Moench) Voss), and jack pine (Pinus banksiana, Lamb.) (Johnson et al. 1995; Kaheil
and Creed 2009). Despite the prevalence of wetlands and clear differences among wetland classes, our understanding of how wildlife react to this habitat gradient in time and space is limited.

Northern Alberta is largely within the boreal forest ecozone and is experiencing extensive land-use change with the expansion of the energy and forestry sectors. As of the late 1990’s, more land had been cleared for seismic lines, pipelines, well sites and roads than for forestry (Foote and Krogman 2006). In 2006, linear features, such as roads and seismic lines, encompassed 0.5 – 3.5km per km² of Alberta’s boreal forest. The number of linear features continues to increase as the industries expand. Alberta is a hub for petroleum extraction and approximately 23% of the provincial area has been used to support the industry (Foote and Krogman 2006). There are more than 80 oilfields and over 300 natural gas fields, resulting in >88000 well sites in Alberta’s boreal forest. Furthermore, forestry is common in the region, adding to habitat loss and fragmentation for some vertebrates (Foote and Krogman 2006).

As industrial activities grow, there is a pressing need for an evaluation of the distribution of vertebrate species to quantify the impact of increased human activity and ensure the continued persistence of these species. Due to the challenges associated with accessing boreal wetlands, these areas are understudied in comparison to more accessible upland areas (Foote and Krogman 2006). Furthermore, vertebrates that exist solely in wet areas are less understood. There is a gap in the scientific literature on how boreal wetlands and associated vertebrates are responding to these increases in human activity (Foote and Krogman 2006). Moreover, most existing studies have focused on how the energy sector disturbance impacts birds (Northrup and Wittemyer 2013; Bayne et al. 2016), with relatively few focused on amphibians (Northrup and Wittemyer...
2013). Amphibians require wetlands to breed, thereby, it is important to discern the type of wetlands they use and how this habitat is affected by anthropogenic changes to the landscape.

1.1.2 Amphibian Declines

Global declines of amphibian populations are well described in the scientific literature, and have been attributed to a number of factors, including predation, disease, pollution, acid rain, global warming, habitat loss, and anthropogenic features (Blaustein and Wake 1990; Blaustein et al. 1994; Alford and Richards 1999; Houlahan et al. 2000; Becker et al. 2007). Due to their complex life cycle, pond-breeding amphibians require the use of both aquatic and terrestrial habitats to satisfy larval and adult life stages (Alford and Richards 1999; Joly et al. 2003; Trenham and Shaffer 2005). As eggs and larvae, amphibians are especially susceptible to acidic or polluted aquatic environments, resulting in slowed growth rates and developmental abnormalities (Blaustein and Wake 1990). Because of their permeable skin, adult amphibians also face issues with absorbing toxins from substrates (Blaustein and Wake 1990; Alford and Richards 1999). As well, terrestrial amphibians are threatened during dispersal and migration by anthropogenic infrastructure, such as roads. World-wide declines of amphibians are a major concern, as these animals account for a considerable fraction of terrestrial vertebrate biomass and predator-prey dynamics involving amphibians would be affected by declines in amphibian populations (Burton and Likens 1975; Blaustein and Wake 1990; Gardner 2001).

In habitat altered by human development, populations of amphibians are known to decrease (Blaustein and Wake 1990; Blaustein et al. 1994; Becker et al. 2007). Roads and associated road effects are known to have negative effects on many amphibians (Fahrig et al. 1995; Ashley and Robinson 1996; Hels and Buchwald 2001; Jochimsen et al. 2004; Fahrig and Rytwinski 2009). The level of land-use change in the boreal forest of northeastern Alberta
demands an evaluation of amphibian species composition in impacted and pristine areas to understand how increased human activity has influenced amphibians. This region is home to 6 amphibian species including, 3 frog species: boreal chorus frog (*Pseudacris maculata*, Agassiz 1850), wood frog (*Lithobates sylvaticus*, LeConte 1825), and northern leopard frog (*L. pipiens*, Schreber 1782); 2 toad species: Canadian toad (*Anaxyrus hemiophrys*, Cope 1886) and western toad (*A. boreas*, Baird and Girard 1852); and 1 salamander species: western tiger salamander (*Ambystoma mavortium*, Baird 1850). Bufonidae (true toads, i.e. *Anaxyrus* sp.) is one of the most rapidly declining amphibian families, with habitat loss thought to be a major contributor (Stuart et al. 2004). Western toads are listed as sensitive in Alberta, and Canadian toads have the legal status of data deficient in the province (AEP 2017), as their biology is poorly understood. The northern leopard frog is the only frog listed as species of conservation concern in the boreal region, with a legal status of at risk in Alberta (AEP 2017). Efforts must be taken to evaluate the status of rare or uncommon species and to ensure the stability of remaining populations (Blaustein et al. 1994).

### 1.1.3 Bioacoustic Technology

Passive acoustic monitoring is an emerging tool that is becoming a popular way for remotely surveying vocalizing birds, mammals, anurans, and insects (de Solla et al. 2005; Laiolo 2010; Corn et al. 2011; Tampon et al. 2012; Furnas and Callas 2015; reviewed by Shonfield and Bayne 2017). Surveys can be done with human observers or with autonomous recording units (ARUs) that can be left at sampling locations and programmed to turn on automatically at scheduled intervals. Bridges and Dorcas (2000) propose that automated surveys are more effective than traditional on-site surveys by human observers, as they can mitigate the disturbance to wildlife by remotely collecting data. More specifically, chances of detecting easily
disturbed vocalizing species increases by removing human influence from the survey (Bridges and Dorcas 2000). Recordings are also extremely accurate records, as the sounds can be played back repeatedly for efficient analysis by more than one human listener (Laiolo 2010; Furnas and Callas 2015; Brauer et al. 2016).

This technology also facilitates monitoring species at larger spatial and temporal scales (Porter et al. 2005), as they can be left unattended in the environment for extended periods and can be deployed to survey multiple areas simultaneously. Elusive species that are challenging to detect visually or at certain hours of the day are particular good candidates for monitoring with this approach (Rognan et al. 2012; Holmes et al. 2014; Holmes et al. 2015; Campos-Cerqueira and Aide 2016). This can be especially important for species that vocalize relatively infrequently or inconsistently, and are not detected easily via other methods.

Sound-producing amphibians in northeastern Alberta were studied using ARUs from 2012 – 2016 to aid in understanding distribution of anurans in response to a changing landscape and determine habitat preferences of uncommon species (Eaton et al. 2011). ARUs have detected the calls of wood frogs, boreal chorus frogs, Canadian toads, and western toads. Acoustic detections of multiple anuran species at a single location are useful, as detections indicate that survey locations likely support breeding populations of one or more anuran species, regardless if target species are present. Furthermore, a lack of detections of a species of conservation concern in areas where other anurans are found, may indicate that the species is not doing as well as others.

1.2 THESIS OUTLINE
This project was developed in conjunction with other undergoing research collected by the Bioacoustic Unit, a collaboration between the lab of Dr. Erin Bayne at the University of Alberta and the Alberta Biodiversity Monitoring Institute. Chapters 2 and 3 examine bioacoustic data collected from a total of 5 field seasons (2012 – 2016), where ARUs were deployed to detect vocalizing anuran species as well as other vocalizing animals. Locations of ARUs were chosen to detect rare or uncommon species, including the Canadian toad, based on proposed habitat requirements and previous detections of target species.

The main objective in Chapter 2 was to identify the distribution and habitat use of the Canadian toad during its breeding season in the boreal forest of the Lower Athabasca Planning Region (LAPR) of northeastern Alberta. This chapter uses a combination of remote sensing data to meet these objectives.

Chapter 3 aimed to understand how roads in the fragmented landscape of the LAPR influenced the relative abundance and calling behaviour of a ubiquitous anuran species, the boreal chorus frog. I used call intensity codes to assess relative abundance of boreal chorus frogs, in relation to roads and indirect road effects (i.e. noise pollution). Furthermore, at a small scale, I examined the calling behaviour of boreal chorus frogs in the presence of road noise through measuring the amplitude (dB) of full frog choruses as vehicles passed.

In Chapter 4, I summarize the findings from Chapters 2 and 3, evaluate the use of bioacoustics for monitoring vocalizing temperate anurans, suggest management strategies for Canadian toads, and discuss implications and future directions for this work.
CHAPTER 2. CANADIAN TOAD HABITAT USE IN NORTHEASTERN ALBERTA

2.1 INTRODUCTION

The process of understanding a species’ ecology is complex but typically begins with an examination of habitat characteristics in locations where the species occurs (Guisan and Zimmermann 2000; Dayton and Fitzgerald 2006). Species-distribution models are useful tools that combine observations of a species’ occurrence with environmental covariates (Guisan and Zimmermann 2000; Engler et al. 2004; McPherson and Jetz 2007). These models are generally used to understand how environmental or anthropogenic features influence a species’ occurrence, abundance or distribution (Guisan and Zimmermann 2000; McPherson and Jetz 2007). Identifying the characteristics of locations where a species does and does not occur within its range is essential for conservation and management efforts, particularly for rare species with relatively specialized habitat requirements.

Some anuran species, such as the Canadian toad (*Anaxyrus hemiophrys*, Cope 1886), may not be easily found without intensive survey effort in a specific area. Existing data suggest that populations of the Canadian toad may have declined since the mid-1980s (Hamilton et al. 1998) in several areas of their range, including the province of Alberta (Eaton et al. 2005a; Fisher et al. 2007; Browne 2009). With some exception to the boreal region in northeastern Alberta (Eaton et al. 2005a; Constible et al. 2010), there are limited survey data for this species. Our current knowledge is inadequate to assess habitat requirements, population sizes, and the extent of their distribution (Eaton et al. 2011). A key reason for our limited knowledge of this species is the challenges associated with surveying for Canadian toad. They are patchily distributed with
relatively low densities (even at breeding sites) do not call consistently between years, and are most often detected by aural cues alone. Furthermore, they vocalize almost exclusively at night in locations that are difficult to survey, and therefore, detection and monitoring require specialized methodologies (Eaton et al. 2011).

Canadian toads, as is typical of temperate anurans, are strongly associated with wetlands. However, we have only a superficial understanding of the specific types of wetlands used by the species for breeding (Breckenridge and Tester 1961; Roberts and Lewin 1979; Hamilton et al. 1998). Early studies noted this species to be the most aquatic of the bufonids in Alberta (Roberts and Lewin 1979; Hamilton et al. 1998). Canadian toads have been observed using flowing river systems for breeding in the boreal forest (Roberts and Lewin 1979). They lay a string of eggs rather than attaching single eggs to vegetation, as many frog species do (Roberts and Lewin 1979). This type of oviposition is thought to allow them to breed successfully in moving water (Roberts and Lewin 1979). As is true of other Anaxyrus species, Canadian toads require terrestrial habitat to forage and overwinter (Breckenridge and Tester 1961; Alford and Richards 1999; Joly et al. 2003; Becker et al. 2007). It is unknown precisely what terrestrial features influence the local occurrence of the Canadian toad. Previous studies indicated this species will dig into the earth to seek refuge during winter months, typically in sandy soils (Breckenridge and Tester 1961; Roberts and Lewin 1979; Kuyt 1991). Therefore, soil texture may be an important characteristic influencing the species’ distribution as it is not freeze tolerant, unlike many other boreal anurans (Storey and Storey 1986; Kuyt 1991; Hamilton et al. 1998).

Previous studies have identified threats posed by human infrastructure to Canadian toads (Roberts and Lewin 1979). Road mortality is a serious concern for anurans that move through fragmented habitats to access breeding and overwintering locations (Fahrig et al. 1995; Joly et al. 2003; Becker et al. 2007). It is unknown precisely what terrestrial features influence the local occurrence of the Canadian toad. Previous studies indicated this species will dig into the earth to seek refuge during winter months, typically in sandy soils (Breckenridge and Tester 1961; Roberts and Lewin 1979; Kuyt 1991). Therefore, soil texture may be an important characteristic influencing the species’ distribution as it is not freeze tolerant, unlike many other boreal anurans (Storey and Storey 1986; Kuyt 1991; Hamilton et al. 1998).
Previous studies have shown that both intermittent and chronic anthropogenic noise have the potential to have negative effects on vocalizing anuran species (Penna and Zúñiga 2014; Shannon et al. 2016), as they rely heavily on acoustic communication to attract mates during the breeding season (Wells 1977; Gerhardt and Huber 2002). The western boreal forest of northeastern Alberta has become increasingly fragmented with the expansion of the energy and forestry sectors. As of the late 1990’s, considerable land has been cleared for seismic lines, pipelines, well sites and roads (Schneider et al. 2003; Foote and Krogman 2006). In 2006, linear features, such as roads and seismic lines, encompassed 0.5 – 3.5 km per km² of Alberta’s boreal forest (Foote and Krogman 2006). The effects of this change in land-use have not been evaluated for the Canadian toad.

We used a combination of passive acoustic monitoring with autonomous recording units (ARUs) and landscape characteristics derived from remote sensing to generate a predictive habitat model for the Canadian toad in northeastern Alberta. The combination of specialized acoustic surveys, precision remote sensing data derived from light detection and ranging systems (LIDAR) describing vertical forest structure, and wetland classifications from Landsat/ Radarsat satellite images provides a powerful set of tools to study Canadian toad habitat use. Our specific objectives were threefold: (1) to quantify Canadian toad use of wetlands and use of uplands to better understand what habitats are important for the species; (2) to determine if soil texture is associated with Canadian toad habitat use; and (3) to evaluate the impact of roads and industrial infrastructure on Canadian toad habitat use.

