

Improving passive acoustic monitoring methods for anuran amphibians
in northern Alberta, Canada

by

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Abstract

The global decline of amphibian populations has been documented since the 1960's. As such, amphibian monitoring programs are becoming increasingly important, in order to inform conservation strategies. Passive acoustic monitoring, i.e. recording the vocalizations produced by animals to detect them, is an increasingly common tool for anuran amphibians, allowing efficient collection of large amounts of data. I used autonomous recording units in wetlands across northern Alberta, Canada, to survey anurans during the breeding season. The overall goal of this thesis is to improve acoustic monitoring programs for anurans.

First, I evaluated a method for visually scanning spectrograms as a more efficient means of processing recordings than the current standard of human listening. I assessed the effectiveness of visual scanning relative to human listening through generating occupancy models for four species of anurans based on data collected via the two techniques. Secondly, I evaluated patterns of calling behaviour in response to different light conditions at night, specifically the lunar phase and presence of light pollution, to help identify environmental conditions more favorable for detecting anurans through recordings. I also conducted a literature review to highlight the current knowledge of the effects of light on anuran breeding behaviour.

I determined that visual scanning is more efficient for processing acoustic data than human listening. Listening to this set of recordings took 6.5 times longer than analysis via visual scanning. Occupancy estimates from models based on visual scanning data were comparable to those using listening data for all four study species. The development of a combination occupancy model, based on data from both analytical techniques, showed that including listening data from a sub-set of recording stations, in addition to data from visual scanning, improved model estimates. Visual scanning can be used to improve efficiency of acoustic processing and

could be applied to non-amphibian species with similar vocalization characteristics, such as a high call rate, or are distinctive because of unique call frequencies or diel activity patterns. My second set of results suggest that lunar phase and light pollution did not have a strong effect on calling behaviour of boreal chorus frog or wood frog. In conjunction with the literature review, my results suggest that light levels could play a role in anuran behaviour but calling behaviour may not be the ideal metric for evaluating their effects. My research can help inform monitoring and conservation plans through improved methodology and a greater understanding of anuran behaviour.

Preface

This thesis is an original work by Jillian Cameron. The research questions were conceptualized by Erin Bayne, Cindy Paszkowski and Jillian Cameron. Data for Chapter 2 were provided by the Bioacoustic Unit and some of the data used in Chapter 3 were provided by Arthur Whiting. Andy Crosby and Richard Hedley assisted with coding and analysis. Weather data were provided by Alberta Agriculture and Forestry and Environment and Climate Change Canada.

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Table of contents

Abstract	ii
Preface	iv
Acknowledgements	v
Table of contents	vi
List of tables	viii
List of figures	ix
List of appendices	xi
Chapter 1: General introduction	1
State of amphibians	1
Passive acoustic monitoring	3
Thesis objectives	4
Chapter 2: Evaluating the efficiency of processing bioacoustic data for an anuran community with visual scanning in comparison to human listening	7
<i>Introduction</i>	7
<i>Methods</i>	10
1 Data collection	10
1.1 ARU stations and deployment	10
1.2 Recording processing – listening	11
1.3 Recording processing – visual scanning	12
2 Comparisons of visual scanning and auditory analysis	12
3 Occupancy models	14
3.1 Single method occupancy models	15
3.2 Combination occupancy models	15
3.3 Reduced listening occupancy models	17
3.4 Model specifications	18
<i>Results</i>	18
Visual scanning comparisons	18
Occupancy models	19
Single method occupancy models	19
Combination occupancy models	20
Reduced listening model	21
<i>Discussion</i>	23
Effort comparison	28
Recommendations	29
Application	30
Conclusion	32
Chapter 3: Changes in calling behaviour of wood frogs and boreal chorus frogs in response to lunar phase and artificial light pollution	38
<i>Introduction</i>	38
<i>Methods</i>	43

Study location and acoustic data collection	43
Acoustic data processing	44
Analysis	46
Literature search	47
<i>Results</i>	48
Boreal chorus frog	48
Wood frog	49
Literature review	49
<i>Discussion</i>	50
Conclusion	56
Chapter 4: General conclusions	66
Summary of findings	66
Applications and limitations	67
Literature cited	72
Appendices	82

List of tables

Table 2.1. Summary table of comparisons conducted between human listening and visual scanning methods for processing audio recordings from autonomous recording units to detect anuran vocalizations. Spectrograms varied in duration from 10 seconds, 30 seconds and 60 seconds. Surveys varied in length from 10 seconds, 1 minute and 3 minutes. 36

Table 2.2. Summary of a series of comparisons of anuran detections between methods of processing audio recordings. The number of recordings in which detections were missed using method 2 relative to method 1 were summed out of the 59 recordings processed. Human listening was the standard against which visual scanning was initially compared. Different configurations of spectrograms for visual scanning were compared to each other. Surveys were 10 seconds, 1 minute and 3 minutes in length. Spectrogram lengths were 10 seconds, 30 seconds and 1 minute in length. 37

Table 3.1. The boreal chorus frog (*Pseudacris maculata*) global model was sub-set into candidate models and the resulting models were ranked using AIC. The global model is highlighted. Competitive models were those less than two delta AIC scores and only models with weights above zero are shown. These values are untransformed coefficients. 63

Table 3.2. The wood frog (*Lithobates sylvaticus*) global model was sub-set into candidate models and the resulting models were ranked using AIC. The global model is highlighted. Competitive models were those less than two delta AIC scores and only models with weights above zero are shown. These values are untransformed coefficients. 64

Table 3.3. Summary of literature review results assessing the effects of artificial light and lunar phase on anuran breeding behaviours. 65

List of figures

- Figure 2.1.** Map of northeastern Alberta, Canada indicating where autonomous recording units were deployed at wetlands to record anuran breeding vocalizations, at 346 stations. The base map is the Alberta Biodiversity Monitoring Institute's wetland probability layer. 33
- Figure 2.2.** Probability of occupancy, ψ , estimates from a series of occupancy models for four anuran species at 346 stations. Data collection was either by human listening to audio recordings or visual scanning of spectrograms. The listening and visual scanning models used data from all 346 stations. To compare time investment relative to visual scanning, the reduced listening model used only 50 stations, listening to which takes approximately the same time as visually scanning the 346 stations used in the other models. 95% credible intervals are presented for the listening and visual scanning models. The reduced listening model was resampled 100 times; the average of all the estimates was taken and the error bars are bootstrapped confidence intervals. 34
- Figure 2.3.** Probability of occupancy, ψ , estimates from a series of occupancy models for four anuran species at 346 stations. Data collected from visually scanning spectrograms of audio recordings was used for each station and listening data was included from a sub-set of stations, either 14%, 50% or 75% of stations. The grey lines represent the occupancy estimate and 95% credible intervals from the model using only listening, the standard to which all other models are compared. Each model was resampled 100 times; the average of all the estimates was taken and the error bars are bootstrapped confidence intervals. 35
- Figure 3.1.** Map of northern Alberta, Canada indicating where autonomous recording units were deployed at wetlands to record anuran breeding vocalizations. Fifty-eight locations were chosen to be within or outside areas of anthropogenic light pollution, indicated by a gradient from white to black, respectively. The base map was created by NOAA's National Geophysical Data Center. 58
- Figure 3.2.** The probability of boreal chorus frogs (*Pseudacris maculata*) calling as a function of percent relative humidity. The left panel represents the new moon phase, <10% lunar illumination, and the right represents the full moon phase, >90% lunar illumination. The absence of light pollution is represented by the red lines and presence of light pollution is the blue line. This is the global model used in the logistic regression that also includes all of the variables: relative humidity and day of year as covariates, with lunar phase and light pollution as predictors. Shading represents 95% confidence intervals. 59
- Figure 3.3.** The probability of wood frogs (*Lythobates sylvaticus*) calling as a function of percent relative humidity. The left panel represents the new moon phase, <10% lunar illumination, and the right represents the full moon phase, >90% lunar illumination. The absence of light pollution is represented by the red lines and presence of light pollution is the blue line. This is the global model used in the logistic regression that also includes all of the variables: relative humidity and day of year as covariates, with lunar phase and light pollution as predictors. Shading represents 95% confidence intervals. 60

Figure 3.4. Summary of literature review showing anuran amphibian calling behaviour responses to variation in moonlight and artificial light conditions. The number of species that increase calling behaviour in dark or light conditions, or have no response, are indicated on the x-axis.

61

Figure 3.5. Summary of literature review showing anuran amphibian breeding behaviour responses to variation in moonlight and artificial light conditions. The breeding behaviours represented are migration, oviposition and amplexus. The number of species that increase calling behaviour in dark or light conditions, or have no response, are indicated on the x-axis.

62

List of appendices

- Appendix 2.1.** Spectrogram images showing the difference in default settings (top) and with adjusted settings (bottom). The contrast was adjusted from the default 120 dB to 90 dB using the -z option in the Sound eXchange program to improve the signal to noise ratio. The x-axis is time in seconds; the y-axis is the frequency in kHz and the dBFS scale, or colour scale, indicating the amplitude of the sound. Spectrograms are of boreal chorus frog (*Pseudacris maculata*) vocalizations at the 3 kHz frequency. 82
- Appendix 2.2.** Spectrogram images illustrating different frame lengths (10, 30 and 60 seconds, top to bottom) for the same recording. The x-axis is time in seconds; the y-axis is the frequency in kHz and the dBFS scale indicating amplitude of the sounds. Spectrograms are of boreal chorus frog (*Pseudacris maculata*) vocalizations at the 3 kHz frequency. 83
- Appendix 2.3.** The hierarchical model for the combination model using a Bayesian framework. Human listening data was included at a subset of stations in addition to visual scanning data. The first section of code describes the model in WinBugs and the second section is in R that links to WinBugs to run the model. 84
- Appendix 3.1.** Terms used in Scopus and Web of Science for the literature search. A complete list of anuran genera (452 genera; amphibiaweb.org 2018) was used in the terms under the anuran subject. 86
- Appendix 3.2.** The final papers used in the literature review. 87
- Appendix 3.3.** Results of the importance function for each species, the boreal chorus frog and the wood frog. The importance function takes the sums Akaike weights from the models' sub-set from the global models and outputs the relative importance of each variable. Each variable is ranked in relative importance and is assigned a value based on the number of models that contain it. The variables are listed in order of importance for each species. 89

Chapter 1: General introduction

State of amphibians

Since the 1960's, amphibian species have been undergoing population declines and species extinctions at higher rates than the average long-term background rates for this group of vertebrates (Houlahan et al. 2000, Alroy 2015). Of all the amphibian species assessed in a global study, 22.5% are data deficient, and cannot be assigned an accurate conservation status, and 43.2% are undergoing some kind of population decline (Stuart et al. 2004). While there is not a single cause to these declines, human activities are considered to be a driving force. Human activities and human population density are thought to be causing population declines through the loss and degradation of quality habitat, illegally trafficking of individuals, propagation of diseases, and environmental effects associated with climate change, such as changing weather patterns (Beebee and Griffiths 2005; Sodhi et al. 2008). To complete their lifecycle, many species of amphibians use both aquatic and terrestrial habitats. These species are exposed to stressors in each of these habitat types, such as water-borne pollutants (Blaustein and Wake 1990) or conflict with human infrastructure, such as roads (Glista et al. 2008).

An additional and recently emerging ecological concern is the spread of anthropogenic light pollution across the landscape (Longcore and Rich 2004), commonly known as artificial light at night, or ALAN. Light pollution has been shown to affect animal behaviour, for example, insects and fish can be attracted to light (Longcore and Rich 2004) and birds can be disoriented by light (Ogden 1996; Le Corre et al. 2002). These behaviours can lead to exhaustion and entrapment. As many amphibians are nocturnal and adapted to the dark, visual recovery from light at night can take a long time and could affect their behaviour (Buchanan 1993) and fitness.

Amphibians are also a relatively understudied group of animals. New species are still being described; as many as one quarter of the known amphibian species were discovered between 2005 and 2014 (Catenazzi 2015). This contributes to the high numbers of amphibian species that are data deficient (22.5%, Stuart et al. 2004), meaning population trends are unknown and statuses cannot be assigned. This is important because conservation resources are allocated based on this type of information, which is determined through species monitoring. With ongoing population declines and limited resources, monitoring programs are necessary and in demand, and there is need for efficient and cost-effective methods for monitoring.

My thesis focuses on amphibian species in Alberta, Canada. There are ten species of amphibians in Alberta, only three of which are considered “secure” based on their general status, (boreal chorus frog (*Pseudacris maculata*), wood frog (*Lithobates sylvaticus*), western tiger salamander (*Ambystoma mavortium*); Government of Alberta 2012, 2019). Three species are listed provincially as “sensitive”: Columbia spotted frog (*Rana luteiventris*), long-toed salamander (*Ambystoma macrodactylum*), western toad (*Anaxyrus boreas*). Three more “may be at risk”: Canadian toad (*Anaxyrus hemiophrys*), great plains toad (*Anaxyrus cognatus*), plains spadefoot (*Spea bombifrons*). Of these species, four are federally listed by COSEWIC as “special concern” (great plains toad, northern leopard frog, western toad, western tiger salamander; Government of Canada 2017). The northern leopard frog (*Lithobates pipiens*) and is provincially listed as “at risk”, with a legal, detailed status of “threatened”. The Canadian toad’s legal, detailed status is “data deficient” by the province because sufficient information about their biology and populations in Alberta are lacking.

Rapid changes are occurring in northern Alberta’s boreal forest, which is home to half of the province’s amphibian species. Urbanization and industrialization, from oil and gas and

forestry, are altering this landscape (Foote and Krogman 2006). Loss of habitat along with the disproportionate effects of climate change in the north (Leduc et al. 2016) could contribute to further instability in these amphibian populations. Given the vulnerable statuses of Alberta's amphibians, research and monitoring has an important role in the conservation of these species. Additionally, because amphibians are often used as indicator species, good monitoring programs can allow us to use them as sentinels for wetland health (Wyman 1990; Wake 1991).

Passive acoustic monitoring

Passive monitoring is becoming a widespread strategy for conducting wildlife surveys. Passive acoustic monitoring relies on the sounds that animals make to determine which species are present at a given location and time. Autonomous recording units (ARUs) are one such tool for conducting passive acoustic monitoring and are preprogrammed to record on a schedule for any duration desired. Technological advancements have enabled these units to become smaller, easier to deploy, and more robust, allowing them to withstand long deployments in the field. They are able to collect large numbers of survey recordings with only two visits to a site, once for deployment and once for retrieval during the period when the focal species are vocalizing, unless batteries and SD cards need replacement during long deployments. Non-experts are able to conduct this field work, removing the requirement of experienced assistants.

ARUs are comparable to human acoustic surveys for avian taxa. The discrepancies that arise are largely attributed to processing methods (Shonfield and Bayne 2017), detection radius (Wilgenburg et al. 2017) and ARU model specifications (Rempel et al. 2013; Turgeon et al. 2017). Despite these variations, surveys conducted with ARUs have been shown to have equivalent or better species detections than human surveys in the field (Shonfield and Bayne 2017).

ARUs are particularly useful for surveying anuran amphibians that call nocturnally. Males vocalize to communicate with conspecifics, forming distinct choruses of calling individuals during the breeding season. Units can be deployed during the day and scheduled to record during the night to avoid having to visit sites in the dark, when anurans are vocalizing. This reduces the risk of navigating wetlands at night and of disrupting behaviour. Additionally, the timing of anuran breeding behaviour is largely dependent on environmental conditions. Long-term deployments ensure that surveys capture the breeding calling period, alleviating the concern of missing the time when amphibians are vocalizing.