2.2 METHODS
2.2.1 Study Area

Our study took place within the Lower Athabasca Planning Region (LAPR) of northeastern Alberta, extending from Cold Lake, AB (54.4642°N, 110.1825°W) to north of Fort McMurray, AB (56.7264°N, 111.3803°W) (Figure 2-1). The LAPR covers 93,212 km² of the northeastern corner of the province (AB ESRD 2012). The terrain is largely undulating, resulting in a mosaic of extensive wetlands (fens and bogs – dominated by black spruce (*Picea mariana*, (Mill.) Britton, Sterns and Poggenb.) and tamarack (*Larix laricina*, (Du Roi) K. Koch); swamps and marshes – dominated by willow (*Salix* sp.), birch (*Betula* sp.), alder (*Alnus* sp.), sedges (*Carex* sp. and *Eriophorum* sp.) and grasses (*Poaceae*) and uplands (dominated by aspen (*Populus tremuloides*, Michx.), balsam poplar (*Populus balsamifera*, L.), white spruce (*Picea glauca*, (Moench) Voss), and jack pine (*Pinus banksiana*, Lamb.)) (Johnson et al. 1995; Kaheil and Creed 2009). Soils are mostly gray luvisols in upland aspen stands, eutric brunisols in sandy uplands, and organics and gleysolics in wet areas (Strong 1992). The most notable disturbance agent within the LAPR is wildfire (Bonan and Shugart 1989; AB ESRD 2012).

Many industrial companies focused on harvesting natural resources have operations located in the LAPR. The oil sands in the LAPR are the third largest petroleum reserve in the world (AB ESRD 2012). This development has resulted in a series of linear features (i.e. roads, cutlines, pipelines) and associated infrastructure. As well as the oil and gas industry, commercial forestry and agriculture have contributed to the extensive land-use change in the LAPR (Foote and Krogman 2006).

Sites were selected using SPOT imagery and land cover layers in geographic information systems (GIS) and chosen based on accessibility (foot, ATV, and some helicopter) and habitat classification. Site selection was influenced by habitat type and a goal to survey each wetland
type (marshes, fens, swamps and bogs) as well as upland habitat. Minimum distance between sampled sites was 600 m. Sites were composed of 5 sampling stations: 4 ARUs in a square formation, spaced 600 m apart at the corners, with a fifth station in the center (Figure 2-2).

2.2.2 **Acoustic Survey Data**

Acoustic surveys for Canadian toads were conducted using Song Meters (Models: SM2+ and SM3; Wildlife Acoustics, Inc., Maynard, Massachusetts, USA), a commercially available autonomous recording unit (ARU). We deployed a single ARU at each sampling station, so each site had 5 ARUs (Figure 2-1; Figure 2-2). We conducted acoustic surveys over 4 years (2012 – 2015). New sites were added each year, and previous sites with Canadian toad and other rare species detections were resampled in the subsequent years (41 sites resampled in 2013, 16 sites in 2014, and 33 sites in 2015) (Table 2-1). ARUs were deployed and retrieved between the following dates (Table 2-1): 09 April 2012 – 12 July 2012 (n = 477); 09 May 2013 – 02 August 2013 (n = 427); 24 April 2014 – 06 July 2014 (n = 365); and 03 May 2015 –30 June 2015 (n = 489). ARUs were fastened approximately 1.5 m above ground to trees or attached to wooden posts when trees were unavailable. ARUs were moved on a bi-weekly rotational system to survey all sites during the toad breeding season (Eaton et al. 2011). They were stratified in sampling by having 3 crews of 2 workers deploying ARUs across different areas of the LAPR at the same time. The ARUs were programmed to turn on and record the surrounding environment for the first 10 min of every hour daily.

A subset of recordings was processed using a standardized listening and data entry protocol. Recordings were selected at times when Canadian toads are known to be vocally active (22:00 – 04:00) to maximize detectability for the species (Eaton et al. 2011). A minimum of 4 and maximum of 14 recordings were processed during these hours for every station (mean =
4.19). Target recordings were randomly selected for human listening, spanning across the days that the ARUs were out. The program Adobe Audition (2012) was used to visualize the recording as a spectrogram to help locate and identify species calls during listening. A minimum of 4 recordings was chosen to ensure that data could be processed and analyzed before additional data were collected during the next field season. Human listening to raw recordings was time-consuming and a single season of acoustic data took approximately 355 hours to process a minimum of 4 recordings per station. Therefore, other processing methods were developed to decrease time spent listening and increase toad detections.

A computer-automated acoustic recognizer was used to identify Canadian toad vocalizations in the recordings collected by the ARUs. Recognizers scan through recordings gathered in the field and locate targeted vocalizations of species of interest (Wildlife Acoustics Inc 2011). The software program Song Scope, developed by Wildlife Acoustics Inc. (2011), uses sophisticated digital signal processing algorithms to compare and identify target vocalizations in recordings. The recognizer was trained on annotated clips of the Canadian toad call and the settings were chosen based on the unique characteristics of the species’ vocalization (Appendix 1). Canadian toads produce a long soft trill (1 – 7 s) that sits in the 1.5 kHz – 2.5 kHz frequency range. The recognizer is constructed to incorporate these unique details based on the metrics: max song length, frequency minimum and frequency range (Appendix 1). Canadian toad calls detected from the recognizer were validated by a human listener to confirm true positives and remove false positives from the results. A season of data can be validated in <20 hours by a human listener.
2.2.3 *Habitat Data*

We examined stations with Canadian toad detections (used) and stations where Canadian toads had never been detected over the length of the study (unused). Binary data reflecting use versus non-use are appropriate when interpreting results to determine the predicted proportional probability of use (Boyce et al. 2002). Used stations included instances when a Canadian toad was detected at every observation as well as only a single detection over the years of the study. The designation of a station as unused required a lack of toad detections by both acoustic processing methods: (1) through human listening by trained observers to a minimum of 4 nighttime (22:00 – 04:00) recordings collected during the toad breeding season (May – July); and (2) with use of the computer automated recognizer to search for Canadian toad calls on all recordings taken from that station. Furthermore, we only considered locations where other anuran amphibians had been detected on recordings to ensure that the habitat was suitable for amphibian breeding activities and likely larval development. Stations were randomly sampled across the LAPR over the length of the study to diversify the dates sampled at each station across years. Via a combination of listening and automated recognition we sought to minimize detection error. Adopting a strict occupancy approach was not appropriate for our analysis, as data were not collected from a closed system and individual males could enter or leave the breeding location or the call chorus (Royle and Nichols 2003; Mackenzie 2005; Royle et al. 2005; Lele et al. 2012).

Adopting a strict occupancy approach was not appropriate for our analysis, as data were not collected from a closed system and individual males could enter or leave the breeding location or the call chorus (Royle and Nichols 2003; Mackenzie 2005; Royle et al. 2005; Lele et al. 2012). Using automated recognition to detect Canadian toads also precludes the use of
conventional occupancy methods because what represents a ‘visit’ is no longer clear. For example, if we had used a 3 min listening period as a replicate, the recognizer approach would have resulted in hundreds to thousands of repeated samples at each station. This is challenging to solve computationally with current software. As well, 3 min segments that are closer together in time (i.e. multiple 3 min repeated surveys in the same 10 min of a recording) are less likely to be independent of each other, which is another assumption of classical occupancy. In contrast, using an entire day of recordings or a week as the unit of replication is possible with a recognizer but is not practical with a human listener.

Used and unused stations were buffered by 150 m in GIS and spatial attributes from the various GIS layers estimated. We used a 150 m buffer, as we could not be certain that the detected toad was directly below the ARU, and we needed to account for the immediate space around the ARU where the toad may have been calling.

Spatial data layers used in our study were as follow.

*Land Cover.* – Habitat classes from Ducks Unlimited’s Enhanced Wetland Classification (DUEWC; Ducks Unlimited Canada 2011) layer were reclassified, reducing 29 classes to 5 major classes (marsh, fen, bog, swamp, and other) in GIS (ESRI 2015) to highlight dominant wetlands in our study area (Table 2-2; Table 2-3). Proportions of broad habitat classes were calculated in geospatial modelling environment (GME; Beyer 2015; Table 2-3). Distance to upland/wetland edges were determined based on classifications from the DUEWC (2011) data (Table 2-3). Pixels for land cover covariates were 30 m x 30 m.

*LIDAR Vegetation Density.* – LIDAR data were collected (2003 – 2014) using pulsed lasers with density ranges 1 – 4 returns per m² to attain accurate distance measurements (Guo et al. 2017).
We used return proportions for 6 different vegetation heights (strata): below 0.15 m, 0.15 m – 1.37 m, 1.37 m – 5 m, 5 m – 10 m, 10 m – 20 m, and 20 m – 30 m (Table 2-3). These layers related the canopy height density of vegetation calculated at each strata (percentage of LIDAR returns) and were used to explore vertical vegetation structure (McGaughey 2016; Guo et al. 2017). Pixels for LIDAR covariates were 30 m x 30 m.

_Elevation._ – A digital elevation model (DEM; Government of Canada et al. 2012) was used to isolate areas of low elevation that may indicate potential breeding locations and was measured at 100 m x 100 m pixels (Table 2-3).

_Soil._ – Soil texture, drainage, and percent sand were extracted from the Soil Landscapes of Canada (SLC; Schut et al. 2011) spatial data layer (Table 2-3). Soil texture ranged from very coarse to very fine, drainage ranged from very rapidly drained to very poorly drained, and sand percentages were taken at a depth of 1 m. Pixels for soil covariates were 30 m x 30 m.

_Human Disturbance._ – Paved and gravel roads were used to generate a distance to roads layer (Table 2-3). Noisy permanent industrial facilities, including compressor stations and industrial plants, were identified from an Alberta facilities layer, and were used to generate a distance to chronic noise layer (Table 2-3). Pixels for human disturbance covariates were 30 m x 30 m.

### 2.2.4 Modeling

Generalized linear models (GLM) using a binomial error family and logit link (hereafter: logistic regression) were used to relate the presence of Canadian toads to different landscape characteristics in the program Stata (Boyce et al. 2002; StataCorp 2013). These models have the capacity to predict the use of the landscape by animals, and what habitat features drive use (Boyce et al. 2002; Lele and Keim 2006).
We used a forward-addition, stepwise model-building approach to understand effects of each covariate on Canadian toad habitat use. Covariates were added in model building to tease out predictive variables related to use by Canadian toads. The frequency of use based on the dominant habitat class within 150 m of the ARU indicated differential habitat use (Figure 2-3), specifically extensive use of fens. Therefore, models evaluating only land-cover characteristics were generated to further examine the type of fens at a finer scale (graminoid, shrubby, and treed). Different land cover and soil covariates were evaluated individually prior to model building to determine which co-linear terms were most predictive when selecting candidate variables for building the final model.

Model selection was based on Akaike’s Information Criterion (AIC) (Burnham and Anderson 2002). Models were ranked using AIC, and the model with the lowest AIC and fewest parameters was chosen as the best model to describe Canadian toad use of the landscape. We considered models with AIC values <2 apart ($\Delta$AIC <2) to be equally supported if they had the same number of parameters (Burnham and Anderson 2002). The accuracy and predictive power of models were evaluated using the receiver operating characteristic (ROC) classification approach with the associated area under the curve (AUC) values (Metz 1978; Swets 1988; Manel et al. 2001). AUC is a widely-used metric to determine model accuracy, where values <0.5 suggest no predictive power or accuracy, >0.5 – <0.7 suggest low predictive power and accuracy, >0.7 – <0.9 suggest good predictive power and indicate useful applications, and >0.9 – 1.0 suggest perfect predictive power and accuracy (Swets 1988; Manel et al. 2001).

2.3 RESULTS
2.3.1 Acoustic Survey Data

Canadian toads were detected at ARU stations across all study years (2012 – 2015). Periods of detections ranged from 14 May 2012 – 15 June 2012 (n = 4), 15 May 2013 – 06 July 2013 (n = 73), 20 May 2014 – 20 June 2014 (n = 50), and 07 May 2015 – 12 June 2015 (n = 59). Toads were detected at some stations across multiple years, so a total of 142 stations were declared as used in our study area. Of these stations, 106 were identified through human listening to field recordings, and 38 stations were added by the automated recognizer. Detections by the automated recognizer overlapped with human listening detections for 57 stations (Table 2-4). Stations where human listening and the recognizer failed to ever detect a Canadian toad during peak calling days and hours were defined as unused and included a total of 524 stations for analysis.

When toads were detected on a recording, they were typically either single individuals or in low abundance and individual vocalizations were easy to identify. However, some sites did yield many toads (n = 25), and most recordings at these locations detected full choruses of Canadian toads where individuals could not be distinguished. At stations with Canadian toad detections, we examined the number of days that toads were detected using recognizer results (n = 516 cases) and found that for the days that ARUs were deployed, analysis of recordings from 4 days, following our protocol, was sufficient to detect the presence of toads 65.3% of the time. This finding indicates that intensive listening to 4 nighttime recordings successfully identified locations with high Canadian toad activity, and thus higher likelihood of use.

2.3.2 Habitat Data and Modeling

Canadian toads were detected most frequently in fen (62.7% of detections) and upland (21.8% of detections) habitat (Figure 2-3a), but detections of toads occurred disproportionally at
some of the other habitats surveyed (Figure 2-3b). Habitats dominated by open water (n = 6) were used at a higher proportion than expected based on our sampling effort (Figure 2-3b). Toads were detected on soils with coarser grain-size more frequently (Figure 2-4a) and at higher proportions (Figure 2-4b) than on soils with finer grain-size. In fen habitat, toads were found in areas that varied in vegetation structure: graminoid fen (15.5% of detections), shrubby fen (12% of detections), and treed fen (20.4% of detections) (Figure 2-5a). Though most toad detections came from treed fen, the highest proportion of detections relative to locations surveyed, indicated highest use of graminoid fen (Figure 2-5b).

The most parsimonious model illustrating Canadian toad use of the landscape used LIDAR-derived data to explain vegetation structure and supported the use of both wetland and upland habitats by toads (pseudo $r^2 = 0.2840$, AUC = 0.8459; Table 2-5; Appendix 2; Appendix 3). Proportion upland expressed as a quadratic term provided the best fit and highlights toad use of edge habitat between uplands and wetlands (marsh, fen, bog, swamp). Areas with coarser soil textures were identified as receiving high use by Canadian toads. LIDAR strata data showed use of areas with vegetation below a height of 1.37 m. As vegetation height (as measured by LIDAR data) increased, models showed a lower likelihood of use, indicating that Canadian toads were calling in habitat with short trees and shrubs, or in open grassy areas. Predicted use was higher at stations located nearer to linear features used by motor vehicles, and of stations nearer open water bodies. Areas at lower elevations were also more likely to be used.

2.4 DISCUSSION

2.4.1 Habitat Use

Upland Use
We found that edge habitat between upland and wetland landscapes is important for Canadian toads based on the quadratic relationship of the proportion upland covariate. Models consistently showed use of wetlands directly adjacent to uplands. Toads are known to typically leave wet areas to forage in uplands following breeding, so proximity of breeding sites to upland habitat seems to be important (Roberts and Lewin 1979; Hamilton et al. 1998; Hannon et al. 2002; Bull 2006; Long and Prepas 2012). Our results agree with findings of Browne and Paszkowski (2010) regarding western toads, *A. boreas* (Baird and Girard 1852), in Alberta, which indicated that an approach based solely on understanding wetland conditions is not sufficient to identify required suitable habitat.

Browne and Paszkowski (2010) found that western toads traveled nearly 2 km to reach hibernation sites in the boreal forest. Canadian toads have been recorded to move up to 1.5 km from the breeding wetland and use upland habitats for overwintering (Garcia et al. 2004; Constible et al. 2010; Paszkowski pers. comm.). While not physically observed, it can be inferred that neighboring upland habitats are used by Canadian toads in our study area for post-breeding foraging and for access to overwintering habitat. Historically, many viewed the Canadian toad as the least terrestrial of the bufonids found in western North America (Breckenridge and Tester 1961; Roberts and Lewin 1979). It was previously thought not to be a forest inhabitant (Breckenridge and Tester 1961; Roberts and Lewin 1979). However, our results and studies over the past 2 decades suggest this toad is quite terrestrial and have documented the use of upland forests following breeding by both males, females and young of the year (Hamilton et al. 1998; Garcia et al. 2004; Eaton et al. 2005a; Constible et al. 2010; Paszkowski pers. comm.).

*Wetland Use*
We found that habitats dominated by open water, such as lakes or smaller water bodies, had the highest proportion of use (Figure 2-3b). Previous studies have found these habitats to be suitable breeding sites for the Canadian toad (Hannon et al. 2002; Eaton et al. 2005b) and this result is consistent with the afore-mentioned characterization of the species as highly aquatic (Breckenridge and Tester 1961; Roberts and Lewin 1979). However, open water wetlands were rarely sampled in our study (n = 6) and only accounted for 0.9% of the locations. Under-sampling of this habitat type could reflect the general pattern of ARU placement on the landscape and the challenges associated with surveying within 150 m of open water. If open water was a habitat feature that was highly important to Canadian toad, we would have expected station use to be strongly influenced by the minimum distance to an open water body, which was not seen in our analysis.

Models consistently showed positive coefficients for wetlands with a high proportion of graminoid fen, while all other wetland types (treed fen, shrubby fen, bog and swamp) had negative coefficients. This suggests that there is a reduced likelihood of detecting Canadian toads in habitat surrounded by a greater proportion of other types of wetlands. Fens are minerotrophic and rich in nutrients derived from groundwater feeding into peatlands (Karns 1992). These wetlands have low acidity (pH >5.0) and are highly productive (Karns 1992). Graminoid fens in particular are characterized by low vegetation and are the wettest of the fen types (National Wetlands Working Group 1997). The water table is typically above the surface in graminoid fens and is below the surface in shrubby and treed fens (National Wetlands Working Group 1997).

In comparison to fens, bogs have very low productivity. Bogs are ombrotrophic peatlands that collect water through nutrient-poor precipitation, contributing to their acidity (pH <4.5) (Bonan and Shugart 1989; Karns 1992). Karns (1992) looked at larval development and survival
of six amphibian species in acidic bog water (pH = 4.2) and neutral water (pH = 7.5). Wood frog, *Lithobates sylvaticus* (LeConte 1825), was the only species that had eggs hatch successfully and larvae survive in acidic water (Karns 1992). Other species, including the American toad (*A. americanus*, Holbrook 1836), did not hatch or survive as larvae in bog water, but did in neutral water (Karns 1992). Our results indicate that bogs are less likely to be used by Canadian toads.

**Soil Use**

We found soil texture to be important in understanding Canadian toad use of the landscape. Locations with coarser soils were used more than locations with fine soils; the top model indicated that predominance of coarser soils was associated with high use of stations by Canadian toads. Coarse, rocky soils have poor water holding capacity and drain much quicker than fine-grained soils (Dayton and Fitzgerald 2006). This property, reduces the number of ephemeral breeding pools on a landscape, but coarser soils should allow toads to dig into the earth to overwinter. Migration distances of Canadian toad to access overwintering habitat are variable, ranging between 0.6 km – 1.5 km (Garcia et al. 2004; Constible et al. 2010; Paszkowski pers. comm.), and information on soil texture was collected at this scale (Schut et al. 2011).

Canadian toads are not freeze-tolerant and need to get below the frost line to survive the winter (Storey and Storey 1986; Hamilton et al. 1998; Russell et al. 2000). The co-occurring western toad will use pre-existing cavities, made by mammals or plant root systems, to hibernate, rather than digging their own burrows (Bull 2006; Browne and Paszkowski 2010). However, Canadian toads have not been documented using existing burrows and records of this species’ overwintering habitat agree that it uses areas with sand or other loose soil types (Breckenridge and Tester 1961; Roberts and Lewin 1979; Kuyt 1991; Garcia et al. 2004; Constible et al. 2010).
**Human Disturbance**

We found no effect of noisy human infrastructure (i.e. compressor stations and industrial plant facilities) on Canadian toad habitat use. However, the distance to roads used by motor vehicles (i.e. primary and secondary roads) was a significant predictor, with toads using areas closer to roads. Most roads in this region are built in uplands but do regularly cross into fens. The boreal forest of northeastern Alberta has a high density of roads (Foote and Krogman 2006) that continues to grow with development. Increased use of areas near roads by Canadian toads may be a sampling artefact. The difficulty of moving in the boreal forest required that all ARUs were no greater than 10 km from a road, however, there was a reasonable spread (0 km to 9.08 km with a mean and SD of 1.97 and 1.84, respectively) of distances. Alternatively, while the road surface themselves may not be suitable toad habitat, environmental features near roads may be.

The vegetation alongside roads is short (<1.37 m – as indicated by LIDAR data), providing warm, open areas for toads to forage. Soils near roads are often loose and coarse in texture, which may provide easy access to overwintering locations. Kuyt (1991) noted two occasions of Canadian toads migrating to breeding locations at the sides of roads in the boreal forest of Wood Buffalo National Park in the Northwest Territories. Though suitable hibernation sites may exist in a ditch or road bed, mortality is a large concern if these animals are forced to travel across busy roads (Kuyt 1991; Fahrig et al. 1995). Kuyt (1991) noted multiple road-killed Canadian toads at his sites.

### 2.4.2 Implications

We found our model to have good predictive power and model accuracy (AUC = 0.8459; Appendix 3), indicating its usefulness in identifying Canadian toad breeding habitat in northeastern Alberta. There are currently very few monitoring programs in place designed to
survey this species in the LAPR, and most detections are a by-product of other research. Our model provides monitoring projects with the information needed to efficiently and effectively survey for this species.

While it would serve as a tool for locating potential Canadian toad breeding locations, we advise against extrapolating these results to other ecoregions for management purposes. Habitat use of this species is known to change across its range (Eaton et al. 2005a), thus, suitable habitat determined by this model, may not be used elsewhere. Likewise, we did not survey all habitat types known to support breeding Canadian toads in Alberta, specifically, shallow (<3 m deep), productive lakes (Hannon et al. 2002; Eaton et al. 2005b).

Use of passive acoustic monitoring for this study was essential in our data collection and discovery of Canadian toad breeding locations. We thereby support previous studies that advocate the use of bioacoustic technology for detecting rare species like toads (Rognan et al. 2012; Holmes et al. 2014; Holmes et al. 2015; Campos-Cerqueira and Aide 2016). Through the combination of acoustic monitoring and remote sensing spatial data, we generated a predictive habitat model for the Canadian toad without interacting with or disturbing the animals themselves.

2.4.3 Conclusions

Our study reveals that habitat use by Canadian toad is influenced by both wetland and upland features during the breeding season. It contributes to a growing dataset (Garcia et al. 2004; Browne 2009; Constible et al. 2010; Paszkowski pers. comm.) indicating that this species is not as aquatic as once thought (Breckenridge and Tester 1961; Roberts and Lewin 1979). Furthermore, our study identified the importance of coarse soils for Canadian toads, information possibly linked to its overwintering requirements.
Our model successfully identifies breeding habitat used by an uncommon toad in the boreal forest of northeastern Alberta. To develop the full picture of the habitat required by this species, future studies should broaden the scope of surveys to incorporate post-breeding habitat use by Canadian toad. We were only able to hypothesize why upland habitats are important, and future research should build on our model to include these unmeasured variables and activity periods.
2.5 TABLES

**Table 2-1.** Detailed ARU deployment information over the 4 years of the study (2012 – 2015).

Each site is composed of 5 ARU stations. No sites were revisited in 2012 as it was the pilot year.

<table>
<thead>
<tr>
<th>Year</th>
<th>First Deployment Date</th>
<th>Last Retrieval Date</th>
<th>Number of Sites Monitored</th>
<th>Number of Sites Revisited</th>
</tr>
</thead>
<tbody>
<tr>
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<td>9 April</td>
<td>12 July</td>
<td>477</td>
<td>NA</td>
</tr>
<tr>
<td>2013</td>
<td>9 May</td>
<td>2 August</td>
<td>427</td>
<td>41</td>
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<td>2014</td>
<td>24 April</td>
<td>6 July</td>
<td>365</td>
<td>16</td>
</tr>
<tr>
<td>2015</td>
<td>3 May</td>
<td>30 June</td>
<td>489</td>
<td>33</td>
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</table>
Table 2-2. Reclassified wetland types using Ducks Unlimited’s enhanced wetland classification data (2011).

<table>
<thead>
<tr>
<th>New Classes</th>
<th>Original Classes</th>
</tr>
</thead>
<tbody>
<tr>
<td>marsh</td>
<td>emergent marsh, meadow marsh</td>
</tr>
<tr>
<td>fen</td>
<td>graminoid rich fen, graminoid poor fen, shrubby rich fen, shrubby poor fen, treed rich fen, treed poor fen</td>
</tr>
<tr>
<td>bog</td>
<td>open bog, shrubby bog, treed bog</td>
</tr>
<tr>
<td>swamp</td>
<td>shrub swamp, hardwood swamp, mixedwood swamp, tamarack swamp, conifer swamp</td>
</tr>
<tr>
<td>other / unclassified</td>
<td>open water, aquatic bed, mudflats, upland conifer, upland deciduous, upland pine, upland other, cutblock, agriculture, anthropogenic, cloud, cloud shadow, burn</td>
</tr>
</tbody>
</table>
Table 2-3. Descriptions of covariates used in model building.

<table>
<thead>
<tr>
<th>Covariates</th>
<th>Description</th>
<th>Type</th>
<th>Mean</th>
<th>Std. Dev</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Land Cover</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>domwetland</td>
<td>Dominant wetland classes (n = 5)</td>
<td>Categorical</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>upland</td>
<td>Proportion of upland</td>
<td>Continuous</td>
<td>0.168</td>
<td>0.260</td>
<td>0 - 1</td>
</tr>
<tr>
<td>dstwat</td>
<td>Minimum distance to open water bodies (km)</td>
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<td>0.994</td>
<td>1.04</td>
<td>0 - 4.55</td>
</tr>
<tr>
<td>dstup</td>
<td>Minimum distance to upland (km)</td>
<td>Continuous</td>
<td>0.165</td>
<td>0.296</td>
<td>0 - 1.99</td>
</tr>
<tr>
<td><strong>LiDAR Vegetation</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>below0pnt15</td>
<td>Return density proportions below 0.15 m</td>
<td>Continuous</td>
<td>0.521</td>
<td>0.249</td>
<td>0 - 0.999</td>
</tr>
<tr>
<td>0pnt15to1pnt37</td>
<td>Return density proportions between 0.15 m-1.37 m</td>
<td>Continuous</td>
<td>0.156</td>
<td>0.105</td>
<td>0 - 0.597</td>
</tr>
<tr>
<td>1pnt37to5</td>
<td>Return density proportions between 1.37 m-5 m</td>
<td>Continuous</td>
<td>0.128</td>
<td>0.0997</td>
<td>0 - 0.532</td>
</tr>
<tr>
<td>5to10</td>
<td>Return density proportions between 5 m-10 m</td>
<td>Continuous</td>
<td>0.0789</td>
<td>0.0870</td>
<td>0 - 0.707</td>
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<tr>
<td>10to20</td>
<td>Return density proportions between 10 m-20 m</td>
<td>Continuous</td>
<td>0.0563</td>
<td>0.0988</td>
<td>0 - 0.688</td>
</tr>
<tr>
<td>20to30</td>
<td>Return density proportions between 20 m-30 m</td>
<td>Continuous</td>
<td>0.00970</td>
<td>0.0431</td>
<td>0 - 0.536</td>
</tr>
<tr>
<td><strong>Elevation</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>elev</td>
<td>Elevation from digital elevation model (km)</td>
<td>Continuous</td>
<td>0.480</td>
<td>0.143</td>
<td>0.200 - 0.719</td>
</tr>
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<td><strong>Soil</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tsand</td>
<td>Total sand at a 1m depth (%)</td>
<td>Continuous</td>
<td>58.8</td>
<td>23.0</td>
<td>14.8 - 90.1</td>
</tr>
<tr>
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<td>NA</td>
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<tr>
<td>drain</td>
<td>Soil drainage classes (n = 5)</td>
<td>Categorical</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Human Disturbance</strong></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dst2road</td>
<td>Minimum distance to linear features used by motor vehicles (km)</td>
<td>Continuous</td>
<td>1.97</td>
<td>1.84</td>
<td>0 - 9.08</td>
</tr>
<tr>
<td>dst2chnoise</td>
<td>Minimum distance to industrial chronic noise source (km)</td>
<td>Continuous</td>
<td>9.59</td>
<td>11.3</td>
<td>0 - 97.6</td>
</tr>
</tbody>
</table>
Table 2-4. Comparison of the performance of the automated computer recognizer versus human listeners. The performance was compared based on the number of sites and stations where the Canadian toad (*Anaxyrus hemiophrys*) was detected using each method. The percentage of sites and stations with detections was calculated based on a total of 1013 stations within 203 sites surveyed in 2013 – 2015. Sites and stations missed by the recognizer had detections based on human listening, and sites and stations added by the recognizer were not detected by human listeners who analyzed a subset of recordings.