A limiting factor associated with ARUs is the vast amounts of recordings collected that then need to be processed. With the increasing popularity of ARUs, methods and supporting technology to assist in processing are constantly improving. Automated computer programs are available to build algorithms that recognize and automatically detect species based on their unique vocalization signature, known as recognizers. Recognizers are able to process large amounts of data, but they do have some limitations. There is still a large time investment associated with collecting quality training data to build the recognizer with and validating the detections from the recognizers, without knowing the inherent rate of false negatives. However, more accessible programs are also becoming available, through freeware, that help in reducing costs and do not require as much expertise to operate. While large, long-term datasets are valuable, the large time investment required to extract data from all the recordings can be prohibitive, especially for monitoring initiatives with limited resources.

Thesis objectives

The focus of my thesis was to improve acoustic monitoring for anuran amphibians using bioacoustic technology. I looked to improve the efficiency of processing recordings and to

inform recording selection to increase the likelihood of species detection. I used ARUs deployed in wetlands across northern Alberta, Canada to target four anuran amphibian species: Canadian toad, western toad, wood frog and boreal chorus frog. These anuran species can be used as model systems to improve acoustic monitoring programs for several reasons. First, the boreal chorus frog and wood frog are common species, and thus the study objectives were not limited by sample size. They were known to be present at the treatment stations, allowing me to survey them and assess their behavioural patterns. This will contribute to further understanding of their biology with the objective of informing acoustic monitoring strategies. Second, the Canadian toad and western toad are rarer species, with a lower frequency of occurrence, providing the opportunity to assess whether lower occurrence rates change conclusions or recommendations about the most effective ways to monitor. This will contribute to more effective monitoring tools and encourage continued monitoring efforts for these rarer species.

This thesis contains two data chapters aimed at its overall thesis objectives. The objective of Chapter 2 is to improve acoustic data collection efficiency and to assess the effectiveness of new methods using occupancy models. I implement a standardized method for processing recordings by visually scanning spectrograms of recordings and compare this to human listening, the current processing standard. I use occupancy models, comparing estimates between the two processing methods, to evaluate the accuracy of visual scanning relative to human listening. I also develop an occupancy model to demonstrate how visual scanning can be improved by incorporating listening data at a sub-set of the stations into the models to account for the occasional calls that were missed by visual scanning. The goal of Chapter 3 was to improve the understanding of anuran calling behaviour so that recording selection can target conditions when calling is more likely to occur. Using the visual scanning method, I assess patterns of calling

behaviour under different light conditions at night for the boreal chorus frog and the wood frog, specifically under different lunar phases and anthropogenic light pollution conditions. I also conduct a literature review to further understand how anuran breeding behaviours are affected by light. The implications and limitations of this work are discussed in the conclusion of this thesis (Chapter 4).

Chapter 2: Evaluating the efficiency of processing bioacoustic data for an anuran community with visual scanning in comparison to human listening

Introduction

Advances in bioacoustic technology now allow for the widespread passive monitoring of vocal and non-vocal taxa, including birds, bats, anuran amphibians, canids, and insects (Sugai et al. 2019). Autonomous recording units (ARUs) are passive monitoring devices preprogrammed to record audio on a desired schedule and are increasingly being used to replace human conducted point counts (Sugai et al. 2019). An advantage over human point counts is that ARUs can be deployed for long periods allowing repeated surveys while human time spent in the field is reduced. Each ARU location only needs to be visited twice, once for deployment and once for retrieval of the devices. The ability to conduct more and longer surveys makes ARUs especially useful for cryptic species that call infrequently or occur at low densities (Lambert and McDonald 2014, Measey et al. 2017, Rogers et al. 2013).

ARUs are a particular improvement for conducting nocturnal acoustic surveys for anurans because it is safer and easier to visit sites in daylight to deploy ARUs rather than navigating wetlands in the dark. Additionally, many more site surveys can be conducted throughout the season using ARUs, decreasing the chances of missing calling episodes. This is particularly important for anurans because calling behaviour is often reliant on environmental conditions, such as temperature and humidity, causing the timing of calling to be unpredictable (eg: Saenz et al. 2006). For explosive breeders like many anuran species, there is a short season for breeding, so the likelihood of capturing this calling period can be increased by being able to conduct more surveys using ARUs despite variability in weather and timing of calling onset between years.

The biggest challenge with passive monitoring using ARUs is the vast amount of acoustic data that are collected, both spatially and temporally (Shonfield and Bayne 2017). While more data reduces the error of statistical estimates, there is cost associated with the concomitant increase in time required to process the data. The conventional method of analyzing audio recordings has been to listen to recordings and identify vocalizing species by the unique sounds they make. In many projects, this is complemented by visualizing the sound through spectrograms. Human listeners identifying and transcribing detections with this method is the common standard for many monitoring programs, however, the process is very time intensive. Transcribing recordings for community data, i.e. identifying all the species present on a recording, takes two to three times as long as the duration of the recording being processed, depending on methodology (Wimmer et al. 2013, Alquezar and Machado 2015, Rocha et al. 2015). Computer-based processing of audio through recognizers – algorithms that recognize and automatically detect species vocalizations – are increasingly being used for processing large volumes of audio data without human listeners interpreting all recordings. There is also a large time investment to use this method, in learning how to build and train recognizers for species in a particular area. In addition, the most advanced recognizers currently in use in this field can create a large number of false positives and an unknown number of false negatives (Shonfield and Bayne 2017). Thus, in most monitoring programs validation of positive recognizer hits, the sounds it classifies as a species detection, is a required step which is also time consuming. While recognizers do hold promise and will eventually reduce the amount of time investment required as they become easier to build and more reliable, their current ability to produce data for multiple species may not be as time efficient as other approaches (Joshi et al. 2017).

Visually scanning spectrograms of recordings is an alternative method for processing acoustic data that might improve efficiency. Manually scanning through spectrograms to identify spectral signatures of calls, visually without listening to the recordings, can improve the speed of processing without a great loss to accuracy (Truskinger et al. 2013). Visual processing can be faster than auditory processing by humans and can produce data for multiple species in some circumstances (Swiston and Mennill 2009, Joshi et al. 2017). Visual scanning can also be more accurate than automatic computer recognition. Human scanners can generate more detections especially for species that vocalize infrequently (Swiston and Mennill 2009). However, this approach may result in false negatives, when calls are distant or masked by other sounds, but still at a lower rate than automatic recognizers (Digby et al. 2013).

The goal of this study was to assess the efficiency of human analysis of recordings via visual scanning compared to listening. I collected detection data using both processing methods, for a community of four anuran species: two frog species that are abundant and two toad species that are rarer in this study area. The first objective was to determine the most appropriate spectrogram configuration for visual scanning for the study species. To do this I compared species detections from visual scanning against detections from human listening, from the same recordings. For the visual scanning, the spectrogram images created were varied in recording length for each survey and the duration of each spectrogram image, i.e. how many seconds of a recording were included in each image. I compared visual species detections among the various configurations of recording and spectrogram durations and to human listening to assess the accuracy of each approach. I chose the best visual scanning method and applied it to a new data set for the remaining objectives. For the second objective, I used occupancy models to assess how model estimates change using human listening versus visual scanning. Third, I created a

modified occupancy model that incorporated both data types to optimize the time-efficiency of visual scanning and the accuracy of human listening. To account for false negatives resulting from visual scanning, I investigated a method of incorporating listening data with visual scanning data in order to use a sub-set of the stations with available human listening data as a confirmation tool to improve estimates from visual scanning. The rationale was that by combining data from these two processing methods, I can achieve an efficient approach to collecting more data by visual scanning, which will produce smaller confidence intervals associated with model parameter estimates and ensure a high level of accuracy with less time and lower cost investments. Finally, I compare occupancy model estimates when the same amount of time for processing the data is allocated to each processing method, to assess the trade-off between the efficiency and accuracy of visual scanning relative to human listening, the slower but more accurate method. By increasing the efficiency of processing acoustic data, this visual scanning method can improve monitoring programs that are constrained by the challenges of big data.

Methods

1 Data collection

1.1 ARU stations and deployment

All ARU deployments across northeastern Alberta, Canada (Figure 2.1) followed a standardized protocol (Lankau 2015). They were deployed between 2013 and 2017, during the anuran breeding season from April to June. The boreal forest of northeastern Alberta is a complex of wetlands intermixed within uplands stand of *Populus*, *Picea*, *Pinus*, and *Larix* species (Norton et al. 2000). All ARUs used in this study were in areas designated as wetlands,

which can include open water, fens, bogs, and swamps based on the Alberta Biodiversity Monitoring Institute wetland probability layer (Hird et al. 2017). The study species that inhabit this area are the boreal chorus frog (*Pseudacris maculata*), the wood frog (*Lithobates sylvaticus*), the Canadian toad (*Bufo hemiophrys*) and the western toad (*Anaxyrus boreas*). These species aggregate in large numbers in wetlands to breed, with the males forming choruses of calling individuals. Wildlife Acoustic Song Meter SM2 and SM4 model ARUs (Wildlife Acoustics Inc., Maynard, MA, USA) were secured approximately 1.5 meters high and they record with a sampling rate of 44.1 kHz. ARUs were placed at wetlands with a variety of monitoring objectives and, therefore, had variable recording schedules and deployment durations. However, each ARU recorded multiple times per night and the average deployment length was 29 days.

1.2 Recording processing – listening

Human listeners, trained acoustic taxonomists, processed ARU recordings following a standardized protocol (Bioacoustic Unit 2015). Using audio software such as Audition or Audacity, observers listened to the recordings while simultaneously viewing the spectrograms to identify vocalizations to species. Listeners documented the first instance at which each species was detected, for every minute of a three-minute recording. Anuran calls were identified to species and an associated calling intensity value from 1-3 was assigned according to North American Amphibian Monitoring Program guidelines (Weir et al. 2005; Weir and Mossman 2005). A calling intensity of 1 indicates there are one or more individuals calling but calls do not overlap. A calling intensity of 2 means there are overlapping calls but individuals are still distinguishable from each other. A calling intensity of 3 signifies that there is a full chorus and individual calls are indistinguishable.

1.3 Recording processing – visual scanning

To create images for visual scanning of the recording spectrograms, I used the freeware program Sound eXchange (SoX; Bagwell 2014). SoX was called through R using tuneR (Ligges et al. 2018) and seewave (Sueur et al. 2008) R packages using a .wav format. SoX processed each .wav audio file and created spectrogram images, saved as .png files. The spectrogram function in SoX uses a Discrete Fourier Transform algorithm, with a Hann window to create the spectrograms. The sampling rate for these images was set to 18 kHz to visualize anuran vocalizations from 0-9 kHz and to remove the higher frequencies that are audible in the recordings but anuran vocalizations do not reach. The contrast was adjusted from the default 120 dB to 90 dB using the -z option in SoX to improve the signal to noise ratio (Appendix 2.1). The spectrogram frame durations used for each recording were set at 10 seconds, 30 seconds and 60 seconds long (Appendix 2.2). Ten second spectrogram frames are similar to what is used when listening and the longer frames reduced the number of images to be processed for each recording. This allowed me to weigh changes in detection accuracy against increased efficiency, by reducing the number of images being processed per recording. I viewed each image to identify the occurrences of the spectral signatures of the four target anuran species. When species were identified, I assigned a calling intensity index value, as in the listening process. If the species identification or calling intensity was uncertain, I listened to the recording for confirmation.

2 Comparisons of visual scanning and auditory analysis

To pilot visual scanning as a method, I first had to determine which spectrogram configurations would be the most suitable. I randomly selected a sample of wetland recordings that had three-minute point counts processed by human listeners. I considered detections based on human listening to be absolutely correct in terms of species identification and calling intensity

index; they served as a standard to which I compared the detections obtained from visual scanning. I used 59 nighttime recordings: 20 in which no anuran species were detected and the remaining contained at least one detection of an anuran species. All of these recordings were then processed using visual scanning.

I then conducted a variety of spectrogram comparisons to select the best specifications for visually scanning audio recordings for anurans (Table 2.1). First, I compared detections from visual scanning to detections from human listening from the same three-minute recordings. I used 10 second spectrogram frames (18 spectrograms for each three-minute recording) because this frame extent is similar to the spectrogram resolution commonly used for auditory analysis. This comparison was intended to inform differences in detection between these two methods of processing recordings. Second, I compared samples of recordings differing in duration using visual scanning. Using 10 second spectrogram frames, two comparisons were made to determine if shorter samples affected the detection of anurans in a recording. I compared detections from the first minute of a recording to detections based on the full three-minute recording. Then, detections for the first 10 seconds of a recording were compared to detections from the first minute. Third, I compared the duration of the spectrogram frames. Spectrograms of the first minute of each recording were made with 10-second and 60-second frame durations, and the number of detections compared between them. The goal was to determine how brief a spectrogram frame could be used while still maintaining detection accuracy with the goal of reducing the volume of images requiring processing. I conducted a final, intermediate comparison of detection patterns between visual analysis of one-minute samples using 30 second spectrogram frames and three-minute listening recordings to test the pairing expected to be most effective.

3 Occupancy models

I used occupancy models to assess if model estimates are affected when calling episodes are missed by visual scanning, and the trade-off between accuracy and efficiency. Occupancy models measure the proportion of sites occupied by a species to determine the probability of occupancy (Mackenzie et al. 2018). These models use detection over multiple surveys to estimate the true occurrence of a species of interest when the species is not detected in each survey. This approach takes into account detectability because if a species is not detected during a sampling event it does not mean it is absent, rather it could simply have gone unrecorded. Occupancy models are generally suitable for anuran surveys. The ARU-based monitoring met the assumptions behind occupancy modelling (Bailey et al. 2014, Mackenzie et al. 2018): 1) the probability of detection was constant across sampling events because I chose recording dates within the species' breeding seasons, 2) site closure was assumed, in that occupancy did not change within a season, due to aggregate breeding behaviours, 3) the probability of occupancy was constant across sites because all stations were selected to be suitable anuran breeding habitat, and 4) surveys were independent of each other because all surveys were conducted on different days. I did not include visit or station covariates in the models because the objective was to compare estimates between methods, not to determine what factors influence occupancy and detection.

I used a second dataset that appropriately constrained the recording selections for occupancy models. The result was 346 randomly selected wetland stations, where an individual ARU is deployed, the maximum stations available that suited the following criteria. Each station had a minimum of four surveys in one year, processed by human listeners. When stations had more than one year of data, the most recent year was chosen. For this dataset, I selected only point counts, a single recording, that occurred during the anuran breeding season, between 15

April and 15 June, in any year from 2013 to 2017. Only nighttime recordings between 00:00 and 04:00 were selected, when the focal anuran species are vocally active. These recordings were processed by the visual scanning method using one-minute point counts made into two 30 second spectrogram frames.

3.1 Single method occupancy models

I ran two basic occupancy models, without any covariates, for the 346 stations, one model using human listening data and the other model using visual scanning data. I compared the probability of occupancy and detectability estimates between the two models to assess how each of the two processing methods performed.

3.2 Combination occupancy models

The goal of the combination models using both types of data was to determine whether there was a ratio of listening to visual scanning data collection that maximized efficiency by reducing the amount of time-intensive listening required to produce smaller standard errors. This hierarchical model is based on the observation confirmation model from Chambert et al. (2015). Chambert et al. (2015) accounted for false positives by using a second, more accurate form of species detection as confirmation data. The model used confirmation data at a subset of sites to account for species being misidentified. I adapted the model of Chambert et al. (2015) to account for false negatives, where species were present and vocalizing at a station but were not detected using the visual scanning approach. False negatives were more important to model than false positives because visual scanning can result in missed calls, as was observed in the first objective. Therefore, in addition to the visual scanning data approach at all stations, I used

human listening data as the confirmation data because, as it is currently the standard for processing acoustic data, it would account for calls that were missed by visual scanning.

In this two-part model, the first part used visual scanning data from all stations, and the second part used listening data from a subset of stations. The model assumes ψ , the probability of occupancy, is equal between the two datasets. The τ parameter was the probability of calling, given that the site was occupied, and was modelled directly from the listening data at the predetermined subset of stations. Detectability was modelled as the probability of detection given calling, ρ . ψ , τ and ρ were assumed to be constant across all sites.

In this framework, detecting the species at site i during survey j is the outcome of three conditionally dependent Bernoulli trials. Occupancy is the outcome of a Bernoulli trial with the probability of success of ψ . In the first part of the model, $z1$ is a binary indicator for occupancy estimated from the visual scanning data so that

$$z1_i \sim \text{Bernoulli}(\psi)$$

at each of the i stations.

$$(\delta[i, t] | z1[i]) \sim \text{Bernoulli}(\tau[i, t] * z1[i])$$

for $t, 1:T$

$$(v[i, t] | \delta [i, t]) \sim \text{Bernoulli}(\rho * \delta)$$

For the subset of stations that use listening data in addition to visual scanning data, I modelled ψ as the outcome of a Bernoulli trial, $z2$, from the subset of stations, J . τ is the probability of calling given the outcome of occupancy, in S sample visits.

$$z2[j] \sim \text{Bernoulli}(\psi)$$

for $j, 1:J$

$$(v[j, s] | z2[j]) \sim \text{Bernoulli}(\tau [i, t] * z2[j])$$

for $s, 1:S$

Detection probability parameters of the combination models, ρ and τ , were multiplied to compare their estimates to the single method models detection parameter, ρ . In this case, ρ is the total probability of detection, including the probability of calling and detectability, which are represented by τ and ρ , respectively, in the combination models.

I varied the number of stations that would include confirmation data in the models by using listening at 14% (50 stations), 50% (173 stations) and 75% (259 stations) of the stations. This allowed me to assess how the model estimates changed with increasing amounts of confirmatory listening data for each species. I ran each combination occupancy model 100 times, taking a random sample of the listening data for the respective number of stations from the original 346 stations for each iteration. The means of these estimates were calculated for each species, for each combination.

3.3 Reduced listening occupancy models

The goal of the reduced listening models was to compare efficiency between listening and visual scanning when equal time was invested in data processing, since listening is more accurate but processing takes longer. I tracked the time it took to visually scan all the recordings and compared that to mean listening processing times. Then, I compared single method occupancy model estimates between visual scanning and human listening at a reduced number of stations, to equalize time of investment in processing. The visual scanning model used data from all 346 stations. The human listening model used data from 50 stations, which would take approximately the same amount of time to process as visually scanning the 346 stations. I ran

this reduced listening occupancy model 100 times with a random sample of 50 stations from the original 346 stations for each iteration. The means of these estimates were calculated for each species.

3.4 Model specifications

I analyzed all the models, both single method and combination models, with a Bayesian approach using program Winbugs (Spiegelhalter et al. 2003; version 1.4.3), with program R (R Core Team 2018; version 1.1.463) through the R2WinBUGS package (Sturtz et al. 2005; version 2.1-21; Appendix 2.3). It ran three chains of 1000 iterations each, with a burn-in of 100 and a thinning rate of 2. Uninformed priors were used with uniform distributions between 0 and 1. I evaluated convergence using the Rhat statistic (Gelman et al. 2004).

Results

Visual scanning comparisons

When comparing listening and visual scanning, visual scanning had fewer detections than listening, missing detections in nine recordings out of 59 (Table 2.2). The missed calls were at calling intensity 1 and usually involved a single calling event. These differences most often arose due to distant calls not being detected or calls being masked by other sounds. There were some observer discrepancies as species indicated by the listeners could not always be detected when I used visual scanning or listened to the recordings for confirmation. These observer identification discrepancies were excluded when evaluating effectiveness of this method.

Visual scanning comparisons of sample durations showed a small increase in detections in recordings based on longer samples. When comparing 10 seconds to one-minute surveys,

there were three recordings where detections were missed in the shorter samples. Five detections were missed in one-minute recordings compared to three-minute recordings.

Longer duration of spectrogram frames resulted in an increase in missed detections. Of the 59 recordings, 60-second spectrogram frames missed calling events in five additional recordings relative to 10-second frames, particularly with western toad detections. In the intermediate comparison of one-minute listening samples against one-minute visual scanning using 30 second spectrograms, there was a difference in detection in six recordings where listening detected calls and visual scanning did not. I determined visual scanning of one-minute surveys using two 30 second spectrogram frame lengths to be the most efficient spectrogram specifications because I could process more recordings, using fewer images, without a large loss of accuracy.

Occupancy models

All occupancy models had Rhat values between 1 – 1.04, which is within the range that supports model convergence.

Single method occupancy models

Estimates from the single method occupancy model for the probability of occupancy and probability of detection were similar between the two processing methods but varied among species (Figures 2.2). The occupancy models for visual scanning estimated the probability of occupancy to be lower for all four species than the estimates for the human listening models. The largest differences in occupancy estimates were for the western toad ($\psi[\text{listen}] = 0.15 \pm 0.03$, $\psi[\text{visual}] = 0.08 \pm 0.02$) and the wood frog ($\psi[\text{listen}] = 0.41 \pm 0.03$, $\psi[\text{visual}] = 0.36 \pm 0.03$), and smaller estimate differences for the boreal chorus frog ($\psi[\text{listen}] = 0.74 \pm 0.02$, $\psi[\text{visual}] =$

0.73±0.02) and the Canadian toad ($\psi[\text{listen}] = 0.26 \pm 0.03$, $\psi[\text{visual}] = 0.25 \pm 0.03$). The probability of detection estimates were the same in both models for the boreal chorus frog ($\rho[\text{listen, visual}] = 0.66 \pm 0.02$) and the Canadian toad ($\rho[\text{listen, visual}] = 0.41 \pm 0.03$) but differed between models for the western toad ($\rho[\text{listen}] = 0.24 \pm 0.05$, $\rho[\text{visual}] = 0.36 \pm 0.06$) and the wood frog ($\rho[\text{listen}] = 0.47 \pm 0.02$, $\rho[\text{visual}] = 0.42 \pm 0.03$). The standard deviations were very similar for both human listening and visual scanning models; no values differed by more than 0.01.

Combination occupancy models

When compared with results of models for a combination of listening data with visual scanning data, the occupancy estimates from the human listening models still had the highest probability of occupancy for all four species (Figure 2.3). Overall, the combination models had lower occupancy estimates than human listening and converged with human listening models as more confirmation data were included. The western toad and wood frog occupancy estimates improved most with increasing amounts of confirmation data in the models. Including confirmation data at all levels in the western toad models ($\psi[14\%] = 0.09 \pm 0.008$, $\psi[50\%] = 0.10 \pm 0.009$, $\psi[75\%] = 0.11 \pm 0.007$) moved estimates from the combination data closer to estimates from the listening model ($\psi[\text{listen}] = 0.15 \pm 0.03$) than from the visual scanning model ($\psi[\text{visual}] = 0.08 \pm 0.02$). For the wood frog, the estimates also approached the listening model estimate ($\psi[\text{listen}] = 0.41 \pm 0.03$) with the inclusion of increasing number of listening stations ($\psi[14\%] = 0.37 \pm 0.010$, $\psi[50\%] = 0.37 \pm 0.010$, $\psi[75\%] = 0.38 \pm 0.008$). All of these estimates were higher than the estimate from the visual scanning model ($\psi[\text{visual}] = 0.36 \pm 0.03$). For the two remaining species, the inclusion of listening data in the models did not result in the same level of change in occupancy estimates. The boreal chorus frog estimates ($\psi[14\%] = 0.73 \pm 0.007$, $\psi[50\%] = 0.73 \pm 0.008$, $\psi[75\%] = 0.73 \pm 0.006$) matched the visual scanning ($\psi[\text{visual}] = 0.73 \pm 0.02$)

estimate and were slightly lower than the human listening estimate ($\psi[\text{listen}] = 0.74 \pm 0.02$). The same pattern was observed for the Canadian toad estimates where combination occupancy estimates ($\psi[14\%] = 0.25 \pm 0.009$, $\psi[50\%] = 0.25 \pm 0.09$, $\psi[75\%] = 0.25 \pm 0.006$) matched results for the visual scanning ($\psi[\text{visual}] = 0.25 \pm 0.03$), and were lower than the human listening, model estimates ($\psi[\text{listen}] = 0.26 \pm 0.03$).

The probability of detection estimates from the combination models also converged on estimates from the human listening model as more confirmation data were included. The western toad detectability estimates were higher than the human listening estimate ($\rho[\text{listen}] = 0.24 \pm 0.05$) and were lowered with the inclusion of more listening data across combination models ($\rho[14\%] = 0.32 \pm 0.018$, $\rho[50\%] = 0.29 \pm 0.020$, $\rho[75\%] = 0.28 \pm 0.013$). The visual scanning estimate was higher than any of the combination models ($\rho[\text{visual}] = 0.36 \pm 0.06$). The wood frog detectability was similar for all combination models ($\rho[14\%] = 0.42 \pm 0.005$, $\rho[50\%] = 0.41 \pm 0.005$, $\rho[75\%] = 0.41 \pm 0.003$) and lower than the human listening ($\rho[\text{listen}] = 0.47 \pm 0.02$) and approximately equal to the visual scanning models ($\rho[\text{visual}] = 0.42 \pm 0.03$). The boreal chorus frog estimates were similar across all models, with the combination models ($\rho[14\%] = 0.65 \pm 0.004$, $\rho[50\%] = 0.65 \pm 0.04$, $\rho[75\%] = 0.65 \pm 0.003$) being slightly lower than the human listening and the visual scanning models, which yielded the same estimate ($\rho[\text{listen, visual}] = 0.66 \pm 0.02$). The Canadian toad combination models were the most similar to the listening and visual scanning estimates ($\rho[\text{listen, visual}] = 0.41 \pm 0.03$) with only slightly lower values ($\rho[14\%] = 0.40 \pm 0.007$, $\rho[50\%] = 0.40 \pm 0.009$, $\rho[75\%] = 0.40 \pm 0.005$).

Reduced listening model

To evaluate the efficiency of visual scanning, I compared its processing time to that of human listening processing times. There were 1384 recordings to process for this study and I

used only the first minute of each recording. Two spectrograms were produced for each recording, a total of 2768 images. Scanning the images took 6 hours and 51 minutes to process, including listening to the recordings when identification confirmation was required. Standard community listening takes an average of two to three times as long to process than the length of the recording (Wimmer et al. 2013, Alquezar and Machado 2015, Rocha et al. 2015). This would result in the listening taking 46 hours 8 minutes to complete; over 6.5 times longer than visual scanning.

It took 9 hours and 42 minutes for SoX to make the spectrograms, however some of this was done with a remote connection which slows the processing speed. This process does not need to be monitored and can run independently so it is not included in the effort comparison.

With visual scanning, more recordings can be processed than with human listening, given the same allotment of time. I ran a reduced listening model with a subset of the stations to determine how lower efficiency of human listening compares to the estimates generated by visually scanning more stations (Figure 2.2). Relative to estimates from the visual scanning models, the reduced listening models overestimated probability of occupancy for all species but detectability estimates were more similar between model types. Standard deviations were larger for all reduced listening estimates. The western toad occupancy estimate ($\psi[\text{reduced}] = 0.27 \pm 0.13$) from reduced listening was larger than for visual scanning ($\psi[\text{visual}] = 0.08 \pm 0.02$) with standard deviations almost seven times as large. Detectability ($\rho[\text{reduced}] = 0.24 \pm 0.15$) for this species had a lower estimate than from the visual scanning model ($\rho[\text{visual}] = 0.36 \pm 0.06$), again with larger standard deviations. Compared to the human listening models, the reduced listening model had larger occupancy estimates ($\psi[\text{listen}] = 0.15 \pm 0.03$) with standard deviations over four times greater and an equal detectability estimate, but with greater standard deviations

(over twice as large). For the wood frog, occupancy ($\psi[\text{reduced}] = 0.42 \pm 0.06$) and detectability ($\rho[\text{reduced}] = 0.46 \pm 0.06$) from the reduced listening estimates were higher than the visual scanning estimates ($\psi[\text{visual}] = 0.36 \pm 0.03$, $\rho[\text{visual}] = 0.42 \pm 0.03$). The estimates were closer to the human listening estimates ($\psi[\text{listen}] = 0.41 \pm 0.03$, $\rho[\text{listen}] = 0.47 \pm 0.02$). The reduced listening standard errors were at least twice as large compared to the visual scanning and human listening standard errors. Based on the reduced listening models, boreal chorus frog had an occupancy estimate ($\psi[\text{reduced}] = 0.74 \pm 0.06$) higher than ($\psi[\text{visual}] = 0.73 \pm 0.02$) and a detectability estimate ($\rho[\text{reduced}] = 0.66 \pm 0.05$) equal to visual scanning models ($\rho[\text{visual}] = 0.66 \pm 0.02$). Reduced listening estimates were also similar to values for the human listening model ($\psi[\text{listen}] = 0.74 \pm 0.02$, $\rho[\text{listen}] = 0.66 \pm 0.02$). Associated standard deviations were three and two times larger for occupancy and detectability reduced listening model, respectively. The Canadian toad probability of occupancy ($\psi[\text{reduced}] = 0.28 \pm 0.06$) from the reduced listening model was overestimated ($\psi[\text{visual}] = 0.25 \pm 0.03$) but detectability ($\rho[\text{reduced}] = 0.41 \pm 0.08$) was estimated equivalently compared to the visual scanning model. Compared to the human listening model, occupancy was again overestimated ($\psi[\text{listen}] = 0.26 \pm 0.03$) and detectability was equivalent. Both estimates had standard deviations at least twice as large estimates from the other two models.

Discussion

This study was initiated to find more efficient methods for processing large amounts of acoustic data for anuran monitoring. Based on the comparison of detections between listening point counts and visual scanning, visually scanning one-minute point counts in two 30 second spectrogram frames was determined to be an effective way to process anuran recording data, based on the spectral signatures and chorusing behaviours of the four anuran species present in

the study area. Thirty-second frames were sufficiently detailed to display spectral signatures of the species effectively but still allow for quick processing. The use of longer spectrogram durations per image produces fewer images for processing, thus more surveys can be conducted with recordings at additional dates and times. Given the chorusing behaviour of the four species, a one-minute point count was found to be as successful at detecting anurans present as a three-minute point count. Investing less time processing each point count, by shortening the length from three minutes to one minute, also contributes to the ability to process more data and conduct more station visits at distinctly different times. Although the visual scanning approach did involve some missed detections, all of them had calling intensities of 1 and were usually single calls. The importance of missing single calling events such as these should be evaluated for each study species and could be especially relevant for rare species.

Visual scanning has been assessed for its effectiveness for recordings of mammals (Rocha et al. 2015), marsupials (Truskinger et al. 2013) and birds (Joshi et al. 2017, Digby et al. 2013, Swiston and Mennill 2009) but, to my knowledge, this is the first study that assesses the method for recordings of anurans. It is also unique in that it compares visual scanning to human listening; most studies regarding the efficacy of visual scanning compare the method to automated recognizers. However, in support of the results of my research, these studies also demonstrated that visual scanning is a promising method for effectively processing acoustic data. It has consistently proven to be equal or more accurate in signal detection than recognizers (Joshi et al. 2017, Digby et al. 2013, Swiston and Mennill 2009, Rocha et al. 2015) and usually requires equal or less time for processing data (Joshi et al. 2017, Digby et al. 2013, Swiston and Mennill 2009).

To determine if the trade-off between quick processing time and missing some detections, that is true of visual scanning, would impact model estimates, I compared occupancy model estimates generated by human listening versus visual scanning. Occupancy estimates were similar between methods but consistently higher when using the listening data. Results for detectability models were more varied across the species. Since I found differences between model estimates, I wanted to determine if visual scanning estimates could be improved by incorporating additional data into models. I used visual scanning data combined with listening data from a subset of stations as confirmation data to account for any false negatives resulting from the visual scanning process. False negatives need to be accounted for because, while visual scanning is a much faster approach to processing audio recordings, there were instances where calling events recorded by listening were missed. By including some listening data, the current benchmark for the truth of acoustic data processing, in models, I hoped to account for missed detections.