<table>
<thead>
<tr>
<th>Listening</th>
<th>Recognizer</th>
<th>Missed by recognizer</th>
<th>Added by recognizer</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. sites</td>
<td>No. stations</td>
<td>No. sites</td>
<td>No. stations</td>
</tr>
<tr>
<td>35 (18%)</td>
<td>100 (9.9%)</td>
<td>34 (17%)</td>
<td>95 (9.4%)</td>
</tr>
</tbody>
</table>
Table 2-5. Logistic regression output for the top model of Canadian toad (*Anaxyrus hemiophrys*)
habitat use in northeastern Alberta. Significance shown: $p < 0.001$ ‘***’, $p < 0.01$ ‘**’,
$p < 0.05$ ‘*’, $p < 0.1$ ‘.’.

| Variable                  | β coefficient | Standard Error | z    | P > |z| | 95% confidence interval |
|---------------------------|---------------|----------------|------|-----|---|------------------------|
| domwetland: marsh         | 1.328         | 1.111          | 1.19 | 0.232 |   | -0.852, 3.507          |
| domwetland: fen           | 0.557         | 0.950          | 0.59 | 0.558 |   | -1.305, 2.418          |
| domwetland: bog           | 0.139         | 1.045          | 0.13 | 0.894 |   | -1.910, 2.188          |
| domwetland: swamp         | -0.039        | 0.922          | -0.04| 0.966 |   | -1.846, 1.768          |
| upland                    | 1.364         | 0.401          | 3.40 | 0.001 |   | 0.577, 2.150           **|
| upland ^ 2                | -0.834        | 0.417          | -2.00| 0.046 |   | -1.652, -0.016        |
| 0pnt15to1pnt37            | 0.374         | 0.134          | 2.79 | 0.005 |   | 0.111, 0.636           *|
| 1pnt37to5                 | -0.532        | 0.149          | -3.57| 0.000 |   | -0.824, -0.240        ***|
| elev                      | -2.216        | 0.292          | -7.59| 0.000 |   | -2.788, -1.644        ***|
| soiltext: coarse          | 2.845         | 0.607          | 4.69 | 0.000 |   | 1.656, 4.034          ***|
| soiltext: moderately coarse| 3.550        | 0.598          | 5.93 | 0.000 |   | 2.378, 4.723          ***|
| soiltext: medium          | 2.453         | 0.636          | 3.86 | 0.000 |   | 1.207, 3.698          ***|
| soiltext: moderately fine | 1.454         | 0.746          | 1.95 | 0.051 |   | -0.008, 2.916         |
| soiltext: fine            | -0.058        | 0.566          | -0.10| 0.918 |   | -1.169, 1.052         |
| dst2road                  | -0.673        | 0.178          | -3.77| 0.000 |   | -1.022, -0.323        ***|
Figure 2-1. Map of the province of Alberta, identifying the Lower Athabasca Planning Region and Canadian toad (*Anaxyrus hemiophrys*) survey locations (2012 – 2015).
Figure 2-2. Diagram of a single site layout. Each site is composed of 5 ARU stations (circles): center (CT), northwest (NW), northeast (NE), southwest (SW) and southeast (SE).
Figure 2-3. Dominant habitat within 150 m bufferzones around ARUs deployed to detect Canadian toads (*Anaxyrus hemiophrys*). Unused stations (n = 524) are where Canadian toads were not detected. Used stations (n = 142) are where Canadian toads were detected. (a) Count of used and unused stations where Canadian toads were detected. (b) Proportion of used and unused stations where Canadian toads were detected.
Figure 2-4. Dominant soil texture within 150 m buffer zones around ARUs deployed to detect Canadian toads (*Anaxyrus hemiophrys*). Unused stations (n = 524) are where Canadian toads were not detected. Used stations (n = 142) are where Canadian toads were detected. (a) Count of used and unused stations where Canadian toads were detected. (b) Proportion of used and unused stations where Canadian toads were detected.
Figure 2-5. Dominant fen wetland habitat within 150 m bufferzones around ARUs deployed to detect Canadian toads (*Anaxyrus hemiophrys*). Fen habitats broken down into sub-habitat categories. Unused stations (n = 524) are where Canadian toads were not detected. Used stations (n = 142) are where Canadian toads were detected. (a) Count of used and unused stations where Canadian toads were detected. (b) Proportion of used and unused stations where Canadian toads were detected.
CHAPTER 3. LARGE SCALE AND SMALL SCALE RESPONSES OF BOREAL CHORUS FROGS TO TRAFFIC ON ENERGY SECTOR ROADS

3.1 INTRODUCTION

Roads are among the most extensive anthropogenic features in most landscapes. An increasing body of evidence suggests roads have a significant impact on biodiversity (Fahrig and Rytwinski 2009). Direct impacts of road mortality on wildlife have been extensively documented (Fahrig et al. 1995; Ashley and Robinson 1996; Trombulak and Frissell 2000; Carr and Fahrig 2001; Hels and Buchwald 2001; Coffin 2007) and patterns suggest that roads are more dangerous for amphibians than birds and mammals (Ashley and Robinson 1996). This increased risk is thought to occur because amphibians are slow-moving in comparison to other vertebrates, congregate in large numbers, and require extensive movement between multiple habitats to complete their complex life-cycle (Joly et al. 2003). While increased amphibian mortality has been observed near roads, whether or not this results in population consequences is not fully understood. Furthermore, the indirect effects of roads such as habitat loss, fragmentation, and edge effects caused by the presence of roads (Jochimsen et al. 2004) also remain unclear. Altered light levels, noise, hydrology, vibrations, and pollution are examples of edge effects indirectly caused by roads that might affect amphibians and result in areas near roads supporting lower abundances of amphibians (Coffin 2007).

Noise pollution is an indirect impact of roads that requires investigation for many species (Fahrig et al. 1995; Jochimsen et al. 2004; Herrera-Montes and Aide 2011). Traffic noise has been shown to have an impact on vertebrates in urban environments and on those that find
suitable environmental conditions near roadsides (Herrera-Montes and Aide 2011). Noise pollution from roads has a detrimental impact on acoustic communication for mammals and birds; however, few studies have evaluated such an effect on amphibians (Vargas-Salinas and Amezquita 2013). Males of many species of frogs and toads rely on acoustic communication to attract females during the breeding season, so effective production of acoustic signals is crucial (Wells 1977; Gerhardt 1994; Gerhardt and Huber 2002). Some vertebrates employ different mechanisms to compensate for acoustic interference, such as altering the amplitude, calling rate, frequency, timing and length of vocalizations (Herrera-Montes and Aide 2011; Penna and Zúñiga 2014). Many vocalizing amphibians have been shown to respond to anthropogenic noise by making changes to their vocalizations or patterns of vocalizing (central Thailand, Sun and Narins 2005; eastern Canada, Cunnington and Fahrig 2010; southern Chile, Penna and Zúñiga 2014).

While traffic noise may cause acoustic interference for some vocalizing amphibians, it is a part of a larger effect that includes the vehicle itself. Vertebrates may respond to anthropogenic disturbance as a stimulus that is perceived as a predation risk (Frid and Dill 2002). Stimuli that generate noise and move rapidly may be particularly likely to be perceived as threatening (Frid and Dill 2002). A passing vehicle therefore may be perceived as a predation risk and an anti-predator response might be expected. However, it is possible that vehicles are too new on the landscape for vertebrates to have evolved a perception of them as predators (Frid and Dill 2002). The perception of road traffic as a predation risk has been well studied in birds (Gill et al. 1996; Ward and Low 1997; Nordell 2016) and mammals (Mace et al. 1996; Nellemann and Cameron 1998; Dyer et al. 2001; Nellemann et al. 2001; Papouchis et al. 2001), but is unstudied in amphibians.
While most studies have found negative effects of roads on amphibians, the majority have been done on roads with high traffic volume in urbanized and rural landscapes. It is less clear if roads in forested areas in recently developed industrial landscapes have a similar influence (Ashley and Robinson 1996; Hels and Buchwald 2001). Loss of habitat can result from areas becoming less suitable for amphibians, rather than through direct destruction. Air and water pollution from roads can have an impact on the attractiveness of adjacent wetland habitats. Vehicle emissions release volatile chemicals into the environment, polluting both air and aquatic systems (Coffin 2007), and may be considerably higher in industrial landscapes with extensive energy development due to the heavy-duty equipment used. Road run-off is a well-documented source of pollution (Coffin 2007) that is known to have serious negative impacts on surrounding aquatic populations, including amphibians (Sanzo and Hecnar 2006; Dorchin and Shanas 2010).

We examined the indirect impact of roads on the relative abundance (large-scale) and calling behaviour (small-scale) of boreal chorus frogs (*Pseudacris maculata*, Agassiz 1850) as a function of roads in a rapidly developing industrial landscape in northeastern Alberta. The main objectives were to: (1) determine if the relative abundance of vocalizing boreal chorus frogs is related to the proximity to roads and (2) evaluate measurable changes in the amplitude of full boreal chorus frog choruses when a vehicle passed. Road noise is an issue for many animals, and therefore, we would expect fewer boreal chorus frogs to occur at breeding sites near roads and/or their calling behavior to differ. Specific predictions for our first objective are: (1) fragmented habitats with high road density will support lower relative abundances of vocalizing boreal chorus frogs and (2) habitats close to paved roads with highest traffic volume, and thus highest noise level, will have the lowest relative abundance compared with habitats near gravel roads and all-terrain vehicle (ATV) trails. Specific predictions for the second objective are: (1) chorus
amplitude will decrease during vehicle passage, (2) chorus amplitude will decrease immediately following a vehicle passage, and (3) chorus amplitude will display a refractory period before returning to levels existing prior to the vehicle passage.

3.2 METHODS

3.2.1 Study Area

This study took place in the Lower Athabasca Planning Region (LAPR) of northeastern Alberta (Figure 3-1). The LAPR covers 93,212 km² of the northeastern corner of the province (AB ESRD 2012). The boreal forest is the dominant natural ecoregion in this area. The terrain is largely undulating, resulting in a mosaic of extensive wetlands (fens and bogs – dominated by black spruce (*Picea mariana*, (Mill.) Britton, Sterns and Poggenb.) and tamarack (*Larix laricina*, (Du Roi) K. Koch); swamps and marshes – dominated by willow (*Salix* sp.), birch (*Betula* sp.), alder (*Alnus* sp.), sedges (*Carex* sp. and *Eriophorum* sp.) and grasses (Poaceae)) and uplands (dominated by aspen (*Populus tremuloides*, Michx.), balsam poplar (*Populus balsamifera*, L.), white spruce (*Picea glauca*, (Moench) Voss), and jack pine (*Pinus banksiana*, Lamb.) (Johnson et al. 1995; Kaheil and Creed 2009). Soils are mostly gray luvisols in upland aspen stands, eutric brunisols in sandy uplands, and organics and gleysolics in wet areas (Strong 1992). The most important natural disturbance agent within the LAPR is wildfire, which shapes the pattern of vegetation and wildlife (Bonan and Shugart 1989; AB ESRD 2012).

The LAPR has experienced extensive land-use change with the growth of industry (Foote and Krogman 2006). Much of the landscape has been transformed to suit the needs of forestry and oil and gas operations (AB ESRD 2012). Linear features, such as pipelines, seismic lines, cut lines, trails and roads network across the LAPR and have densities of between 0.5 – 3.5 km of linear features per km² of Alberta’s boreal forest (Foote and Krogman 2006). Road traffic has
increased in volume throughout the region with the expansion of infrastructure (AB ESRD 2012). Two main highways exist in the LAPR, Hwy 63, extending south of Boyle to north of Fort Mackay, and Hwy 881, stretching from Lac La Biche to south of Fort McMurray. Both highways have high traffic volumes over the 4 years of our study (Hwy 63: averaged annual daily traffic = 350-63090; Hwy 881: averaged annual daily traffic = 300-9570) (Alberta Transportation Strategy and Policy Branch 2016).

3.2.2 Study Species

The boreal chorus frog is very common throughout the prairie provinces of Canada and ranges from northeastern British Columbia to southwestern Quebec and north to Great Bear Lake in the Northwest Territories (Russell et al. 2000; Fisher et al. 2007). This species is not an explosive breeder and has a long breeding season from April to June (Russell et al. 2000; Fisher et al. 2007). Males are known to begin calling before the snow melts (Russell et al. 2000), which is typically early April in the boreal forest of northeastern Alberta. Boreal chorus frogs will call both day and night, allowing for greater potential overlap between choruses and high traffic periods.

The advertisement call of the boreal chorus frog is unique and easily distinguishable. It is a short (1 – 1.5 s) trill with a rising inflection, almost resembling the noise created by a finger running across the edge of a fine comb (Russell et al. 2000). Large group choruses characterize the reproductive behaviour of the boreal chorus frog, as is typical of most pond-breeding, temperate anuran species. Males advertise in groups to increase the impact of their acoustic signal and attract females (Wells 1977; Rehberg-besler et al. 2016). Boreal chorus frogs vocalize between 3 kHz – 4 kHz, which is in the upper range of typical traffic noise. When multiple individuals are calling, they create a strong band within this frequency range (Figure 3-2). Frogs
belonging to the family Hylidae, including boreal chorus frogs, hear frequencies up to 4.5 kHz, but also frequencies as low as 100 Hz – 600 Hz, through a combination of three endorgans (Smotherman and Narins 2000).

### 3.2.3 Acoustic Survey Data

Passive acoustic data were taken from autonomous recording units (ARUs) located at varying distances from roads (0 km to 20.6 km). ARUs were deployed on a rotational system for the months of April – August (2012 – 2016), and were left out at a single station for a minimum of 3 days. ARUs used in this study were deployed and retrieved on the following dates: 14 June 2012 (n = 1); 07 May 2013 – 19 August 2013 (n = 372); 26 April 2014 – 11 July 2014 (n = 376); 24 April 2015 – 09 July 2015 (n = 372); and 16 May 2016 – 06 July 2016 (n = 192). Repeat visits occurred between years but only 1 year was used in this analysis with a total of 993 stations analyzed here. The ARUs were fastened approximately 1.5 m above ground to trees or attached to wooden posts when trees were unavailable. The ARUs were programmed to turn on and record the surrounding environment for the first 10 min of every hour daily, generating 24 stored recordings per day.

Human listeners processed full 10 min recordings. All anuran detections included abundance information in the form of the commonly used Wisconsin frog call index (Weir 2001; Weir and Mossman 2005; Paloski et al. 2006; here referred to as call intensity) where: 1 = calls do not overlap and individuals can be distinguished; 2 = calls overlap, but individuals can be distinguished; 3 = full chorus and individuals cannot be distinguished (Table 3-1). Because frogs congregate during the breeding season and vocalizations are often indistinguishable between individuals, call intensity was used to provide a rough idea of the size of the calling population at a given time and place. This method is used commonly for anuran monitoring.
3.2.4 Relative Abundance Models

We used the call intensity codes (Weir 2001; Weir and Mossman 2005; Paloski et al. 2006) recorded for boreal chorus frogs through human listening of 10 min recordings collected from acoustic surveys with ARUs (2012 – 2016). Recorded call intensity codes for anuran surveys have been shown to be good indicators of relative abundance (Nelson and Graves 2004; Stevens and Paszkowski 2004). We ran multinomial logistic regression in Stata (StataCorp 2013) to understand the nominal responses of each call intensity (0, 1, 2, 3) to road metrics. We determined the maximum call intensity recorded for a given station and extracted associated landscape metrics for that station from geographic information systems (GIS) (ESRI 2015). All station data were collected within 150 m buffers around the exact ARU station location. Buffers were used because frogs are likely not vocalizing immediately adjacent to the ARU, thus we must consider the landscape characteristics relating to the area around the ARU and not the specific point. Along with road information, we extracted data relating to both wetland and upland habitats. Covariates used in this analysis were: month, time of day (night/day), minimum distance to water (km), proportion upland, minimum distance to paved roads (km), minimum distance to gravel roads (km), minimum distance to all-terrain vehicle trails (km), and total paved and gravel road length (km) density calculated under the buffer (Table 3-2).

3.2.5 Amplitude Analysis

Stations between 0 km to 1.5 km from a road used by motor vehicles were processed further to better understand the behavioural responses of boreal chorus frogs to passing traffic. Noise from roads has been documented to extend >4 km from the road (Parris et al. 2009), so this distance was chosen for our study to ensure that noise pollution would exist at this distance. Recordings chosen to evaluate this question included instances of passing traffic (hereafter:
vehicle passage) and full choruses of boreal chorus frogs (call intensity = 3). The recordings were selected only if no other biotic or abiotic interruptions existed, ensuring that the loudest noise on the recordings were generated by either the frog chorus or the vehicle passage. If other species were vocalizing, or if weather conditions were not ideal, recordings were not included. Recordings (n = 188) were clipped into 2 min files within the program Adobe Audition CS6 version 5.0 (2012) to capture specific occurrences:

1. frogs only (FO): full boreal chorus frog choruses with no other acoustic signals,
2. vehicle only (VO): single vehicle passages with no other acoustic signals, and
3. frogs and vehicle (FV): full boreal chorus frog choruses with a single vehicle passage (Figure 3-2).

In total, 356 clips were made to satisfy the above categories (FO: n = 86; VO: n = 91; FV: n = 179). Recordings including a vehicle passage were clipped to center the vehicle in the clip, leaving 1 min before and 1 min after the vehicle noise reached a maximum amplitude.

Within Adobe Audition CS6 software (2012), a specified FFT filter (Fast Fourier Transform) was applied to all of the clipped recordings to isolate the frequency of the boreal chorus frog advertisement call (Figure 3-2). The FFT filter was used to eliminate all frequency <2.5 kHz and >4.5 kHz, ensuring that the only acoustic signals in each clip were within the frequency of the frog vocalization. Road noise from passing traffic generates low frequency acoustic interference (<2.5 kHz) that falls within frequencies that hylid frogs can detect (Smotherman and Narins 2000). Acoustic energy from traffic has been shown to decrease at frequencies higher than 3 kHz – 4 kHz (Bee and Swanson 2007); therefore, the vehicle’s measureable amplitude is decreased when the filter is applied.
Using the packages “seewave” (Sueur et al. 2008) and “tuneR” (Ligges et al. 2016) in the statistical program R (R Core Team 2016), all clipped recordings were run through a loop to extract the amplitude (dB) throughout the recording. The amplitude (dB) was averaged every 10 s and the largest amplitude measurement was extracted in every recording. The largest amplitude measurement was used to identify when the vehicle was present, except for clips without a vehicle passage. Every recording was run through the loop unfiltered to get the raw amplitude and then again with the FFT filter applied to get the isolated amplitude.

Mixed effect linear regression models using maximum likelihood estimation were generated in the statistical program Stata version 13.0 (StataCorp 2013) to better understand the effect of passing traffic on frog chorus amplitude. Models were generated for FO, VO and FV filtered recordings. The ARU station was used as a random effect in the models to account for any patterns driven by recordings taken from the same location. Other covariates used were: date, time, distance to the nearest road (km), and the time before/after the vehicle passage (timetotruck) (Table 3-3). For FO analysis, “timetotruck” was still used, but did not represent an actual vehicle passage. Top models were determined by using Akaike’s Information Criterion (AIC) (Burnham and Anderson 2002). We considered models with AIC values <2 apart (ΔAIC) to be equally supported (Burnham and Anderson 2002).

Pairwise comparisons were made in Stata (StataCorp 2013) to measure any significant differences in the amplitude of the full frog choruses before and after the vehicle passage in the FV data. This was done for FO data as well to assess whether full choruses in the absence of a vehicle passage remained at the same amplitude level during a comparable time period. Comparisons were done for the VO data to understand the amplitude generated by a vehicle passage and ensure that the highest amplitudes occurred at the vehicles’ closest passage points.
(at the center of the clips). Frogs could either decrease chorus amplitude or stop calling entirely when a vehicle passes, or they could increase chorus amplitude to compensate for the vehicle noise. Either response could be caused by two phenomena – the number of frogs calling or the loudness of calls of individual frogs.

3.3 RESULTS

3.3.1 Relative Abundance Models

Our model included: month, time of day, distance to open water, proportion upland, and the minimum distance to paved, gravel and trail roads (Table 3-2; Table 3-4). None of our covariates were significantly correlated. Month and time of day were used as fixed effects to account for seasonal and daily calling intensity. The effect size and direction of the coefficients informed the impact of the covariate on boreal chorus frog call intensity (Table 3-4). We found that the highest call intensity (call intensity = 3), indicating full frog chorus and highest relative abundance, was likely to occur at stations closer to any road type (paved, gravel and trails; Figure 3-3; Appendix 4; Appendix 5; Appendix 6). Therefore, it was concluded that distance to roads influences the relative abundance of vocalizing boreal chorus frogs, such that shorter distances result in higher numbers of frogs.

We used the margins command in Stata (StataCorp 2013) to predict the probabilities of the highest call intensity occurring at increasing distances from each road type (Table 3-5; Figure 3-4). There was little difference regarding the type of road and the predicted probability of a full chorus. The highest predicted probability for a full chorus occurred at the minimum distance to each road type (Table 3-5; Figure 3-4). The lowest predicted probability was seen for gravel roads (gravel: 0.337; trails: 0.368; paved: 0.370). There was a weak negative association between
road length density and call intensities (Figure 3-5), with stations in areas with lower road density tending to record higher calling intensities. However, only 6% of stations had a value >0 for this covariate, indicating that this metric was not appropriate to use with our buffer size of 150 m. This covariate was not explored any further.

The model predicted the highest call intensity to occur at greater distances to open water, likely a result of many small temporary pools of water not measured in available coarse GIS layers (Figure 3-6). Predicted call intensity was greater with increasing proportions of upland habitat surrounding a station (Figure 3-7).

### 3.3.2 Amplitude Analysis

Removing frequencies outside of the 2.5 kHz – 4.5 kHz range decreased the amplitude of sound generated by vehicles, but did not change the amplitude produced by frog choruses (Figure 3-8). The majority of the spectral signature generated by the vehicle event remained under 2.5 kHz, though in some cases, the vehicle signature extended into the 2.5 kHz – 4.5 kHz frequency range; however, in these cases the boreal chorus frog signature was still louder.

Mixed effect linear regression models generated with FV data had a lower AIC when the “timetotruck” covariate was expressed as a quadratic equation. The quadratic model had a lower AIC compared to the linear model for the FV (ΔAIC = 32.8) data. The FO data had a lower AIC (ΔAIC = 1.92) as a linear model, indicating that vehicle noise was responsible for the quadratic relationship. The VO data represents no biological information; however, the VO data was modeled to confirm that the vehicle passage resulted in the improved fit of the quadratic term in terms of frog calling amplitude (ΔAIC = 285.1).
Pairwise comparisons of the averaged amplitude at 10 s intervals before and after the vehicle passage showed no significant differences ($p > 0.05$) for the FO and FV comparisons (Table 3-6; Figure 3-9a; Figure 3-9c). Furthermore, there was no measurable difference in amplitude (dB) of full frog choruses before, after or during a vehicle event (Figure 3-9c; Figure 3-10). As expected, there was also no significant change in full frog choruses in the absence of a vehicle event (Figure 3-9a).

The amplitude measurement immediately before (position -1) and after (position 1) the vehicle event differed significantly in the VO data ($\chi^2 = 17.97$, $p = 0.0000$; Table 3-6; Figure 3-9b). This significant result does not relate to the behaviour of the frogs, but provides insight into noise-production by vehicles. Amplitude is expected to be at maximum at the point of closest passage of the vehicle (McBeath and Neuhoff 2002), and the higher amplitude immediately following the vehicle event is likely a result of this phenomenon.

3.4 DISCUSSION

We examined large-scale and small-scale behavioural responses of boreal chorus frogs to roads and traffic with the expectation that we would find a negative response for at least one scale. Furthermore, if we could not detect a large-scale response of roads on relative boreal chorus frog abundance, we then expected to find a small-scale change in chorus amplitude to accommodate acoustic interference by road traffic. This was not the case for our study. We found boreal chorus frogs to have a positive behavioural response to roads and a neutral behavioural response to traffic.
3.4.1 Relative Abundance

In our study area, the landscape is fragmented (Foote and Krogman 2006; Schneider and Dyer 2006; AB ESRD 2012). While boreal chorus frogs may not be the most mobile animals, landscape connectivity is still important and we should consider how this fragmented environment influenced boreal chorus frog abundance in our models, so that we may better understand the potential impacts to less abundant boreal anurans. We found boreal chorus frogs to have the highest predicted relative abundance, as measured by calling intensity, closest to paved roads, followed by all-terrain vehicle trails and lastly gravel roads. All road types were strong positive predictors of high frog relative abundance. It is important to note that we are not discussing frog use of roads themselves, but instead, are referring to vegetation conditions near roads. Our study also indicated that upland habitat is important for this species, similar to other temperate anuran studies (Hannon et al. 2002; Houllahan and Findlay 2003; Eigenbrod et al. 2008). Many temperate species require the use of wetlands for breeding, but also travel into upland areas to forage and overwinter (Fahrig et al. 1995; Joly et al. 2003). We saw a weak association with distance to open water bodies; however, the spatial scale of the data was not fine enough to account for smaller pools of standing water on the landscape, and distances to open water was limited to permanent bodies of water (>30 m wide).