Overall, occupancy estimates from the combination models, were improved, i.e. became closer to estimates from human listening models, as more confirmation data were included. This occurred because more false negatives were accounted for and error estimates were decreased. The level of improvement of the combination models varied by species. The boreal chorus frog and Canadian toad models were least improved via the combination approach, whereas the wood frog and western toad models displayed larger improvements. These differences could be due to the acoustic characteristics of these species' calls. The latter two species have less distinct calls than the former two, which have relatively unique call frequencies and call at high amplitudes. The wood frog and western toad calls are less distinguishable making them more likely to be confused with or masked by other sounds, compared to the boreal chorus frog and Canadian

toad. They call at lower amplitude, making them more difficult to detect at a distance or in the presence of other sounds. They also call at lower frequencies, thus calls are easily masked by ambient noise and other animal sounds. In the case of the western toad, there were also fewer instances where a full chorus of calling individuals was recorded. These differences in call characteristics could be a contributing factor for the differences in model performance among species. This could result in calling events that are missed at low calling intensities (Brauer et al. 2016) because when calling with high intensity, the anurans were reliably detected. Thus, the combination models were best at improving detection estimates of the species with the least distinct calls.

The visual scanning method created some variation in the probability of detection estimates. Visually scanning the sample dataset created no false positives, only false negatives. Based on this information, I developed a model to account for the false negatives. However, in the occupancy dataset, false positives did occur, creating heterogeneity in detection probabilities and resulting occupancy estimates that were biased toward lower values (Bailey and Adams 2005). This was observed in all the models compared to the human listening models, across all the species. Low occupancy estimates can be further enhanced when species have low detection probabilities (Bailey and Adams 2005); the western toad illustrates this pattern. Low probability of detection for this species could be due to its rarity relative to the other anurans: it was present at the fewest number of stations and there were fewer individuals based on call intensity data. Full choruses were not recorded and, as discussed, western toad calls are missed during processing due to acoustic characteristics that decrease chances of detection. Additionally, a contributing factor to low occupancy estimates for the western toad could be due to false positive detections, which in turn decreases occupancy estimates as the model accounts for these false

positives. Compared to the calls of the other three species, western toad calls are more difficult to differentiate from the vocalizations of non-anuran species, such as Wilson's Snipe (*Gallinago delicata*) winnowing, Sora (*Porzana carolina*) calls and some shorebird calls.

The combination models were built to account for false negatives, which were considered to be more relevant to the visual scanning method. To adjust for this, the next step for the building of better models is to compare model estimates with and without the false positives to see what their effects. This will inform the causes of the lower occupancy estimates generated by the combination models. If the false positives are a large contributing factor, then the combination models can be improved by accounting for the occurrence of false positives in future iterations of the model.

The assessment of the series of combination models was a valuable exercise for several reasons. They show that combining visual scanning and listening methods can improve estimates, allowing for the efficiency and the accuracy of the different processing methods to be retained. Based on characteristics of calls, the amount of listening required can be adjusted to improve the estimates. For instance, including listening in addition to visual scanning will be most effective for species with less distinct calls. Furthermore, the combination models for the boreal chorus frog and the Canadian toad show little change in the estimates with the inclusion of listening. This suggests that models based on visually scanning alone can be equally as effective as combination models and would increase efficiency by removing the need to listen to recordings. Finally, combination models offer the advantage of achieving better estimates of occupancy with lower levels of uncertainty. With the combination models, you can get separate estimates of τ and ρ , where τ is the probability of calling given occupancy, and ρ is the probability of detection given calling. Similar to my results, a study comparing between manual

and automated detections showed that a mixed approach of acoustic data processing was the most accurate and efficient method (Rocha et al. 2015). In this case, the investigators used automated recognition followed by visual scanning, whereas I used human listening combined with visual scanning.

Effort comparison

Visual scanning offered a much faster processing time than traditional listening methods, which allowed more data to be collected and increased the precision of estimates. Using 30 second spectrogram frames to process one-minute point counts visually was much faster than listening to recordings; visual scanning took about one-seventh of the time required for listening. Reduced processing time allows more data to be collected, with only a small loss to accuracy in detection. When more data are collected by visiting more stations, estimates and associated error are improved. As observed for the reduced listening models, when fewer stations were analyzed, occupancy was often over-estimated and there were higher levels of error associated with both occupancy and detectability estimates. This suggests that estimates from visual scanning, while lower than the human listening, should be more accurate than listening to recordings from fewer stations. This is highlighted by the models for the rarest species, western toad, where reduced listening models overestimated occupancy by over three times. It has been cautioned that high occupancy estimates based on low detection probabilities should be considered carefully (Mackenzie et al. 2002). For this study system, it would be problematic to draw conclusions regarding the distribution of rare species from estimates generated by models from limited listening data, rather it would be beneficial to generate models based on visual scanning.

Recommendations

Overall, I recommend using visual scanning for processing large amounts of bioacoustic data for anurans, particularly for nocturnal species that vocalize in choruses. Visually scanning spectrograms is a fast and standardized method for processing recordings. This method allowed me to identify that one-minute point counts are as effective as three-minute point counts in detecting anuran choruses. It is a relatively accurate method for collecting large amounts of data, increasing the precision of estimates compared to more time-intensive methodologies. Based on the models estimates from my study, even if single calling events are missed, visually scanning more recordings is a more accurate method to assess occupancy than listening to recordings from fewer stations. However, by including some listening data in models based on visual scanning data, I can reach a balance between accuracy and efficiency.

I found for anurans that combining listening data with visual scanning data is most important when modelling rarer species and/or species that are more difficult to detect or identify. In such cases, time invested in listening may be well spent and bring estimates closer to those of the human listening estimates. However, as seen in the reduced listening models, sacrificing inclusion of too many stations in favor of listening to more recordings from fewer stations may inflate model estimates and generate higher levels of error. For species with low occupancy, allocating effort to survey more stations less frequently is recommended versus conducting many visits to fewer stations (Mackenzie et al. 2005). Additionally, prior knowledge of rare species, such as calling behaviours or likely occupancy, will help guide listening strategies and improve study inferences (Mackenzie et al. 2005).

During visual scanning, spectrograms were configured to facilitate visual detection of all four species of interest. When applying this method to other species of anurans, some of the

spectrogram specifications may need to be adjusted to suit other spectral signatures. The spectrogram frame length selected does alter the appearance of the spectral signatures and while calls of the four study species were still discernable in 30-second frames, other minimum time frames may be required for other calling signatures. Users should adjust spectrograms to best suit their species of interest, recognizing that these changes will affect the time investment in scanning as more or fewer images are created.

Visual scanning did miss some vocalizations, either a single calling individual or a single calling episode. In application it would be important to consider the relevance of such calls. Since single calls may change the outcome of model estimates, one would have to decide whether or not to include them, based on research objectives. For example, if anuran breeding is the metric, does a single calling individual indicate the occurrence of breeding? Considering the biology of the study species can improve model building and interpretation.

Application

Anurans are currently a widely studied group of animals because they are used as environmental indicators that are reported to be experiencing high rates of population declines and species extinctions worldwide (Stuart et al. 2004, Adams et al. 2013). As such, there is potential to apply this method to all types of acoustic monitoring initiatives to improve efficiency. The visual scanning method is ideal for bioacoustic datasets that are of large temporal or spatial extents and can also be applied to questions other than occupancy patterns, such as studies concerning phenology, range mapping or population trends. This approach is also suitable for improving and prolonging monitoring programs because it is cost-effective, using only freeware, and time effective, allowing resources to extend further. Learning the programs and processing the data does not require high expertise and it is not computationally demanding

to produce or process the spectrograms, particularly in comparison to automatic species recognizers.

Automated processes for collecting data from audio recordings are sought after to reduce the amount of manual processing required. However, there is still some manual processing required to obtain high quality clips for training computer programs. The visual scanning method offers an efficient way to look through recordings to locate clips of the target species. This property is particularly important for improving automated processing for rare species with low detections rates, which often lack the amount of sound samples needed for good training (Priyadarshani et al. 2018). Also, by processing more audio data, more information can be collected that improves automated systems but can be difficult to obtain, such as sound pressure (dB) (Llusia et al. 2011).

This visual scanning method is particularly functional for anurans because of their calling patterns. A benefit in analyzing nocturnal calling anurans in this study area is that, as has been observed in previous visual scanning approaches (see Truskinger et al. 2013), when few other organisms are vocalizing at this time it reduces overlap of sounds and increases the detectability of anuran calls. Choruses of calling anurans also increases the ability to detect their calls because they vocalize consistently and with a high amplitude. However, this method could still be applicable for other vocalizing species. It will likely be most effective for calls or songs that are not easily masked by other sounds, such as nocturnal species, species that call at relatively unique frequency compared to the sounds of their surroundings or those that vocalize in groups, with high amplitude or high calling rate.

Conclusion

Scanning spectrogram images is a standardized way of visually processing acoustic data. Conducting one-minute point counts was shown to be as effective as the conventional three-minute surveys in detecting anurans calling at higher abundances. In some instances, visually scanning missed single calls. Combination models were developed to account for calls missed by visual scanning. These models were particularly effective in improving occupancy estimates for species with less distinct vocalizations, that had higher rates of missed detections. However, for species with more unique calls, including data from listening in the models produced little improvement, suggesting that visual scanning alone would be sufficient. There is still room for improvement in modelling to address occurrences of false positives. Finally, given the same time constraints, using visual scanning to process more data rather than listening to recordings from fewer stations resulted in more precise occupancy model parameter estimates.

This method can help improve monitoring programs that use acoustic surveys by increasing efficiency of processing and helping solve the problem of processing big data. Additionally, many monitoring programs do not have the means to buy software, invest the time in training or have experts on hand to analyze acoustic data. This method is readily accessible and requires limited training for detecting the target species in spectrograms. It is an accurate and efficient method that is particularly useful for anuran amphibians and has potential to be effective for other vocalizing species, given they have high visual detectability in spectrograms.

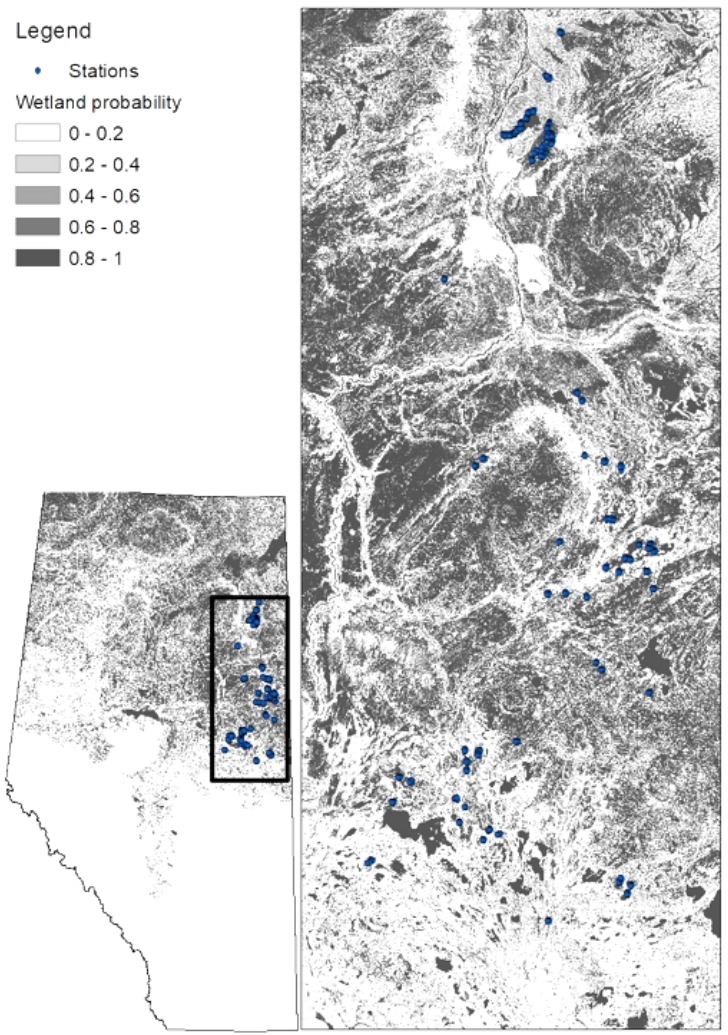


Figure 2.1. Map of northeastern Alberta, Canada indicating where autonomous recording units were deployed at wetlands to record anuran breeding vocalizations, at 346 stations. The base map is the Alberta Biodiversity Monitoring Institute’s wetland probability layer.

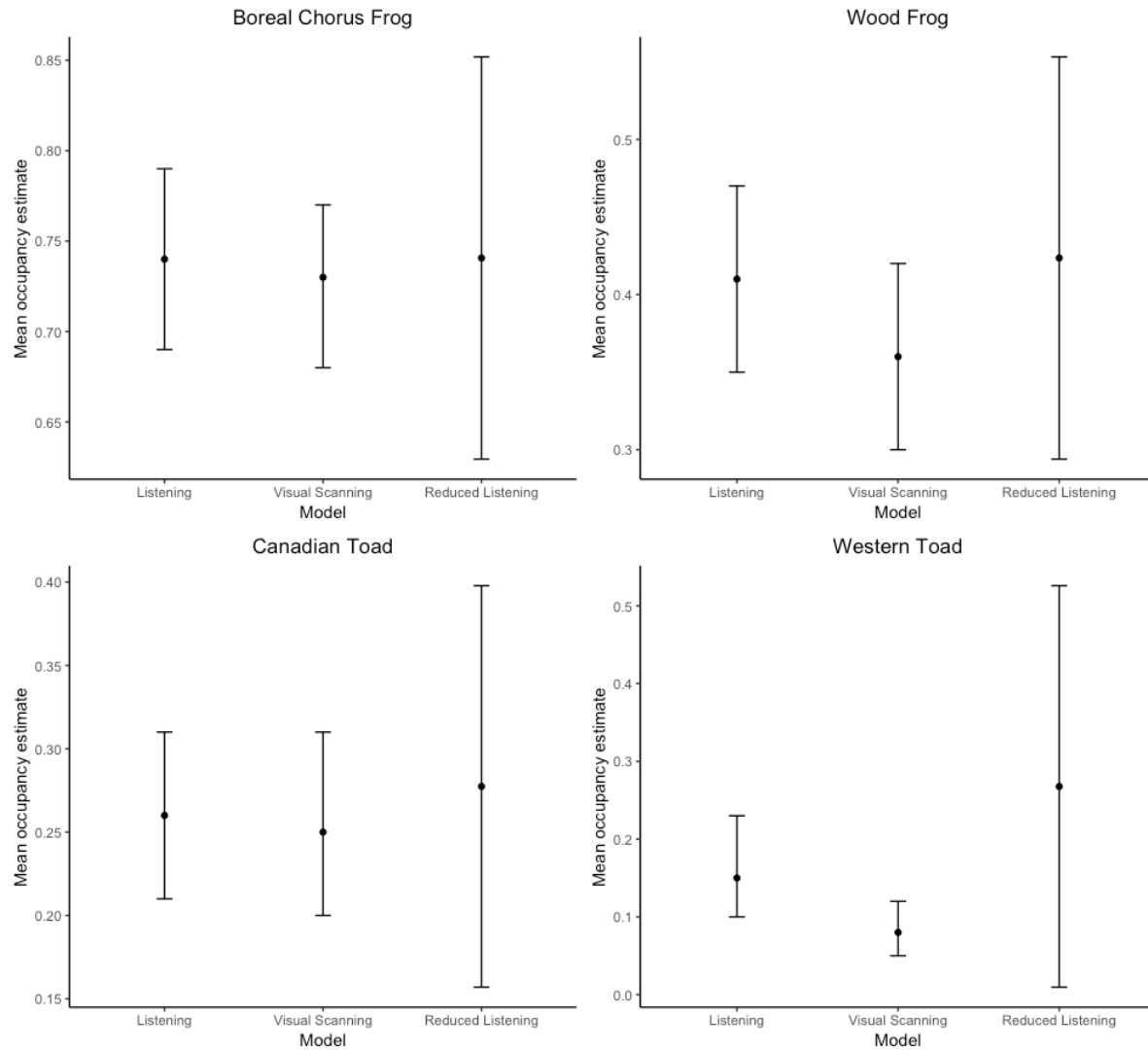


Figure 2.2. Probability of occupancy, ψ , estimates from a series of occupancy models for four anuran species at 346 stations. Data collection was either by human listening to audio recordings or visual scanning of spectrograms. The listening and visual scanning models used data from all 346 stations. To compare time investment relative to visual scanning, the reduced listening model used only 50 stations, listening to which takes approximately the same time as visually scanning the 346 stations used in the other models. 95% credible intervals are presented for the listening and visual scanning models. The reduced listening model was resampled 100 times; the average of all the estimates was taken and the error bars are bootstrapped confidence intervals.