Our study is not the first to find a positive relationship between boreal chorus frog abundance and roads (Browne et al. 2009). Newer studies have suggested that many temperate anuran amphibian species, including boreal chorus frogs (Mushet et al. 2012), are less sensitive to environmental change than has been proposed previously. Wood frogs (*Lithobates sylvaticus*, LeConte 1825) have been recorded to show a positive relationship with paved roads (presence-absence from call surveys, Trenham et al. 2003; calling intensity as relative abundance metric,
Eigenbrod et al. 2008) and other species have been recorded to lack responses to roads (*Hyla versicolor* (LeConte 1825), *L. clamitans* (Latreille in Sonnini de Manoncourt and Latreille 1801), Eigenbrod et al. 2008). The discrepancy in road effects are likely species specific and connected to the reproductive rates, population size, ability to avoid oncoming traffic and the benefits of being near a road (i.e. food source, breeding habitat) (Fahrig and Rytwinski 2009). It is a common belief that the adjacent roadside habitat drives the positive or neutral response to roads by a handful of anuran species though this habitat may be detrimental to certain populations (Trenham et al. 2003). Roadside habitat includes ditches that can be flooded from rain and road run-off, and often is characterized by short upland vegetation, allowing for easy foraging and movements.

Boreal chorus frogs are known to occupy a wide range of habitats (Russell et al. 2000; Mushet et al. 2012) including disturbed sites such as roadside ditches and flooded fields (Russell et al. 2000; Fisher et al. 2007). Individuals may easily move to other breeding locations to avoid any negative effects of being near active roads; however, many anurans do not travel far and have restricted movements to and from breeding locations (Gerhardt and Huber 2002; Smith and Green 2005; Herrera-Montes and Aide 2011), making it a challenge for these animals, in comparison to birds and mammals, to move away from roads (Herrera-Montes and Aide 2011). Furthermore, increased habitat fragmentation due to roads makes it dangerous or impossible for frogs to disperse to other locations (Joly et al. 2003; Jochimsen et al. 2004), and has been noted to increase the risk of road mortality in other temperate anurans (Ashley and Robinson 1996; Hels and Buchwald 2001). Smith and Green (2005) found that while most anurans have low vagility, some individuals will travel >10 km. Maximum dispersal distance recorded for boreal chorus frogs is 685.8 m (Spencer 1964; Smith and Green 2005) and road encounters would be
possible at this scale. Furthermore, considering the fragmented habitat of our study area it is likely that other indirect effects from roads (i.e. noise pollution) would reach the entire extent of their breeding habitat (Parris et al. 2009) even if fragmentation is not an immediate concern.

3.4.2 Calling Behaviour

We found no detectable change in boreal chorus frog full chorus amplitude (dB) in the presence of a passing vehicle. Frogs continued to call, showing no significant change in amplitude before, during and after the vehicle passage, indicating that boreal chorus frogs do not change their behaviour in the presence of roads. Nelson et al. (2016) found similar results regarding pacific chorus frogs (*P. regilla*, Baird and Girard 1852) in Oregon and reported a lack of any vocalization change in response to traffic noise, while other studies on frog calling behaviour near roads have found detectable shifts in vocalizations when vehicle noise is present (*L. clamitans, L. pipiens* (Schreber 1782), *H. versicolor*, Cunnington and Fahrig 2010; *Andinobates bombetes* (Cope 1865), Vargas-Salinas and Amezquita 2013).

Birds have been documented to change their acoustic signal in the presence of noise pollution (Slabbekoorn and Ripmeester 2008). However, it is costly for anurans to change their vocalizations (i.e. call rate, call frequency, call amplitude) (Parris 2002; Parris et al. 2009; Scofield et al. 2011), and may be energetically easier to avoid calling during times of acoustic interference. For frogs to increase their chorus amplitude or make other changes to their vocalizations, they would be required to expend energy (Parris 2002; Scofield et al. 2011), and ultimately, would need to replenish energy reserves through foraging (Scofield et al. 2011), something that may not be possible if food sources are low in the spring. Regarding our results, we saw very little acoustic interference with boreal chorus frog vocalizations generated from on-coming and leaving traffic, as is indicated by the low amplitude measurements in filtered
recordings (Figure 3-9b). This tells us that for the frequency band used by boreal chorus frogs, the vehicle has little impact, other than at its closest point, so the frogs may not respond by adjusting their behaviour for such a brief interruption.

Brooke et al. (2000) noted that male anuran amphibian calling behaviour is self-perpetuating as a positive feedback loop, where males will continue to call once they have been stimulated by other calling males. We measured full chorus behaviour in the absence and presence of vehicles and found this to be true. It is possible that traffic activity was insufficient to affect boreal chorus frog behaviour. Perhaps examining at recordings with multiple vehicle passages, rather than one would have resulted in enough acoustic interference to break the calling feedback-loop. Chronic abiotic noise has been shown to cause anurans from temperate forests in the southern hemisphere to alter their calling behaviour, and in some species (e.g. *Batrachyla taeniata*, Girard 1855) this is done through changing the chorus amplitude (Penna and Zúñiga 2014). On the other hand, chronic noise may ultimately result in habituation of anurans to the noise source (Penna et al. 2005; Shannon et al. 2016). If passing vehicles were constant, we might expect this to be occurring in our study. We looked at single vehicle passages, though we know these roads are subject to increased traffic levels. Therefore, it is possible that habituation has occurred in our study, but it is difficult to justify this response with the traffic volume we specifically looked at.

Jansen (2009) determined population sizes from anuran choruses of 25 species found in Bolivia using sound pressure level measurements (dB) and reported instances when human observers on the site accidentally disturbed calling anurans and reduced calling intensity. If the frogs in our study perceived danger from the passing vehicle, the same response may have been measured, however, it was not. Vehicles generate loud noises and bright light, move rapidly and
cause vibrations (Coffin 2007). No natural predators of temperate frogs (e.g. garter snakes, herons, fish) (Brodman 2008; Rittenhouse et al. 2008) exhibit these characteristics. From an evolutionary standpoint, it is entirely possible that these frogs do not view traffic on roads as a threat (Ashley and Robinson 1996).

3.4.3 Conclusions

Our study shows that boreal chorus frogs are indifferent to roads and associated noise pollution, but that does not eliminate any unseen short-term or long-term negative effects for this species. A positive numerical response to the proximity to roads was observed in boreal chorus frog relative abundance from recorded call intensity codes, and we suggest this is a consequence of potential suitable habitat existing adjacent to roads and the previously documented generalist nature of this species. The observed lack of behavioural change, in terms of chorus amplitude, implies that vocalizing boreal chorus frogs do not react to passing traffic. We suggest that this may be the result of habituation over the breeding season or lack of perception of the passing vehicles.

Our study leaves several questions unanswered: (1) do male frog choruses relay information to females or does the traffic acoustically interfere with this signal, and (2) is road mortality a large risk for this species and have populations adjusted dispersal patterns to other breeding wetlands in response to loss of habitat connectivity? Further studies are necessary to evaluate these questions and explore indirect effects of roads to fully understand their impact on this species and other temperate anuran species. Trombulak and Frissell (2000) note that it is unlikely that many road effects on wildlife will be addressed in any way that results in complete mitigation or remediation, so it is important to conserve natural soundscapes and interior breeding areas that lack road effects.
### Table 3-1. Description of call intensity codes based on the Wisconsin frog call index (Paloski et al. 2006).

<table>
<thead>
<tr>
<th>Call Intensity</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No calls.</td>
</tr>
<tr>
<td>1</td>
<td>Individuals can be counted; there is space between calls (no overlapping of calls).</td>
</tr>
<tr>
<td>2</td>
<td>Calls of individuals can be distinguished but there is some overlapping of calls.</td>
</tr>
<tr>
<td>3</td>
<td>Full chorus. Calls are constant, continuous, and overlapping; individual calls cannot be distinguished.</td>
</tr>
</tbody>
</table>
Table 3-2. Descriptions of covariates used in multinomial logistic regression models.

<table>
<thead>
<tr>
<th>Covariates</th>
<th>Description</th>
<th>Type</th>
<th>Mean</th>
<th>Std. Dev</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>month</td>
<td>Calendar month (n = 5; April, May, June, July, August)</td>
<td>Continuous</td>
<td>6.01</td>
<td>0.657</td>
<td>4 - 8</td>
</tr>
<tr>
<td>timeofday</td>
<td>Categorical time of day (night = 20:00 – 03:00; day = 04:00 – 19:00)</td>
<td>Categorical</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>upland</td>
<td>Proportion of upland</td>
<td>Continuous</td>
<td>0.201</td>
<td>0.285</td>
<td>0 - 1</td>
</tr>
<tr>
<td>dst2water</td>
<td>Minimum distance to open water (km)</td>
<td>Continuous</td>
<td>1.00</td>
<td>1.10</td>
<td>0 - 4.55</td>
</tr>
<tr>
<td>dst2paved</td>
<td>Minimum distance to paved roads used by motor vehicles (km)</td>
<td>Continuous</td>
<td>12.1</td>
<td>10.3</td>
<td>0 - 61.6</td>
</tr>
<tr>
<td>dst2gravel</td>
<td>Minimum distance to gravel roads used by motor vehicles (km)</td>
<td>Continuous</td>
<td>2.94</td>
<td>3.10</td>
<td>0 - 20.6</td>
</tr>
<tr>
<td>dst2roads</td>
<td>Minimum distance to either paved or gravel roads used by motor vehicles (km)</td>
<td>Continuous</td>
<td>2.58</td>
<td>2.85</td>
<td>0 - 20.6</td>
</tr>
<tr>
<td>dst2trails</td>
<td>Minimum distance to trails used by all-terrain vehicles (ATVs) (km)</td>
<td>Continuous</td>
<td>0.547</td>
<td>0.528</td>
<td>0 - 3.27</td>
</tr>
<tr>
<td>rddensity</td>
<td>Total road length (km) under buffer</td>
<td>Continuous</td>
<td>0.0137</td>
<td>0.0602</td>
<td>0 - 0.635</td>
</tr>
</tbody>
</table>
Table 3-3. Descriptions of covariates used in mixed effect linear regression models. Averaged amplitude values differed according to data (frog only, vehicle only, frog and vehicle).

<table>
<thead>
<tr>
<th>Covariates</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>station</td>
<td>Unique ARU location identifier used as a random effect</td>
</tr>
<tr>
<td>date</td>
<td>Julian date</td>
</tr>
<tr>
<td>time</td>
<td>Daily hours (n = 24)</td>
</tr>
<tr>
<td>amplitude</td>
<td>Averaged amplitude (dB) measures taken every 10 s</td>
</tr>
<tr>
<td>timetotruck</td>
<td>Position on recording (10 s bins) either before or after the vehicle passage</td>
</tr>
<tr>
<td>timetotruck2</td>
<td>Quadratic expression of timetotruck covariate</td>
</tr>
<tr>
<td>dst2roads</td>
<td>Minimum distance to either paved or gravel roads used by motor vehicles (km)</td>
</tr>
</tbody>
</table>
Table 3-4. Multinomial logistic regression output informing effects of road types on call intensities of male boreal chorus frogs (*Pseudacris maculata*). Call intensity is the dependent variable. Significance shown: $p < 0.001 \, ***$, $p < 0.01 \, *$, $p < 0.05 \, .$, $p < 0.1 \, .$

|                           | Coef.   | Std. Err. | z      | P > |z|  | 95% Conf. Interval |
|---------------------------|---------|-----------|--------|-----|---|-------------------|
| **Call Intensity: 0** (base outcome) |         |           |        |     |   |                   |
| month                     | -1.172564 | .1258141 | -9.32  | 0.000 |   | -1.41916 -0.92597  *** |
| timeofday                 | -0.0093787 | .3055538 | -0.03  | 0.976 |   | -0.60825 0.58946  |
| dst2water                 | .304987 | .1099779 | 2.77   | 0.006 |   | 0.089434 0.52054  * |
| upland                    | .0021762 | .1174859 | 0.02   | 0.985 |   | -0.22809 0.232444 |
| dst2paved                 | .2615827 | .1198187 | -2.18  | 0.029 |   | -0.49642 -0.02674  . |
| dst2gravel                | .1589864 | .1142243 | 1.39   | 0.164 |   | -0.06489 0.382862 |
| dst2trails                | .035455 | .1013903 | 0.35   | 0.727 |   | -0.16327 0.234176 |
| _cons                     | -.7075596 | .280452 | -2.52  | 0.012 |   | -1.25724 -0.15788  . |
| **Call Intensity: 1**     |         |           |        |     |   |                   |
| month                     | -1.237246 | .1321979 | -9.36  | 0.000 |   | -1.49635 -0.97814  *** |
| timeofday                 | 1.035683 | .4098948 | 2.53   | 0.012 |   | 0.232303 1.839062  . |
| dst2water                 | .3193093 | .1181855 | 2.7    | 0.007 |   | 0.08767 0.550949  * |
| upland                    | .1433577 | .1187044 | 1.21   | 0.227 |   | -0.0893 0.376014  |
| dst2paved                 | .0247732 | .1263778 | 0.2    | 0.845 |   | -0.22292 0.272469 |
| dst2gravel                | -.2256336 | .1503668 | -1.5   | 0.133 |   | -0.52035 0.06908  |
| dst2trails                | .0360084 | .1088233 | 0.33   | 0.741 |   | -0.17728 0.249298  |
| _cons                     | -.1843696 | .3932384 | -4.69  | 0.000 |   | -2.61443 -1.07296  *** |
| **Call Intensity: 2**     |         |           |        |     |   |                   |
| month                     | 2.234936 | .4382225 | 5.1    | 0.000 |   | 1.376036 3.093837  *** |
| timeofday                 | .2369352 | .0969542 | 2.44   | 0.015 |   | 0.046908 0.426962  . |
| dst2water                 | .1705735 | .0980266 | 1.74   | 0.082 |   | -0.02156 0.362702 |
| upland                    | -.1986155 | .103868 | -1.91  | 0.056 |   | -0.40219 0.004962 |
| dst2paved                 | -.687781 | .1081456 | -0.63  | 0.531 |   | -0.27974 0.144181  |
| dst2gravel                | -.098924 | .0915441 | -1.08  | 0.280 |   | -0.27835 0.080499  |
| _cons                     | -2.119054 | .4276636 | -4.95  | 0.000 |   | -2.95726 -1.28085  *** |
Table 3-5. Predicted probabilities of the highest call intensity, indicating full frog chorus, occurring as a response of the distance to different road types. Significance shown: $p < 0.001$ ‘***’, $p < 0.01$ ‘**’, $p < 0.05$ ‘*’, $p < 0.1$ ‘.’.