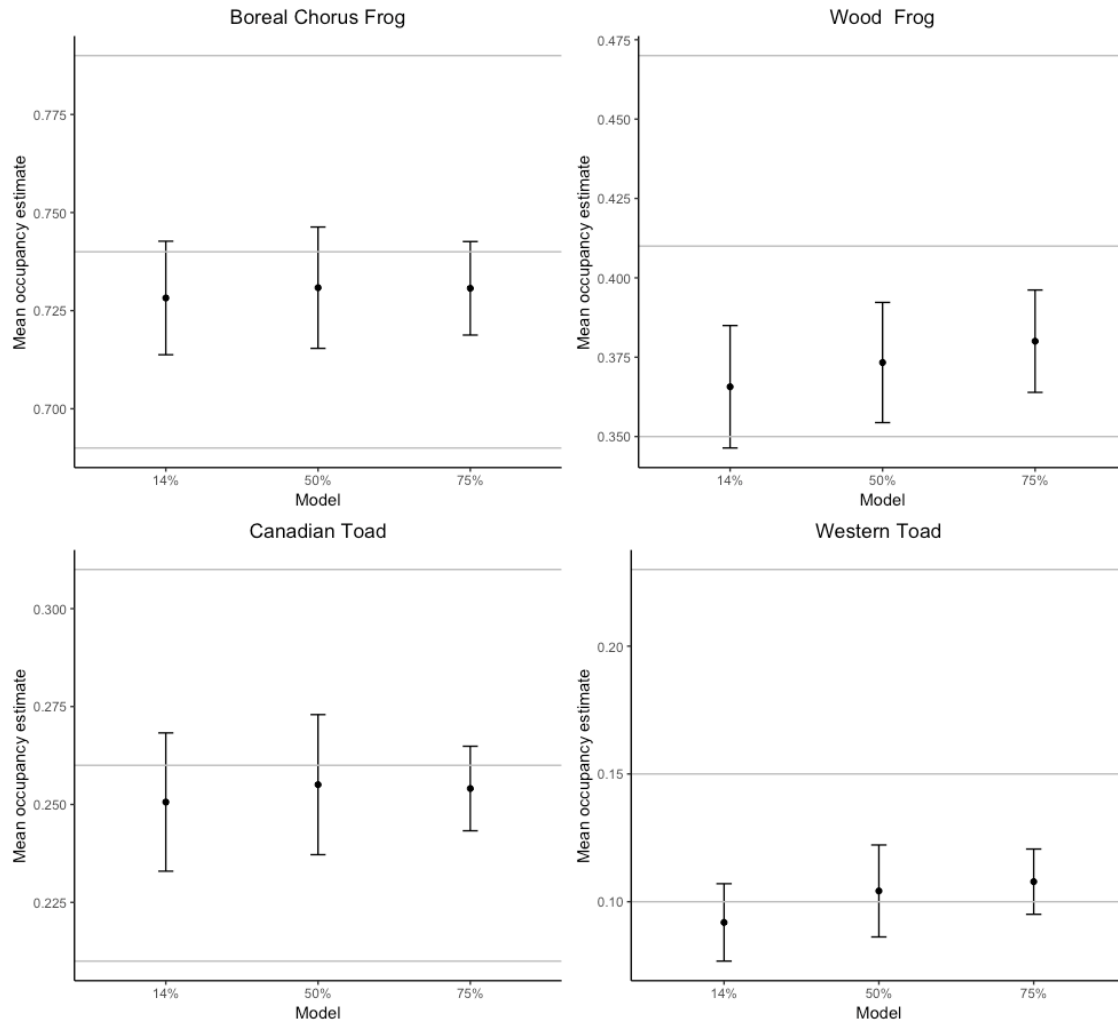


Figure 2.3. Probability of occupancy, ψ , estimates from a series of occupancy models for four anuran species at 346 stations. Data collected from visually scanning spectrograms of audio recordings was used for each station and listening data was included from a sub-set of stations, either 14%, 50% or 75% of stations. The grey lines represent the occupancy estimate and 95% credible intervals from the model using only listening, the standard to which all other models are compared. Each model was resampled 100 times; the average of all the estimates was taken and the error bars are bootstrapped confidence intervals.

Table 2.1. Summary table of comparisons conducted between human listening and visual scanning methods for processing audio recordings from autonomous recording units to detect anuran vocalizations. Spectrograms varied in duration from 10 seconds, 30 seconds and 60 seconds. Surveys varied in length from 10 seconds, 1 minute and 3 minutes.

Method 1	vs.	Method 2	Purpose of comparison
Listening - 3 minutes		Visual scans - 3 minutes	Determining accuracy of visual scanning.
Visual scans - 1 minute		Visual scans - 10 seconds	Determining optimal duration for point counts.
Visual scans - 3 minutes		Visual scans - 1 minute	Determining optimal durations for point counts
Visual scans - 10 second frames		Visual scans - 60 second frames	Determining optimal duration for spectrogram frame.
Listening - 3 minutes		Visual scans - 1 minute, 30 second frames	Testing chosen combination specifications.

Table 2.2. Summary of a series of comparisons of anuran detections between methods of processing audio recordings. The number of recordings in which detections were missed using method 2 relative to method 1 were summed out of the 59 recordings processed. Human listening was the standard against which visual scanning was initially compared. Different configurations of spectrograms for visual scanning were compared to each other. Surveys were 10 seconds, 1 minute and 3 minutes in length. Spectrogram lengths were 10 seconds, 30 seconds and 1 minute in length.

Method 1	Method 2	Recordings with missed detections
Listening - 3 minutes	Visual scans - 3 minutes	9/59 * (15.25 %)
Listening - 1 minute	Visual scans - 1 minute, 30 second frames	6/59 * (10.2 %)
Visual scans - 1 minute	Visual scans - 10 seconds	3/59 (5.1 %)
Visual scans - 3 minutes	Visual scans - 1 minute	5/59 (8.5%)
Visual scans - 10 second frames	Visual scans - 60 second frames	5/59 (8.5%)

*Does not include observer identification discrepancies when a call detected by a listener could not be verified by the investigators.

Chapter 3: Changes in calling behaviour of wood frogs and boreal chorus frogs in response to lunar phase and artificial light pollution

Introduction

Declines in amphibian populations have been documented globally since the 1960's (Houlahan et al. 2000). Higher extinction rates relative to the long-term background average for amphibians are also being documented worldwide (Alroy 2015). These declines have been attributed to a variety of stressors, largely influenced by human activity, such as habitat loss and degradation, facilitation of disease proliferation, competition or predation by introduced species, and climate change (Beebee and Griffiths 2005). The relative importance of stressors on amphibian populations vary regionally and between populations and often consist of multiple threats acting on populations concurrently (Grant et al. 2016, Meredith et al. 2016). In light of these declines, a variety of approaches to conserve populations are being evaluated. Evidence suggests that some conservation strategies can be successful, such as public engagement, managing or modifying roads and ditches, and creating and restoring ponds and wetlands (Smith et al. 2018), but there is not sufficient evidence to draw strong conclusions about the efficacy of these actions (Meredith et al. 2016). Thus, along with conservation efforts, long-term monitoring strategies are needed to ensure that we can detect and effectively reverse declines in amphibian populations (Beebee and Griffiths 2005).

Passive acoustic monitoring is becoming a common approach for monitoring species with distinct vocalizations, as technologies for sampling animal vocalizations are improving, becoming more economical, and are increasingly user friendly. Acoustic surveys using passive monitoring are becoming increasingly common for anuran amphibians (Sugai et al. 2019), which vocalize during the breeding season to communicate with conspecifics. For example, autonomous recording units (ARUs) are passive monitoring devices that are programmed to

record on a daily schedule, in areas where the target organism is thought or known to occur (Shonfield and Bayne 2017). Once the units are retrieved from the field, recordings are processed to identify species based on vocalizations. ARUs more easily facilitate data collection for anurans because non-experts can conduct the field work and field sites do not need to be accessed at night when most anurans are vocally active. Further, the resulting recordings collected by ARUs can be reprocessed to confirm detections or to collect additional data post-survey (Hobson et al. 2002). ARUs promote the collection of large datasets because they can be deployed for long periods of time, many recordings can be collected at a single site and, if multiple units are used, surveys can be conducted at many sites simultaneously. However, processing large volumes of acoustic data is time intensive due to the sheer number of recordings that can be collected with minimal effort in the field and that individual recordings need to be processed by trained observers to identify the focal species. To optimize efforts in processing data, it is imperative to identify the environmental conditions under which anurans are most vocal in order to record or analyze recordings so as to increase the likelihood of detecting species of interest and thus reduce monitoring costs.

Limited studies have examined the effect of light on anuran breeding behaviour. For nocturnal species, the lunar phase is a natural source of variation in light levels at night and amphibian species can have variable responses to moonlight. Moonlight has been shown to have inconsistent effects on anuran behaviour, such as having higher rates of calling or locomotion under different lunar phases, or different lunar light intensities (Grant et al. 2012). In some cases, studies have been conducted in a laboratory setting to simulate lunar phase; the results may or may not reflect how the organisms are responding to variable light conditions in their natural environment (eg: Bonachea and Ryan 2011a).

In addition to variable light conditions due to lunar phase, introduction of artificial anthropogenic light pollution is known to impact nocturnal anuran species (Longcore and Rich 2004, Perry et al. 2008). Anurans using urban wetlands may be exposed to anthropogenic light pollution, at environments such as stormwater ponds, which can be important for urban anurans to complete their reproductive cycle (Brand and Snodgrass 2010). Anurans that are adapted to dark conditions (Cornell and Hailman 1984, Buchanan 1993) may be affected by variation in light conditions at night, especially with the introduction of artificial light, which is generally of higher light intensity than moonlight (Gaston and Bennie 2014). In anuran studies, artificial light has most often been tested experimentally using temporary sources of light, such as flashlights or spotlights, which do not accurately reflect actual light pollution conditions on the landscape, and thus it is difficult to draw conclusions from such studies as to how real-world light pollution affects anuran behavior. Understanding how calling behaviour is affected by environmental factors such as light is important because calling is often used as a metric in studies of population size or site occupancy. If chronic light pollution does affect calling behaviour, it should be taken into account when using calling as a proxy for presence or abundance.

To address the lack of understanding of the effects of variable light in a natural setting on anuran amphibians, this study aims to explore the effects of natural and artificial light at night on the calling behaviour of two frogs species breeding in northern Alberta, Canada, the boreal chorus frog (*Pseudacris maculata*) and the wood frog (*Lithobates sylvaticus*). The first objective of my study was to determine if acoustic monitoring of these two species can be improved by incorporating the effects of natural and artificial light at night on their calling patterns. By building general knowledge about the calling behaviour of these understudied species, I hope to direct acoustic monitoring effort towards locations and times that these species are more likely to

be vocally active. To accomplish this objective, I compared the occurrence of calling under new and full moon conditions, and at sites with and without anthropogenic light pollution.

My second objective was to conduct a literature search to review published studies that document patterns the effects of light on anuran calling behaviour. I also wanted to investigate whether other breeding behaviours, such as migration, oviposition and amplexus, are affected by light, in the event that calling behaviour is not the best metric for assessing the effect of light on anurans. I evaluated papers that investigated the effect of lunar phase and light pollution on breeding behaviours of anurans and compared these results to my findings.

Three hypotheses have been proposed concerning mechanisms behind the effects of light on anuran breeding calling behaviour. First, “the predation hypothesis”, is based primarily on the behaviour of tropical anurans predated by bats, such as *Similisca sila* predation by *Trachops cirrhosus* in Panama. It yields two paradoxical predictions. One suggests that anurans are more active under high light conditions because they can better detect the presence of their predators (Tuttle and Ryan 1982). The second prediction is that anurans are more active under low light conditions in order to avoid detection by predators that rely on vision for foraging (Rand et al. 1997; Bonachea and Ryan 2011b; Da Silva Nunes 1988). The second, “synchronization hypothesis”, proposes that anurans breeding in aggregations may use the lunar phase as a cue to synchronize breeding and ensure appropriate arrival times of individuals on breeding sites (Grant et al. 2009). The third, “visibility hypothesis, proposes that increasing light levels assist individuals in visually finding and assessing mates (Onorati and Vignoli 2017). Therefore, increased breeding activity under higher light conditions might result in more calling.

Boreal chorus frogs and wood frogs, the focus of my study, may not have the same predation pressures as experienced by tropical anuran species. While predation risk for anurans

in the boreal forest is not well known, the potential predators – snakes, mammals, birds, fishes – are different and fewer than those of the previous studies and therefore anti-predator behaviours may also be different. Of these potential predators, few species are truly nocturnal and so may be less likely to affect calling behaviour of anurans. For example, known predators of the closely related western chorus frog (*Pseudacris triseriata*) are snakes (eg: *Thamnophis radix*, *T. sirtalis*; Whitaker 1971). Snake predators of the boreal chorus in northern Alberta (*T. sirtalis*) as largely diurnal, due to their thermoregulatory requirements, and the need for frogs to adopt anti-predator behaviour at night is likely lower than during the day. Similarly, a cue for breeding synchronization may not be necessary, since the breeding season at high latitudes is very short, so emergence and the onset of breeding may occur as soon as the weather conditions, such as appropriate temperatures, are occurring (Whitaker 1971). As such, I propose that in this study system light has the strongest effect on mate selection. For example, wood frogs may use colour for sex recognition of conspecifics and mate choice can be based on size (Banta 1914), both of which could be facilitated by light conditions. Based on the results of other studies, I hypothesized that light affects the breeding behaviour of boreal chorus frog and wood frog, I expected there to be relationships between calling activity and light conditions. I predict that there will be a higher probability of calling under brighter conditions, such as occur under the full moon and in areas with light pollution. Under artificially lit conditions, I expected higher calling intensities during the new moon phases relative to sites with no light pollution, as light pollution masks lunar cues, creating a condition known as “the perpetual full moon” (Longcore and Rich 2004). If the hypothesized patterns do exist, acoustic monitoring can take advantage of these behaviours and improve detection by conducting surveys under optimal light conditions.

Methods

Study location and acoustic data collection

To investigate the effects of variable light condition on boreal chorus frog and wood frog vocal behaviour, this study was conducted at wetlands in northern Alberta, Canada. The study area is in the northern aspen parkland and the boreal forest, in both urban and natural wetlands. The urban wetlands were a combination of stormwater ponds in the cities and more natural wetlands in the smaller urban centers. In the natural areas, wetland complexes occur within a mosaic of mixed stand or black spruce (*Picea mariana*) forests (Norton et al. 2000). The main industries in the area are oil and gas and forestry. Boreal chorus frog and wood frog breed in aggregation in the spring, with wood frog aggregations occurring earlier than the boreal chorus frog. Collectively, calling typically begins in April or early May with some individuals detected into late June or early July. This behavior results in choruses of calling individuals around and within wetlands.