| Delta-method | Margin | Std. Err. | z | P > |z| | [95% Conf. Interval] |
|--------------|--------|-----------|---|-----|---|---------------------|
| **Distance to Paved Roads** |        |           |   |      |   |                     |
| 1 | 0.370006 | 0.039523 | 9.36 | 0.000 | 0.292541 | 0.44747 | *** |
| 2 | 0.346879 | 0.022464 | 15.44 | 0.000 | 0.302851 | 0.390908 | *** |
| 3 | 0.32163  | 0.014139 | 22.75 | 0.000 | 0.293917 | 0.349342 | *** |
| 4 | 0.294823 | 0.022378 | 13.17 | 0.000 | 0.250964 | 0.338683 | *** |
| 5 | 0.267113 | 0.035359 | 7.55  | 0.000 | 0.19781  | 0.336416 | *** |
| 6 | 0.239192 | 0.047662 | 5.02  | 0.000 | 0.145777 | 0.325589 | *** |
| 7 | 0.185367 | 0.066155 | 2.8   | 0.005 | 0.055706 | 0.315028 | *    |
| 8 | 0.160586 | 0.071655 | 2.24  | 0.025 | 0.020149 | 0.301023 | .    |
| **Distance to Gravel Roads** |        |           |   |      |   |                     |
| 1 | 0.336673 | 0.04124  | 8.16 | 0.000 | 0.255845 | 0.417501 | *** |
| 2 | 0.331701 | 0.022901 | 14.48 | 0.000 | 0.286815 | 0.376587 | *** |
| 3 | 0.322908 | 0.014163 | 22.8  | 0.000 | 0.29515  | 0.350667 | *** |
| 4 | 0.310501 | 0.02444  | 12.7  | 0.000 | 0.262599 | 0.358402 | *** |
| 5 | 0.294837 | 0.040254 | 7.32  | 0.000 | 0.21594  | 0.373734 | *** |
| 6 | 0.276417 | 0.056371 | 4.9   | 0.000 | 0.165933 | 0.386901 | *** |
| 7 | 0.255855 | 0.071689 | 3.57  | 0.000 | 0.115348 | 0.396363 | *** |
| 8 | 0.233842 | 0.085526 | 2.73  | 0.006 | 0.066213 | 0.40147  | *    |
| 9 | 0.21109  | 0.097262 | 2.17  | 0.030 | 0.02046  | 0.401719 | .    |
| **Distance to Trails** |        |           |   |      |   |                     |
| 1 | 0.368331 | 0.035049 | 10.51 | 0.000 | 0.299636 | 0.437027 | *** |
| 2 | 0.344463 | 0.020788 | 16.57 | 0.000 | 0.303719 | 0.385208 | *** |
| 3 | 0.321076 | 0.014113 | 22.75 | 0.000 | 0.293416 | 0.348736 | *** |
| 4 | 0.298289 | 0.021307 | 14    | 0.000 | 0.256528 | 0.34005  | *** |
| 5 | 0.276211 | 0.03317  | 8.33  | 0.000 | 0.211199 | 0.341223 | *** |
| 6 | 0.254941 | 0.045042 | 5.66  | 0.000 | 0.166666 | 0.343222 | *** |
| 7 | 0.234564 | 0.055919 | 4.19  | 0.000 | 0.124965 | 0.344163 | *** |
| 8 | 0.215152 | 0.065473 | 3.29  | 0.001 | 0.086827 | 0.343477 | *    |
| 9 | 0.196759 | 0.073572 | 2.67  | 0.007 | 0.05256  | 0.340957 | *    |
Table 3-6. Pairwise comparisons of the recording amplitude averaged every 10 s before and after a vehicle passage to assess changes in boreal chorus frog (*Pseudacris maculata*) chorus amplitude across the recordings and determine if changes were related to vehicle passage. Frog only (FO) data did not have a vehicle passage, however, pairwise comparisons were made as for vehicle only (VO) and frog and vehicle (FV) data to document patterns of natural changes in frog chorus amplitude. VO data did not include any frog chorus, but was used to uncover changes in sound amplitude during the vehicle passages. FO = frog only data (n = 86); VO = vehicle only data (n = 91); FV = frog and vehicle data (n = 179). Significance shown: $p < 0.001$ ‘***’, $p < 0.01$ ‘**’, $p < 0.05$ ‘*’, $p < 0.1$ ‘’.  

<table>
<thead>
<tr>
<th>Data</th>
<th>Before Vehicle Position</th>
<th>After Vehicle Position</th>
<th>$\chi^2$ value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FO</td>
<td>-1</td>
<td>1</td>
<td>0.13</td>
<td>0.76</td>
</tr>
<tr>
<td>FO</td>
<td>-2</td>
<td>2</td>
<td>0.35</td>
<td>0.5569</td>
</tr>
<tr>
<td>FO</td>
<td>-3</td>
<td>3</td>
<td>0.89</td>
<td>0.3456</td>
</tr>
<tr>
<td>FO</td>
<td>-4</td>
<td>4</td>
<td>0.78</td>
<td>0.377</td>
</tr>
<tr>
<td>FO</td>
<td>-5</td>
<td>5</td>
<td>3.46</td>
<td>0.0628</td>
</tr>
<tr>
<td>FO</td>
<td>-6</td>
<td>6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>FO</td>
<td>-7</td>
<td>7</td>
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<td>-</td>
</tr>
<tr>
<td>FO</td>
<td>-8</td>
<td>8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>VO</td>
<td>-1</td>
<td>1</td>
<td>17.97</td>
<td>0.0000  ***</td>
</tr>
<tr>
<td>VO</td>
<td>-2</td>
<td>2</td>
<td>0.35</td>
<td>0.5561</td>
</tr>
<tr>
<td>VO</td>
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</tr>
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<td>VO</td>
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<td>0.01</td>
<td>0.9368</td>
</tr>
<tr>
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<td>7</td>
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<td>0.7369</td>
</tr>
<tr>
<td>VO</td>
<td>-8</td>
<td>8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>FV</td>
<td>-1</td>
<td>1</td>
<td>0.03</td>
<td>0.8719</td>
</tr>
<tr>
<td>FV</td>
<td>-2</td>
<td>2</td>
<td>1.30</td>
<td>0.2543</td>
</tr>
<tr>
<td>FV</td>
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<td>3</td>
<td>1.88</td>
<td>0.1698</td>
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<td>0.2427</td>
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<tr>
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<td>7</td>
<td>1.50</td>
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</tr>
<tr>
<td>FV</td>
<td>-8</td>
<td>8</td>
<td>2.28</td>
<td>0.1313</td>
</tr>
</tbody>
</table>
3.6 FIGURES

Figure 3-1. Map of the province of Alberta, identifying the Lower Athabasca Planning Region and boreal chorus frog (*Pseudacris maculata*) survey locations (2012 – 2016).
Figure 3-2. Spectrograms including boreal chorus frogs (*Pseudacris maculata*) calling in full chorus during a vehicle passage. x-axis = time (s); y-axis = frequency (kHz). Boreal chorus frog calls are 3 kHz – 4 kHz from 0.00 min to 2.00 min. Maximum frequency vehicle event occurs at 0.50 min to 1:10 min, and is between 0 kHz – 2 kHz. (a) Unfiltered spectrogram of boreal chorus frog calls during a single vehicle passage. (b) Filtered spectrogram to isolate the 2.5 kHz – 4.5 kHz frequency range. Sounds were visualized in Adobe Audition CS6 software (2012).
Figure 3-3. Probability of detecting boreal chorus frog (*Pseudacris maculata*) call intensities as a response to the distance to roads (km). CI = call intensity codes (Table 3-1).
Figure 3-4. Predicted probabilities of the highest call intensity (call intensity = 3), indicating full frog chorus, occurring as a response to the distance to different road types. (a) Distance to paved roads; (b) Distance to gravel roads; (c) Distance to all-terrain vehicle (ATV) trails. All distances were standardized to make them easily comparable.
Figure 3-5. Predicted probabilities of the call intensities occurring as a response to gravel and paved road length (km) density. (a) Call intensity = 0; (b) Call intensity = 1; (c) Call intensity = 2; (d) Call intensity = 3.
Figure 3-6. Probability of detecting boreal chorus frog (*Pseudacris maculata*) call intensities as a response to the minimum distance to open water (km). CI = call intensity codes (Table 3-1).
Figure 3-7. Probability of detecting boreal chorus frog (*Pseudacris maculata*) call intensities as a response to the proportion of upland. CI = call intensity codes (Table 3-1).
Figure 3-8. Amplitude envelope (dB) of a single recording of a vehicle passage created by applying a Hilbert transform to the waveform. (a) Unfiltered vehicle only (VO) recording; (b) Filtered vehicle only (VO) recording; (c) Unfiltered frog only (FO) recording; (d) Filtered frog only (FO) recording.
Figure 3-9. Average amplitude measures taken at each 10 s interval from all filtered 2 min recordings (n = 356) to show pairwise comparisons of amplitude measurements before, during and after a vehicle passage. y-axis = amplitude (dB); x-axis = 10 s intervals representing before (-10 to -3), during (-3 to 3) and after (3 to 10) a vehicle passes. At interval 0 the vehicle is at its closest point. (a) Frog only (FO) data (n = 86) showing full chorus behaviour without a vehicle passage, so comparisons were made by making the middle of the recording 0. (b) Vehicle only (VO) data (n = 91) where no frogs are chorusing. (c) Frog and vehicle (FV) data (n = 179).
Figure 3-10. Average amplitude measures taken at each 10 s interval from all frog and vehicle (FV) 2 min recordings (n = 179) to show pairwise comparisons of amplitude measurements before, during and after a vehicle passage. y-axis = amplitude (dB); x-axis = 10 s intervals representing before (-10 to -3), during (-3 to 3) and after (3 to 10) a vehicle passes. At interval 0 the vehicle is at its closest point. (a) Unfiltered FV data. (b) Filtered FV data isolating 2.5 kHz to 4.5 kHz frequency range.
CHAPTER 4. CONCLUSION

4.1 SUMMARY

The main objectives of my thesis were to understand habitat use of an uncommon toad species using acoustic monitoring and to evaluate potential issues caused by roads for vocalizing anurans in a fragmented landscape. I collected field data for my study over 3 years (2013 – 2016), and relied on data collected by other students or Bioacoustic Unit members in the pilot year (2012). Processing of acoustic data collected from autonomous recording units (ARUs) involved personally listening to nearly 72 hours of raw recordings. I used an acoustic recognizer for Canadian toad (Anaxyrus hemiophrys, Cope 1886) (see Chapter 2) to increase detections of toads in the study area.

Chapter 2 investigated habitat use for breeding Canadian toads in wetlands in the boreal forest of northeastern Alberta, and documented that fen is the wetland type used most frequently by this species. Coarse soil texture (i.e. sand) was found to be an important habitat covariate, supporting the proposed overwintering strategy of this species of digging below the frost line (Breckenridge and Tester 1961; Roberts and Lewin 1979; Kuyt 1991). Upland habitats were found to be highly predictive of Canadian toad detections. A non-linear relationship was seen for this covariate, suggesting that the occurrence of upland edge habitat was the driving characteristic of this relationship. The importance of upland habitat in the generated model is consistent with the observations for other studies that Canadian toad is highly terrestrial post-breeding (Hannon et al. 2002; Garcia et al. 2004; Constible et al. 2010). My model failed to detect a negative relationship between toad occurrence and human infrastructure or chronic
noise; in fact, the minimum distance to roads was predictive of Canadian toad occurrence, indicating potential good habitat along road edges, such as loose soil for overwintering (Kuyt 1991) or warm open areas for foraging and travel.

Chapter 3 used an extremely common anuran, the boreal chorus frog (*Pseudacris maculata*, Agassiz 1850), to evaluate potential impacts on relative abundance and calling behaviour in the presence of road impacts. Calling data taken from raw acoustic recordings were used to relate calling intensity to the relative abundance (Nelson and Graves 2004; Stevens and Paszkowski 2004) of boreal chorus frogs, and the minimum distances to roads and all-terrain vehicle (ATV) trails were seen to predict higher relative abundances of this species. Noise pollution, an indirect effect of roads and local traffic, was seen to have no influence over the calling behaviour of this anuran species. Full chorus amplitude (dB) of male boreal chorus frogs was measured before, during and after a vehicle passage, and no increase or decrease in chorus amplitude could be detected, indicating that perception of the vehicle did not alter group behaviour or that frogs simply failed to perceive the vehicle.

4.2 EVALUATION OF BIOACOUSTICS FOR ANURAN MONITORING

Amphibian monitoring programs, such as the North American Amphibian Monitoring Program (NAAMP) (Weir 2001; Weir and Mossman 2005), rely on a collaboration of citizen science and wildlife organizations to conduct aural calling surveys for anuran amphibians along predetermined routes (Steelman and Dorcas 2010; Shearin et al. 2012). Many volunteer-based monitoring programs are not species specific and due to a variety of factors, very rarely result in detections of uncommon species, such as the Canadian toad (Shearin et al. 2012). Detections of anuran species can vary due to numerous biotic and abiotic factors. For instance, variation in
calling activity between different target species may allow for some to be detected while others are undocumented. Weather conditions, such as wind and rain may inhibit observers from detecting calling amphibians and changes in temperature may influence the calling behaviour and thereby the detectability of the species.