ARUs were programed to record between April and July, 2011 through 2013, and in 2018, with a minimum deployment length of 18 days. Acoustic activity was recorded using the Wildlife Acoustic Song Meter units, models SM2 and SM4 (Wildlife Acoustics Inc., Maynard, MA, USA). ARUs were deployed in or adjacent to wetlands at 1.5 meters high and record with a sampling rate of 44.1 kHz (Lankau 2015). They were programed to record multiple times per day. Each station had a variable deployment length because ARUs were deployed for a variety of objectives. From these ARUs, recordings were selected that occurred at midnight (0000h).

To obtain both of the light treatments, artificial and natural light, I first had to select stations in the presence and absence of light pollution (Figure 3.1). Then, of the recordings from these stations, I selected recordings that occurred during the full moon or new moon phases. The

levels of illuminance for the light treatments are variable (Gaston et al. 2013). Under a clear sky, light from the moon can vary from 0.01 lux in a quarter moon to 0.3 lux in a full moon. Light from urban centers can be between 5-15 lux from street lighting.

First, stations selected for wetlands in the presence of light pollution were located primarily in Edmonton (53.544389 x -113.4909267, 15 stations) and Fort McMurray (56.7263796 x -111.3803407, 8 stations), with some surveying done in the towns of Wabasca (56.0144698 x -113.8215527, 4 stations) and High Level (58.516354 x -117.13516, 2 stations). Stations in the absence of light pollution were paired latitudinally with light-polluted stations. Overall, there was a total of 58 stations. Twenty-nine stations were in areas of light pollution, adjacent to sources of artificial light in these cities. The remaining 29 stations were in areas free of direct artificial light sources, including Elk Island National Park, part of a dark sky preserve east of Edmonton. Second, recordings were processed for analysis if they occurred either during a new moon (when the moon was less than 10% illuminated), or during a full moon (when the moon was over 90% illuminated). Lunar illumination and lunar position data were obtained from the *oce* package (Kelley and Richards 2018) in R (R Core Team 2018; version 1.1.463). Of the full moon midnight recordings, only those that occurred when the moon was above the horizon were selected, ensuring that the moon would be visible when the ARUs were recording. Individual ARUs had variable deployment lengths, with a minimum deployment of 18 days, and all available recordings during the described period were processed if they met the illumination requirements.

Acoustic data processing

To detect patterns in wood frog and boreal chorus frog vocalizations within the study area, audio recordings were processed by visually scanning spectrograms of the recordings to

identify and quantify vocal activity of the focal species. Spectrograms were created with the program Sound eXchange (SoX; Bagwell 2014), using a Discrete Fourier Transform algorithm, with a Hann window. The first minute of each recording was converted into two 30-second long spectrograms, to increase scanning efficiency. These images were visually scanned for the calling patterns of both species simultaneously. In each recording, vocal activity was considered to represent breeding behaviour in the form of a calling chorus when I detected several individuals calling (i.e., vocalizations were overlapping): these observations were assigned a score of 1. Vocal activity that consisted of one or a few individuals calling (i.e., calls did not overlap) was not considered to represent breeding activity and was assigned a score of 0, in addition to the times where no calling was observed.

Because the objective of the study was to quantify patterns of breeding call production under different light conditions, and not to quantify the absolute presence or absence of the study species, only those stations where boreal chorus frog or wood frog were detected were used in the model to determine changes in calling behaviour. By only using stations with known species presence and assigning a binary code for the occurrence of breeding choruses, I controlled for the abundance of individuals calling. Boreal chorus frogs were detected at each of the 58 stations sampled. Wood frogs were detected at approximately half of the stations with light pollution, so I used these available stations for light pollution assessments and matched them to unlit controls by latitude, giving a total of 30 stations for the wood frog analysis. The reason the wood frog was detected at fewer stations could be due to ARU deployments missing the breeding period. In two of the four years studied, the earliest ARU deployments occurred at the end of April (2011 and 2013).

Analysis

Logistic regression (lme4 package; Bates et al. 2015) was used to model the presence and absence of breeding vocalizations of the boreal chorus frog and the wood frog in response to lunar phase and the presence of light pollution. Breeding choruses were modelled as a binary response variable, as described above. The light predictors, lunar phase and light pollution, were classified as full moon or new moon and presence or absence of light pollution. Weather data were used as covariates to control for seasonal behavioural responses that occur partly in response to environmental factors, such as temperature and rainfall, which affect ectotherm activity (eg: Canavero et al. 2008). Weather data were obtained from Environment Canada (Government of Canada 2019; weathercan package; LaZerte and Albers 2018) or Government of Alberta (Alberta Agriculture and Forestry 2019) weather stations, based on proximity to the ARU station. A correlation test was conducted between temperature, relative humidity, day of year and photoperiod to determine which weather covariates to include in the models. Temperature and photoperiod had a correlation value higher than 0.7 with day of year. Therefore, day of year and relative humidity were retained in the models to represent seasonality. To control for differences in calling behaviour in the different sampling years and at the different sampling stations, year and station were included as random effects in the models.

A single model was created for each species that included all the variables considered to be important in affecting calling behaviour. These were the global models and included both light treatments as predictors, relative humidity, and day of year as covariates, and station and year as random effects using random intercepts. An interaction term between lunar phase and light pollution was also included. Day of year was scaled to allow for model convergence. Within the global model, I conducted model selection by sub-setting the global models using the

dredge function and using AIC for ranking the sub-set models. Competitive models were those less than two delta AIC values apart. The dredge function in R (MuMIn package; Bartón 2018) allowed me to estimate an importance function (MuMIn package; Bartón 2018), which was used to determine the relative importance of each variable. This function sums Akaike weights from all the sub-set models and outputs the relative importance of each variable. Predictive probability plots were modelled using the global models, to show the effects of lunar phase and light pollution.

Literature search

I conducted a systematic literature search to determine how light has been shown to affect calling behaviour and other breeding behaviours in anurans. Scopus and Web of Science search engines were used for the search. The search included all anuran genus names, 452 genera, and a series of terms for lunar phase and light pollution (Appendix 3.1). The papers retained were those that pertained to anuran breeding behaviour and examined either lunar phase or artificial light. The first pass through papers uncovered by the search engine involved reading the titles and abstracts to determine suitability for the review. In the second pass, I read the whole paper to collect the relevant information and conclude whether would be included in the final reporting (Appendix 3.2). I recorded details about the paper, the species in the study, the treatment types, the response(s) measured and the final results. I did not include papers that were conducted purely in a laboratory setting.

Results

Boreal chorus frog

The predictive probability plots from the boreal chorus frog global model showed that there was an average probability of calling of approximately 50% (Figure 3.2). Overall, boreal chorus frogs were less likely to vocalize when there was more light (Table 3.1). Calling was less likely during the full moon than under the new moon, although the difference was small. Boreal chorus frogs were also less likely to call at stations in areas with light pollution than at unlit stations; the light pollution treatment had a larger effect size than lunar phase. There also was an interaction between lunar phase and light pollution. The difference between the probability of calling in each light pollution condition was greater when the moon was new than when it was full. However, despite these trends, the 95% confidence intervals were overlapping for each of the four light treatments.

During the model selection process, three models were less than two delta AIC apart. The top model for the boreal chorus frog included light pollution, day of year and relative humidity, in order of decreasing effect size. Boreal chorus frogs were less likely to call when artificial light was present, later in the season and as relative humidity increased. The second ranked model was the global model, including all the variables. The third model only included the seasonality covariates of relative humidity and day of year. Across all the sub-set models, the most important variables were relative humidity and day of year (Appendix 3.3). Light pollution was the next variable of importance, followed by lunar phase and the interaction term.

Wood frog

Wood frogs had higher average probabilities of calling than the boreal chorus frog, at 95% (Figure 3.3). The global model showed a decrease in calling activity in the presence of light pollution but an increase in calling under a full moon, with light pollution having a stronger effect (Table 3.2). There was also an interaction between light pollution and lunar phase: under a full moon, light pollution had little effect on calling probabilities but under a new moon wood frogs were less likely to call at artificially lit stations. The 95% confidence intervals were also overlapping for each of the light treatments in this model.

The global model was the best fit and all other models differed by more than two AIC units. The global model included all the variables: day of year, the interaction term between light treatments, light pollution, lunar phase and relative humidity, in order of decreasing effect size. The probability of calling decreased in the presence of light pollution, later in the season and with higher relative humidity, but increased with a full moon. The interaction term had the biggest effect size. Across all the sub-set models, day of year and lunar phase were the most important variables, followed by relative humidity, light pollution and the interaction term (Appendix 3.3). The relative importance of the variables were greater in the wood frog model than in the boreal chorus frog model.

Literature review

I reviewed the literature for studies that have directly tested the effects of lunar phase and light pollution on the breeding behaviour of anuran amphibians (Table 3.3). To compare my results to the literature, I first summarized the effects of the different light treatments on calling behaviour of studies conducted in the field. Six studies looked at the effects of moonlight on a

total of 24 anuran species and three studies looked at the effects of artificial light on total of 8 anuran species. Generally, the anuran species studied called less when their environment was brighter, under full moon or light pollution. Sixteen of 24 species called less under a full moon, 3/24 called more and 5/24 were unaffected by moonlight conditions. The artificial light studies found that 7/8 species called less under lit conditions and 1/8 species called more frequently when it was brighter. However, this review did not identify any studies that looked at the effects of light pollution on calling; instead, researchers introduced artificial light sources through flashlights or spotlights.

Next, I assessed how other breeding behaviours were affected by variation of in light levels; the majority of studies only examined moonlight. The breeding behaviours examined were arrival to i) breeding grounds, ii) amplexus events and iii) oviposition or spawning. These behaviours showed less of a pattern in response to light conditions. For timing of arrival, half of 10 species migrated, emerged or arrived at breeding grounds under bright moon conditions. Nine of the 20 species' arrival movements were not affected by light and 1/20 link migration behaviour most to darker conditions. Fewer studies examined occurrence of amplexus; 1/3 species had lower rates of amplexus when it was brighter and 2/3 species had higher amplexus rates when it was brighter. With respect to oviposition and spawning, 9/23 species laid eggs more frequently in darker conditions, 6/23 species in brighter conditions and there was no effect of light on 8/23 species. One study was conducted that used light pollution as a predictor and found that there was no effect of artificial light on oviposition.

Discussion

I expected that there would be observable patterns in frog calling activity in response to light conditions. This was based on the hypothesis that light would facilitate mate finding and I

would observe higher breeding activity, calling in this case, under brighter conditions. Significant responses were not seen in either species when to changes in light conditions connected to the lunar phase or light pollution from urban centers. The light models for each species produced results with small effect sizes for both light treatments with largely overlapping confidence intervals. This suggests that the trends that did exist were not very strong. In northern Alberta, there is seasonal variation in day length that these frogs could be well adapted to and, as a result, do not rely on light for facilitating mating. There is also potential that mate selection does not rely on visual cues and therefore, is not affected by light. For example, with wood frogs, mate selection by males has been shown to be based on male-male competition (Berven 1981) and so visual selection may not be as important of a factor. However, males do select larger females to mate with (Berven 1981), which could be visually or physically mediated.

According to my results, the difference in light at night between the new moon and the full moon has only a small effect on the probability of calling for the two frog species studied. These species both have northern distributions, which is associated with seasonal weather pressures on the period available for breeding. The breeding season is short relative to other regions and the cold weather is a constraint on the timing of the initiation of breeding and completion of metamorphosis. Therefore, frogs have a limited window of opportunity to mate and may not be as selective with respect to other environmental factors, such as light at night. As mentioned above, the lack of response to light could also be due to the variable photoperiod in the north; anurans are already habituated to seasonal changes in photoperiod that results in long periods of daylight and reduced periods of darkness during the breeding season.

The probability of calling tended to be lower at stations with light pollution than those without. Furthermore, the difference between calling at stations with and without light pollution

was larger under a new moon. In contrast to the light environment under a new moon, the intensity of light pollution may be perceived as greater and cause reduced calling. Frogs may be accustomed to overall changes in environmental brightness, but the high contrast caused by bright light pollution conditions on a dark night could reach a threshold where calling is affected. This notion could be tested by conducting a test under a gradient of light intensity, rather than just presence or absence of light. However, because the study species do not exhibit strong differences in calling in response to variation in natural light, they may not be responding to differences in artificial light per se but rather some other anthropogenic factor that is present at these urban sites. It has been suggested that multimodal anthropogenic pollution, for example, light and noise, could have additive effects (Halfwerk and Slabbekoorn 2015). For instance, hermit crab (*Coenobita clypeatus*) anti-predator behaviour is most impacted when noise and light are combined (Chan et al. 2010). Another study showed interactive effects of light and noise on frog-biting midges (*Corethrella* spp.). With high noise, midge abundance was always low but at low noise, abundance was lower in higher light (McMahon et al. 2017). Because these results do not indicate a strong direct effect of light pollution but show potential for an underlying effect of urban locations on calling behaviour, it is important to determine if such interactions exist. It would be important to consider differences in urban wetlands, such as those that are more natural versus stormwater ponds.

Probability of calling decreased as day of year and humidity increased. This response to the day of year is expected, as calling is less likely to occur later in the season as breeding is completed. However, the response to relative humidity is less intuitive as calling often increases in wetter conditions (eg: Bellis 1962). However, in this area, relative humidity increases later in the season. The decrease in probability of calling could be a response to seasonality rather than

relative humidity itself. With a correlation factor between day of year and relative humidity of 0.44, there is only a moderate degree of collinearity. The fact that the boreal chorus frog and the wood frog have different phenologies could explain the differences in the magnitude of their responses to relative humidity, as relative humidity changes with season.

To compare these results to the existing literature, I reviewed previous studies that directly assessed the effects of moonlight and artificial light on anuran breeding behaviours. The results from the two species in my study were not consistent with the calling behaviour studies from the literature. My results show little effect of moonlight while the literature indicates a trend of less calling in brighter moonlit conditions. However, these studies primarily attributed the changes in calling behaviour to avoidance of bat predators, whereas brighter conditions increased the perceived risk of predation. Variation in light may not similarly affect calling behaviour of the anurans in this study because their perceived risk differs in response to a different suite of predators, making calling responses attributed to this mechanism difficult to compare.

Superficially, my results appear to agree with other light pollution studies in the literature because they also show a reduction in calling under artificially lit conditions. However, the other studies introduced an acute and discrete light source to the system, so the observed changes in behaviour was likely in response to a novel stimulus that requires habituation (Blumstein 2016). These manipulations differed from the chronic presence of light pollution in this study, which anurans could already be habituated to, making behavioural responses incomparable. Overall, calling behaviour is the most frequently studied anuran breeding behaviour with respect to light and appears to be the most consistent in observed responses, which are negative, across the reviewed studies. Given this pattern in the literature, it is possible that light negatively affects

calling behaviour in boreal chorus frogs and wood frogs but more studies looking at both lunar phase and light pollution need to be conducted.

This review assessed whether breeding behaviours other than calling are impacted by light, in case data collected with ARUs are ineffective in determining the effects of light on anuran breeding. Other breeding behaviours – arrival to breeding grounds, amplexus and oviposition/spawning – examined in the literature were less consistent in their responses to moonlight than calling. Amplexus and oviposition or spawning behaviours were not consistently affected by light and displayed similar number of cases of altered behaviour in response to either brighter or darker conditions, as well as examples lacking observed effects. Examples in the literature suggest that arrival at breeding sites does not generally occur under low versus high light levels, and in many cases migrations to wetlands were unaffected by light. It is even more unclear whether light pollution is impacting these breeding behaviours because only one study was found that compared sites in urban versus rural areas to examine effects of light pollution. This study found a reduction in clutches in urban areas, but this was not well explained by light (*Engystomops pustulosus*, Panama City; McMahon et al. 2017). This study is comparable to ours because it also suggests a trend in reduced breeding behaviour in urban locations that is not well explained by light pollution. To my knowledge, this study and the aforementioned study are novel in testing the effects of light pollution on urban anurans. Together they suggest that, while there is not a strong effect of light, looking at discrete measures of fitness in response to urban environments could be the next step for understanding the effects of light pollution on urban anurans.

In terms of monitoring boreal chorus frog and wood frog populations through call surveys, strategizing survey days based on the lunar phase is not likely to increase the likelihood

of detecting these species because the lunar phase has only a small effect on calling behaviour. While a stronger effect of light pollution was observed, the trend is still weak. As such, it is not likely that the probability of detecting these anuran species will change under different light conditions. Despite the fact that calling behaviour may not be the ideal metric for revealing the potential effects of light, I cannot completely disregard the possibility that light has consequences on the breeding success of these species, as it has been observed that there are lower abundances of egg masses, larvae, metamorphs and adults in urban wetlands (Scheffers and Paszkowski 2013, 2016). The importance ranking of the variables in the models suggest that light levels still could be relevant to these species. Light pollution was the third most important variable in the model for the boreal chorus frog and lunar phase was the second most important variable for the wood frog. Additionally, results of the literature review show that there is potential for some other breeding behaviours to be affected by light.

Further studies are needed to understand how different breeding behaviours are affected by light. Interactions between light and other factors could also reveal patterns that could, in turn, affect anuran behaviour. For example, light has been shown to decrease the presence of parasites because there is a higher predation risk in brighter conditions (McMahon et al. 2017). Identifying patterns such as these may help highlight factors contributing to population trends. Such studies could also determine if using light pollution as a proxy for the effects of urbanization is appropriate (eg: Perry et al. 2008).

Cloud cover was not considered in this study and could contribute to the variation in the observed results. It has been shown that clouds have variable effects on moonlight. An interaction between moonlight and cloud cover was found to explain variation in the probability of detection for two of ten anuran species in a study in Maryland, United States (Weir et al.

2005). Similarly, when the calling and mating intensities of an anuran were compared in response to variation in lunar illumination, no effect was found when light levels were corrected for cloud cover (Underhill and Hobel 2018). However, it has also been shown that clouds can have a significant effect on illumination only during certain phases of the moon (Onorati and Vignoli 2017). When the moon is brighter, clouds have a significant effect but there is no effect of clouds when the moon is in the three-quarter or new moon phases. In this study, multiple surveys were conducted for each full moon period, when the moon was >90% illuminated to increase the probability of sampling in both cloudy and clear conditions. It is possible that lunar cues could be masked if there were a lot of cloudy days, increasing variability in these results. However, the intensity of light pollution would be unchanged or increased by clouds as they can exacerbate skyglow through reflection (Kyba et al. 2011). Therefore, I maintain that the presence of clouds is negligible for the light treatments I tested but could lead to the large variation associated with these results.

Conclusion

This research contributes to the relatively poorly studied topic of how light affects anuran behaviour. Alone, these models only show small effects of light on calling behaviour and that it is not necessary to account for light as a factor when selecting recordings for analysis. Calling behaviour may not be a good indication of the effects of light on anurans, however, light could still be an important factor in affecting anuran behaviour. In combination with the reviewed literature, I show that natural light could still have a role in other breeding behaviours. This means there is potential for light pollution to disrupt these natural patterns however, further research about chronic light pollution is needed. By understanding anuran responses to variation

in their environment, monitoring practices and subsequent decision making can become more accurate by accounting for factors that affect behaviour.

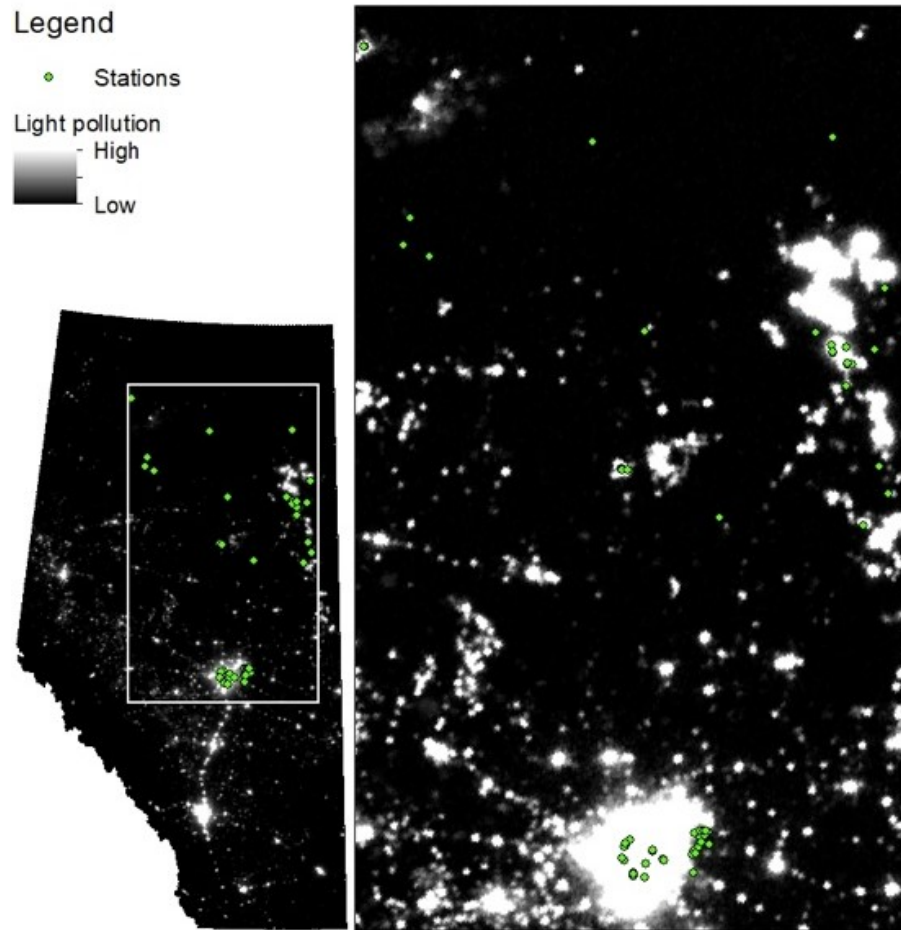


Figure 3.1. Map of northern Alberta, Canada indicating where autonomous recording units were deployed at wetlands to record anuran breeding vocalizations. Fifty-eight locations were chosen to be within or outside areas of anthropogenic light pollution, indicated by a gradient from white to black, respectively. The base map was created by NOAA's National Geophysical Data Center.

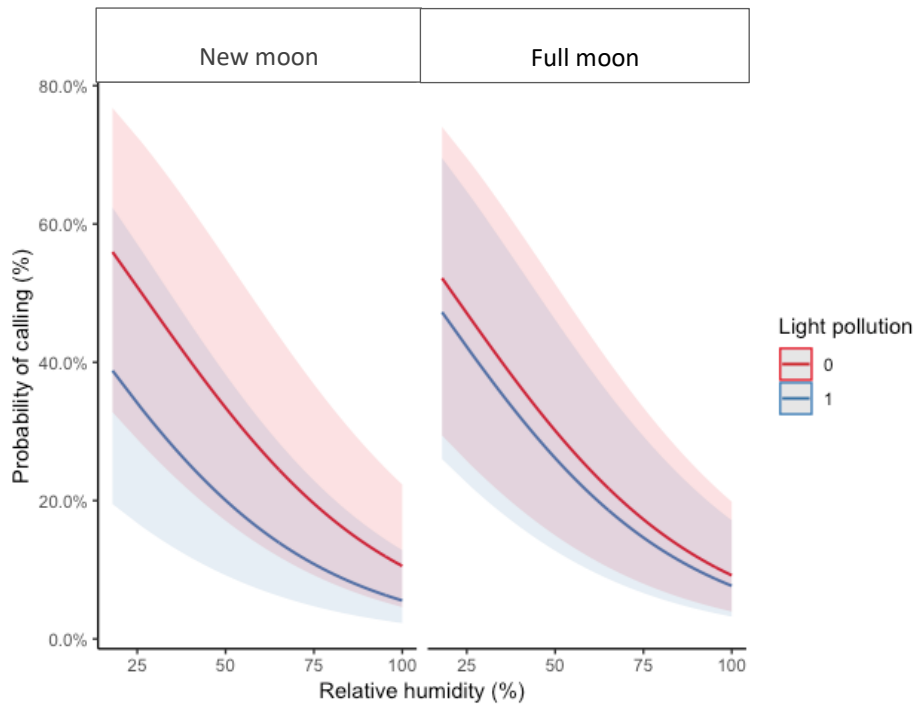


Figure 3.2. The probability of boreal chorus frogs (*Pseudacris maculata*) calling as a function of percent relative humidity. The left panel represents the new moon phase, <10% lunar illumination, and the right represents the full moon phase, >90% lunar illumination. The absence of light pollution is represented by the red lines and presence of light pollution is the blue line. This is the global model used in the logistic regression that also includes all of the variables: relative humidity and day of year as covariates, with lunar phase and light pollution as predictors. Shading represents 95% confidence intervals.

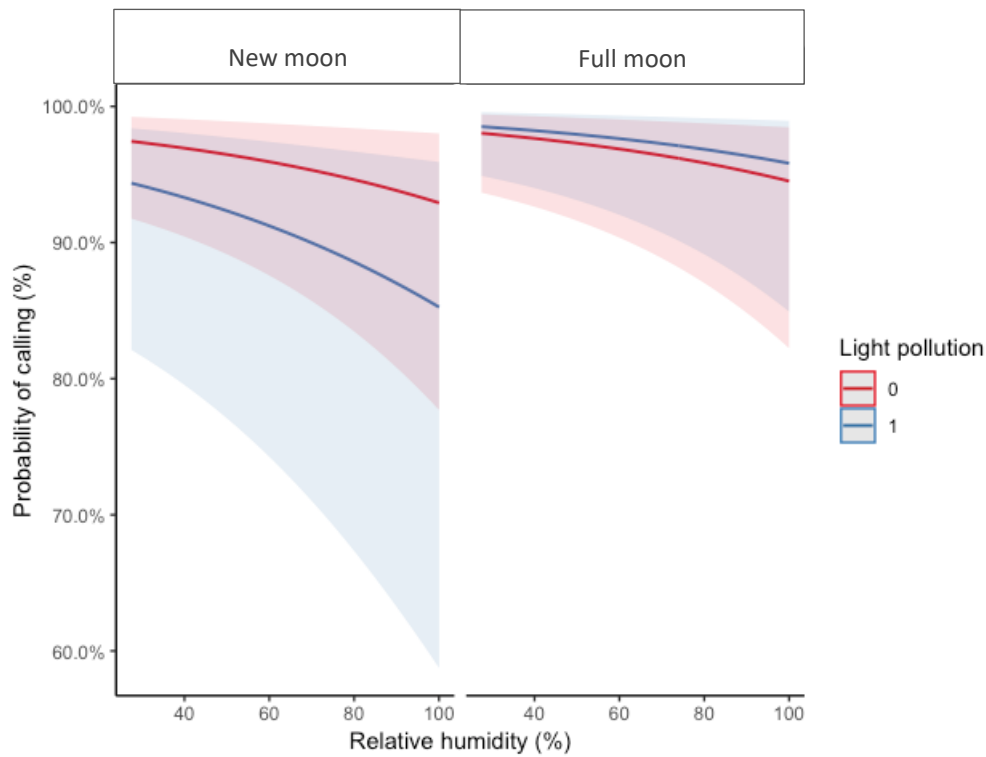


Figure 3.3. The probability of wood frogs (*Lythobates sylvaticus*) calling as a function of percent relative humidity. The left panel represents the new moon phase, <10% lunar illumination, and the right represents the full moon phase, >90% lunar illumination. The absence of light pollution is represented by the red lines and presence of light pollution is the blue line. This is the global model used in the logistic regression that also includes all of the variables: relative humidity and day of year as covariates, with lunar phase and light pollution as predictors. Shading represents 95% confidence intervals.

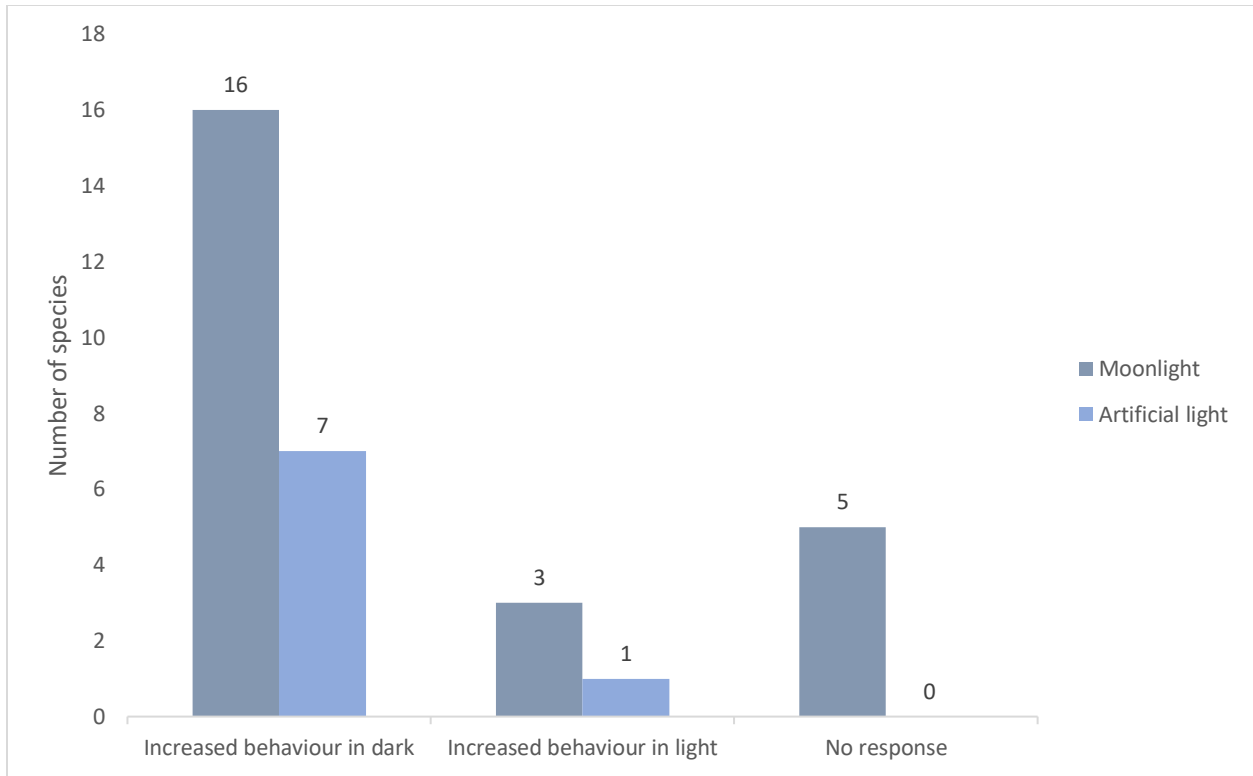


Figure 3.4. Summary of literature review showing anuran amphibian calling behaviour responses to variation in moonlight and artificial light conditions. The number of species that increase calling behaviour in dark or light conditions, or have no response, are indicated on the x-axis.