With the use of passive acoustic monitoring, the issue with species detectability is dealt with to a greater extent (Shearin et al. 2012; Brauer et al. 2016). ARUs can be left on the landscape for long survey periods, and are programmable to record at optimal times when anurans of interest are the most active. More data can be collected over a larger temporal scale (Porter et al. 2005), eliminating any negative detection influences by short-term environmental factors. This is especially important when considering uncommon species (Rognan et al. 2012; Holmes et al. 2014; Holmes et al. 2015; Campos-Cerqueira and Aide 2016).

Over the field seasons relating to this thesis, Canadian toad detections were made ranging across the Lower Athabasca Planning Region (LAPR), covering 93,212 km² of the northeastern corner of Alberta (AB ESRD 2012). This study is the first of its kind to examine Canadian toad habitat use at this regional scale, and to employ passive acoustic monitoring. Without the use of ARUs, the intensity of sampling would have been significantly lower, and it is likely that I would not have successfully detected as many Canadian toad locations (n = 142 unique stations). Furthermore, Canadian toads were only recorded on 1 day out of 7 – 14 days of surveying 29% of the time, demonstrating how crucial use of ARUs was to this study. If I had sampled at a smaller temporal scale, similar to most traditional call survey methods (e.g. NAAMP), I could have easily missed detecting toads that were not consistently vocalizing.

To decrease the amount of time spent listening to acoustic data, automated recognizers were used to identify the target vocalizations of Canadian toads in raw recordings (Wildlife
Recognizers generate false positive detections, requiring human validation of isolated vocalizations to confirm toad presence. False positives produced by the Canadian toad recognizer, included vocalizations produced by other co-occurring anuran species (western toad (*A. boreas*, Baird and Girard 1852), boreal chorus frog, and wood frog (*Lithobates sylvaticus*, LeConte 1825)) and avian species (Common Loon (*Gavia immer*, Brunnich 1764), Bonaparte’s Gull (*Chroicocephalus philadelphia*, Ord 1815), and Common Raven (*Corvus corax*, Linnaeus 1758)). The recognizer also identified some non-vocal avian sounds, such as the winnow produced by modified tail feathers of the Wilson’s Snipe (*Gallinago delicata*, Ord 1825) and the drumming of woodpecker species. Low frequency industrial noise was also flagged by the recognizer, as some noises lie in the similar frequency range as the Canadian toad call (1.5 kHz – 2.5 kHz). The amount of time taken to validate the recognizer flags is minimal in comparison to listening to the entirety of each recording. Upon examining a single year of acoustic data (2013) with both human listening and recognizer validation, I found human listening of <2% of the collected recordings to take 355 hours to complete, whereas, recognizer validations of 100% of the recordings took only 19 hours.

Resources for bioacoustics monitoring methods are becoming more available, and in recent years several statistical packages have been developed to assist in analyzing sound data in an ecological context. Amplitude measurements made in Chapter 3 used a combination of sound packages available in the widely available statistical software, R (R Core Team 2016). I used the packages “seewave” (Sueur et al. 2008) and “tuneR” (Ligges et al. 2016) to extract amplitude measurements throughout recordings. This allowed me to make accurate measurements of the raw soundscape and relate that to the natural biological systems of interest, in this case, the chorus behaviour of boreal chorus frogs.
Passive acoustic monitoring is an advantageous method of wildlife data collection and is extremely useful for species that are rare or uncommon on the landscape (Rognan et al. 2012; Holmes et al. 2014; Holmes et al. 2015; Campos-Cerqueira and Aide 2016). There are many applications for this type of data collection for field research, including studies of temperate anuran species, as demonstrated by this thesis.

### 4.3 MANAGEMENT STRATEGIES FOR CANADIAN TOAD

There are no current management plans for the Canadian toad in Alberta. However, ongoing research and monitoring in northeastern Alberta has the potential to develop such plans for management of this species. The last status report for the Canadian toad in Alberta was conducted nearly two decades ago (Hamilton et al. 1998) and this species still remains poorly understood in the province (Browne 2009). Along with few studies focused on this species, another reason for our limited knowledge is a lack of reported zero data in provincial datasets, indicating areas where the Canadian toad does not occur on the landscape (Browne 2009). My results successfully identify habitats in the boreal forest of northeastern Alberta used and unused by this species, and therefore provides key information on habitat that should be monitored and conserved for the Canadian toad. Habitat loss is likely the largest threat to most amphibians and has been documented to be the likely driver of the decline in Canadian toad. Within northeastern Alberta, land-use change, due to industrial activity, is thought to be a potential threat to the Canadian toad.

The Canadian toad is known to exploit human-modified landscapes (Paszkowski pers. comm.), which my research found to be true as well, considering proximity to roads. This is largely thought to occur due to benefits like foraging, basking, or loose soils existing near some
impacted landscapes. I found a strong positive association of Canadian toad habitat use and edge habitat between wetlands and uplands, suggesting use of uplands following the breeding season. This behaviour has been documented for a few Canadian toad studies in the boreal forest (Hannon et al. 2002; Garcia et al. 2004; Constible et al. 2010; Paszkowski pers. comm.) and for other bufonid species, such as the western toad (Browne and Paszkowski 2010). High proportion of graminoid fen was found to have the largest positive association with Canadian toad when considering other wetland habitats; however, they were detected in all fen types (graminoid, shrubby and treed) more often than in any other wetland class (i.e. marsh, bog, swamp). Furthermore, I found soil texture to strongly influence toad use, in that coarser soils were used more than finer soils.

Management strategies for Canadian toad need to consider the need for both wetland and upland habitat for this species. Though it largely occupies wetlands for breeding, this study and others have shown that upland edge and interior are important for the species. Canadian toads have been recorded to disperse up to 1.5 km from the breeding wetland (Garcia et al. 2004; Constible et al. 2010; Paszkowski pers. comm.), and studies regarding other temperate anuran species suggest movements can be greater (Smith and Green 2005; Browne and Paszkowski 2010). Hence, areas up to 3 km from known Canadian toad breeding locations, identified in my research, should be managed carefully for human activity to allow for safe toad movements into surrounding habitat following the breeding season. Furthermore, areas with sandy or coarse soils within 3 km of Canadian toad locations must consider potential loss of overwintering habitat if impacted by development. When identifying important habitat to conserve for a species, all aspects of its life cycle must be considered. Furthermore, to successfully conserve Canadian toad populations, we must consider breeding wetland habitat, post-breeding upland habitat, and
overwintering habitat. My results reflect breeding habitat for the species and provide insight into habitat requirements following breeding.

I propose that ARU surveys identified 19 populations of Canadian toads based on wetland connectivity assessed with the Ducks Unlimited enhanced wetland classification GIS layer (Ducks Unlimited Canada 2011). Three wetland complexes were major locations for Canadian toad in the LAPR, all together accounting for 60.6% of stations with toad detections: (1) McClelland Lake area (26.1%), north of Fort McMurray; (2) Bohn Lake area (21.1%), east of Janvier; and (3) habitats throughout an industrial area (13.4%), south of Anzac. These latter, developed sites, along with the McClelland Lake and Bohn Lake areas, require management plans to ensure continued existence of the species in this region. The Canadian toad is patchily distributed and loss of a single major population, could be detrimental to the persistence of the species in the region.

Specific strategies for managing Canadian toad populations in the boreal forest of northeastern Alberta include: (1) locate additional breeding locations for Canadian toad by visual or aural surveys in known habitat used by the species (i.e. fen habitat adjacent to upland areas with coarse soils); (2) identification and mitigation of threats to important breeding locations, including the 3 locations mentioned above; (3) continued research to fill in knowledge gaps regarding post-breeding movements into upland habitat and overwintering requirements; (4) development of a management plan and long-term monitoring programs to assess trends in abundance at known Canadian toad locations; and (5) outreach to general public and inform private industries of discoveries, regarding Canadian toad detections and abundance, to implement management strategies cohesively.
Suggested management strategies laid out here for the Canadian toad should not be extrapolated to populations of Canadian toad in regions outside of the boreal forest. Differences in life-history traits are known to exist across populations and have been shown to vary for Canadian toad populations throughout Alberta (Eaton et al. 2005a). Therefore, management of the species should consider variation of population requirements to best conserve the species.

4.4 IMPLICATIONS AND FUTURE DIRECTIONS

Amphibian declines, locally, regionally and globally, have become a serious concern over the past 3 decades (Blaustein and Wake 1990; Blaustein et al. 1994; Houlahan et al. 2000; Gardner 2001; Stuart et al. 2004), with human impacts identified as a major contributing factor. With the expansion of the energy sector in the LAPR of the boreal forest of northeastern Alberta (Schneider and Dyer 2006; AB ESRD 2012), concerns are being raised regarding the stability of amphibian populations in this region (Eaton et al. 2011), especially as it is not yet understood how increased levels of human infrastructure and associated activity influence the distributions and behaviour of these species.

The Canadian toad is a species of conservation concern in Alberta, and is currently poorly understood, resulting in its legal designation as data deficient by the Alberta Endangered Species Conservation Committee. In Chapter 2, I identified habitat used and unused by this species during the breeding season and generated a predictive model (AUC = 0.8459; Appendix 3) that can be used to explore potential locations that may be used by the Canadian toad. There are several gaps in the literature surrounding this species (Eaton et al. 2005a) and this study helps to fill in missing information regarding breeding requirements. Findings from this study provide information needed to survey for Canadian toads in the LAPR efficiently and effectively. This
model is currently being used by the Bioacoustic Unit to find new potential breeding locations for Canadian toads using ARUs. Results from the 2017 field season will be used to validate the predictive capacity of the model.

I looked at potential human impact on anuran amphibians in Chapter 3 by evaluating boreal chorus frog relative abundance and calling behaviour in the presence of roads. I chose to look at boreal chorus frogs due to their high abundance on the landscape and reliable calling behaviour throughout the breeding season (Russell et al. 2000; Fisher et al. 2007). This species is also known to occupy a wide range of habitats (Russell et al. 2000; Fisher et al. 2007; Mushet et al. 2012), ensuring that the species would be detected across many stations. Boreal chorus frogs used habitat near roads and relative abundance was predicted to be highest near roads; furthermore, full chorus of frogs did not increase or decrease calling amplitude (dB) in the presence of passing vehicles. No negative effects of roads were found with this species; however, other species, such as Canadian toad, may experience unforeseen consequences from anthropogenic impacts. While the boreal chorus frog call sits within the 3 kHz – 4 kHz frequency range, the Canadian toad vocalizes in the 1.5 kHz – 2.5 kHz range, and would overlap significantly with traffic noise (<2.5 kHz). Therefore, the Canadian toad call and industrial or traffic noise occupy the same frequency range, as demonstrated by the Canadian toad recognizer. Thus, acoustic interference should be a concern for this species. I propose that methods used in Chapter 3 of my thesis be used to evaluate the effect of noise pollution from roads on the Canadian toad.

My thesis contributes to a framework for understanding the distributions of anuran amphibians by examining environmental features associated with breeding habitat and by assessing impacts from roads and associated noise pollution. I show the value of passive acoustic
monitoring for surveying vocalizing anuran species. However, this is just a beginning, as the
documentation of population trends for amphibian populations in the LAPR and the causes of
changes in the distribution and abundance, will require further monitoring, ideally including the
deployment of ARUs.
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APPENDICES

Appendix 1. Details of the settings used for the automated computer recognizers built in SongScope to detect the breeding call of the Canadian toad (*Anaxyrus hemiophrys*).

<table>
<thead>
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<th>Recognizer settings</th>
<th>Canadian toad</th>
</tr>
</thead>
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<tr>
<td>Min. quality(^a)</td>
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</tr>
<tr>
<td>Min. score(^b)</td>
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<tr>
<td>Sample rate (Hz)</td>
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<td>Max. complexity(^c)</td>
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<td>Max. resolution(^d)</td>
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<tr>
<td>Max. syllable gap (ms)</td>
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<td>Max. song length (ms)</td>
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<td>Dynamic range (dB)</td>
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Recognizer performance statistics

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<td>Total training (% ± SE)</td>
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<td>State usage</td>
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<td>Feature vector</td>
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<td>Mean symbols (n)</td>
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<td>Syllable types</td>
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<tr>
<td>Mean duration of syllable (s)</td>
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<tr>
<td>Sources for annotations</td>
<td>Gavin Berg, ESRD</td>
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</table>

\(^a\) Quality values range from 0 to 100 and indicate signal quality confidence  
\(^b\) Score values range from 0 to 100 and indicate percent match with recognizer  
\(^c\) Number of states used to generate the model for the recognizer  
\(^d\) Size of feature vectors in the recognizer  
\(^e\) Number of sampled used by the Fast Fourier Transform algorithm to generate a recognizer  
\(^f\) Amount of overlap between each Fast Fourier Transform window
**Appendix 2.** Forward-addition stepwise model building, where covariates were added only if model AIC improved. Null model includes no predictive covariates. The top model, with lowest AIC, showing Canadian toad (*Anaxyrus hemiophrys*) habitat use in the boreal forest of northeastern Alberta is identified in the box. ΔAIC shows the difference in AIC between each generated model and the top model. Covariate descriptions can be found in Table 2-3.

<table>
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<th>Model</th>
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<th>ΔAIC</th>
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Appendix 3. Area under the curve (AUC) values from receiver operating characteristic (ROC) approach for Canadian toad (*Anaxyrus hemiophrys*) data.

Area under ROC curve = 0.8459
Appendix 4. Probability of detecting boreal chorus frog (*Pseudacris maculata*) call intensities as a response to the distance to paved roads (km). CI = call intensity codes (Table 3-1).
Appendix 5. Probability of detecting boreal chorus frog (*Pseudacris maculata*) call intensities as a response to the distance to gravel roads (km). CI = call intensity codes (Table 3-1).
**Appendix 6.** Probability of detecting boreal chorus frog (*Pseudacris maculata*) call intensities as a response to the distance to all-terrain vehicle (ATV) trails (km). CI = call intensity codes (Table 3-1).