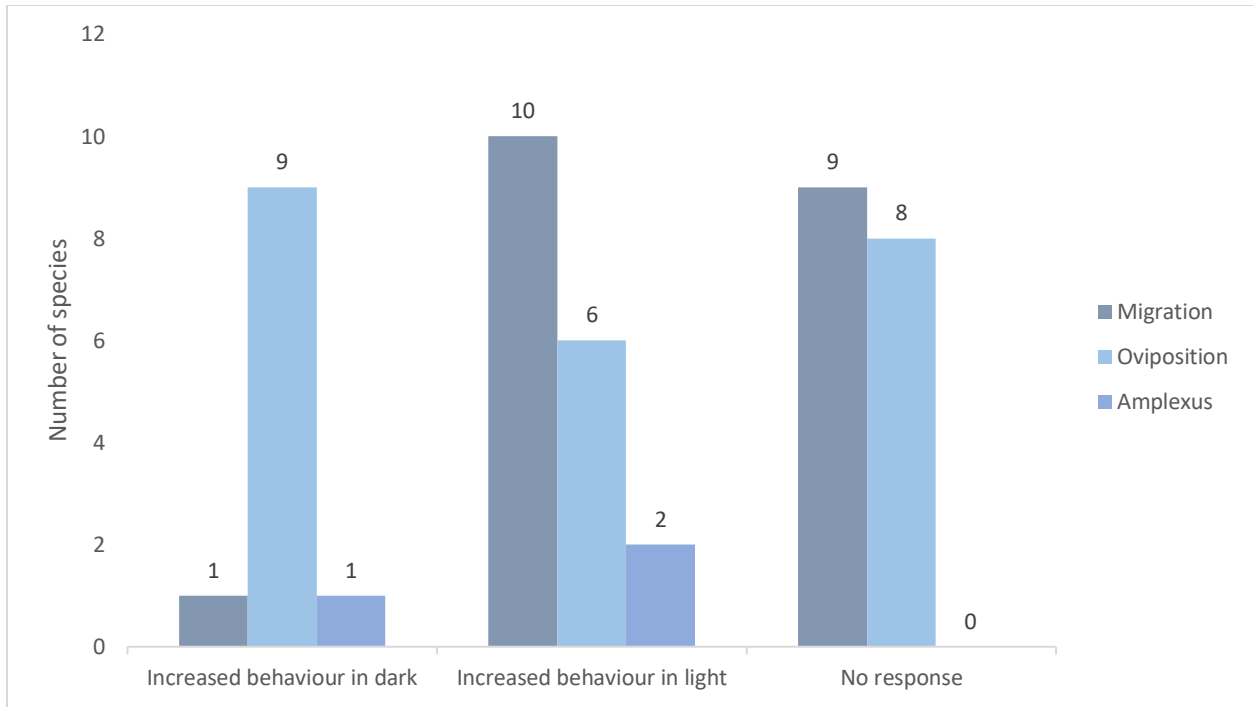


Figure 3.5. Summary of literature review showing anuran amphibian breeding behaviour responses to variation in moonlight and artificial light conditions. The breeding behaviours represented are migration, oviposition and amplexus. The number of species that increase calling behaviour in dark or light conditions, or have no response, are indicated on the x-axis.

Table 3.1. The boreal chorus frog (*Pseudacris maculata*) global model was sub-set into candidate models and the resulting models were ranked using AIC. The global model is highlighted. Competitive models were those less than two delta AIC scores and only models with weights above zero are shown. These values are untransformed coefficients.

Intercept	Light pollution	Lunar phase	Relative humidity	Day of year, scaled	Light pollution* Lunar phase	df	logLik	AICc	delta	weight
0.7334	-0.4425		-0.02953	-0.3203		6	-919.752	1851.5	0	0.329
0.7607	-0.6951	-0.1524	-0.02899	-0.3268	0.4983	8	-918.037	1852.1	0.6	0.244
0.46			-0.02905	-0.3286		5	-921.148	1852.3	0.78	0.223
0.7417	-0.442	-0.01555	-0.02954	-0.3205		7	-919.744	1853.5	2	0.121
0.4707		-0.01899	-0.02907	-0.3288		6	-921.135	1854.3	2.77	0.083

Table 3.2. The wood frog (*Lithobates sylvaticus*) global model was sub-set into candidate models and the resulting models were ranked using AIC. The global model is highlighted. Competitive models were those less than two delta AIC scores and only models with weights above zero are shown. These values are untransformed coefficients.

Intercept	Light pollution	Lunar phase	Relative humidity	Day of year, scaled	Light pollution* Lunar phase	df	logLik	AICc	delta	weight
-1.4	-0.8189	0.2724	-0.01466	-1.122	1.105	8	-336.109	688.4	0	0.717
-1.668		0.7048	-0.01513	-1.111		6	-339.8	691.7	3.33	0.136
-2.501	-0.8069	0.2739		-1.2	1.171	7	-339.446	693	4.64	0.07
-1.558	-0.1777	0.7017	-0.0155	-1.102		7	-339.569	693.2	4.89	0.062
-2.789		0.7291		-1.187		5	-343.465	697	8.63	0.01
-2.735	-0.1178	0.7275		-1.183		6	-343.363	698.8	10.45	0.004
-1.244			-0.01598	-1.108		5	-346.006	702.1	13.71	0.001

Table 3.3. Summary of literature review results assessing the effects of artificial light and lunar phase on anuran breeding behaviours.

Behaviour	Light treatment	Behaviour increased in dark condition	Behaviour increased in bright condition	No effect	# species	# papers	Comments
Calling	Artificial	7	1	0	8	3	More complex calls with light pollution.
Calling	Moonlight	16	3	5	24	6	More complex calls full moon.
Arrival	Moonlight	1	10	9	20	4	
Amplexus	Moonlight	1	2	0	3	3	
Oviposition/ spawning	Moonlight	9	6	8	23	6	
Oviposition/ spawning	Artificial	0	0	1	1	1	More clutches found in rural areas, not attributed to light.

Chapter 4: General conclusions

Summary of findings

I found visual scanning to be a very efficient method of processing acoustic data for four species of anuran amphibians. It was much faster than human listening and only missed calling episodes that involved single individuals, typically a single call. Overall, visual scanning of recordings underestimated occupancy relative to human listening, but only by small amounts. Visual scanning estimates were improved when I incorporated human listening at a sub-set of stations into models, using listening as a source of detection confirmation data. Finally, I compared estimates from data collected with equal time investment for each processing method to compare efficiency. Reduced listening models used data from fewer stations because listening required longer to process. These reduced listening models overestimated the occupancy parameters, suggesting that processing recording data from more stations with visual scanning is superior to having fewer stations processed with the slightly more accurate human listening method.

I assessed the probability of calling for the boreal chorus frog and wood frogs under natural and artificial light conditions, using a logistic regression model. I did not find strong effects of light, either due lunar phase or light pollution, on the presence of calling choruses. Small differences in the light pollution treatments were observed, as both species were less likely to call in areas with light pollution but overlapping confidence intervals for the trends of calling probability indicated that differences were not significant. Despite the inconclusive results, the relatively high importance of light-related variables in my models and findings from my literature review do suggest that effects of light on anuran breeding behaviour deserves further investigation.

The factors I used to account for environmental and seasonal conditions, relative humidity and day of year, were also important in explaining the probability of calling. Changing calling behaviour in response to seasonal variables is expected and suggests that we can continue to monitor anurans as we have been, with respect to seasonality. However, as drivers of calling behaviour, these factors will require ongoing consideration in light of climate change. To maintain high monitoring standards, surveying will have to be adjusted to account for the changes being observed in the abiotic factors that affect behaviour due to climate change.

Applications and limitations

Analysis of recording with visual scanning can be an effective tool for monitoring programs that use passive acoustic monitoring methods. It increases efficiency with short processing time and good accuracy. This method uses freeware programs, meaning large volumes of data can be collected and processed at relatively low cost. Analyzing data collected at more stations using visual scanning, with a small trade-off in accuracy, is an improvement over traditional analysis via listening methods, which are time consuming. Improving the robustness of monitoring designs by implementing recording analysis via visual scanning will benefit studies focused on anurans because sample sizes are often low due to low detection rates.

This approach to processing recordings proved particularly successful with anurans in the boreal because their calls are distinguishable from other sounds. The formation of calling choruses are very distinct signals that have high amplitude on spectrograms and their nocturnality means there are few sounds occurring simultaneously to mask calls. The characteristics that make this method useful for anurans might limit its use for other species. However, the method could be used for species that call in unique frequencies or at unique times of day, and those with high call rates or call in large groups. For example, this method has been

effective in detecting canid vocalizations (Garland 2018). Additionally, this method could be useful for identifying recordings with high quality examples of vocalizations, such as those needed for training automated recognizers.

The combination occupancy model I created produced more accurate occupancy estimates compared to models based only on visual scanning data but could still be improved by accounting for false positives. I had assumed that the calls of the four study species were distinct enough that misidentifications would not occur however, the low biased occupancy estimates suggest this was indeed the case. This could be mitigated in the future by building combination models that account for false positives. This change would also make combination models more widely applicable to other species that may have less distinct vocalizations and thus false positives are more common.

Based on the results of this study of light levels on calling in boreal chorus frog and wood frog, I cannot recommend ways to adjust monitoring to account for behavioural differences under varying light conditions. I used stations that represented suitable habitat for the target species by only analyzing recordings from sites where the species were known to occur. Because I found that, when present, the target species will call under all the tested light conditions, I do not feel that researchers have to account for light levels when choosing recordings for processing. However, I also cannot state that light is unimportant to anuran behaviour. Based my results and findings in the literature, it may be that a more sensitive metric that measures reproductive success is needed, in order to detect an effect of light on amphibian biology. I recommend that continued studies and monitoring should be conducted to fully understand the effects of light and, further, the effects of other urban factors on anuran behaviour and performance of populations.

The visual scanning methodology to process recordings from passive acoustic monitoring is flexible and can be adjusted to suit different monitoring objectives. First, the spectrogram specifications can be altered to facilitate detection of different sounds by changing the frequency range, duration of the spectrogram images and the appearance of the spectrograms, such as contrast. In this research, the 30-second spectrogram images allowed me to easily identify the calls of four anuran species with very different spectrogram signals. Second, I found that conducting 1-minute surveys were equally effective at detecting chorusing behaviour. If single calls are important for monitoring the species of interest, such as rare species where single calls are important, then the duration of the surveys could be increased accordingly. Third, due to the explosive breeding behaviour of these anurans, conducting surveys each night ensures that the breeding period will not be missed. Finally, if you were to target a community of species, moving ARUs throughout the season will increase the probability of detection for each species, if they require different breeding habitats. For example, in northern Alberta, targeting habitats for the early breeding frog species first and then moving ARUs to more suitable habitat for the toad species would allow you to collect more representative data for each species and increase the probability of detection.

When using a passive acoustic approach for monitoring anurans, some design elements require more work. Determining the optimal time to deploy ARUs would reduce the chances of missing the onset of the breeding period. For example, this was observed in the wood frog occupancy estimates that are lower than expected in the study area and could be due to ARUs being deployed after wood frogs stop calling at some stations. To account for this, environmental conditions such as snow cover or date of ice off could be better indicators to predict calling for this species. Determining when breeding is likely to occur is especially important, and difficult,

for species with unpredictable breeding behaviour, that respond to very specific weather events. This is seen in anuran species in southern Alberta, such as the great plains toad, that will not breed until the precipitation conditions are suitable. Research to determine the most effective way of detecting breeding behaviour of such species would inform monitoring design. For example, whether you are more likely to capture the breeding period if ARUs remain in one location for the season or if moving units periodically will increase the chances of surveying under conditions conducive to breeding. A better understanding of what drives the onset of breeding behaviour in anurans will allow monitoring programs to adjust for future changes in weather conditions expected with climate change.

Future work should include maintaining and improving monitoring for anurans. Advancements in the bioacoustics field allows for many new opportunities to improve our ability to detect and survey anuran amphibians. Particularly, passive acoustic monitoring technologies can facilitate the implementation of long-term monitoring. This approach can help determine factors that affect detectability of anurans with the goal to improve acoustic techniques with the long-term goal of improving monitoring strategies to ensure that surveys are conducted at appropriate times and under appropriate conditions. This will lead to proper interpretation of monitoring results and early perception of changing population trends. Ongoing research is critical to maintaining effective monitoring programs for anuran amphibians, to ensure monitoring responds to dynamic conditions due to climate change and human activities.

Passive acoustic monitoring is an effective tool for monitoring sound-producing animals. Accompanying the benefits of autonomous recordings units are the large amount of data that are collected and the issue of processing these data. I demonstrated methods that can be implemented to improve the effectiveness of data processing with the visual scanning technique.

I was also able to contribute to future monitoring efforts by increasing general knowledge and understanding of the behaviour of four anuran species. Together, this improved methodology and greater understanding of anuran calling patterns, can help optimize the use of bioacoustic technology for surveying and monitoring anuran amphibian populations.

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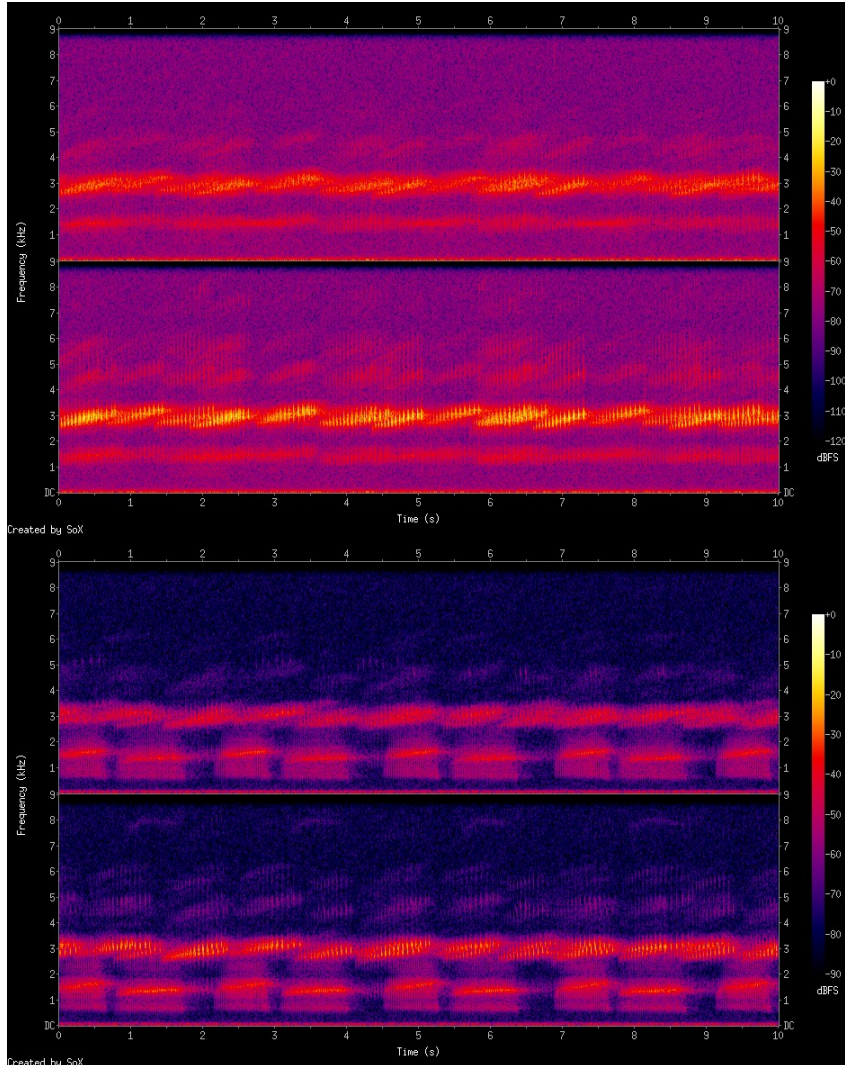
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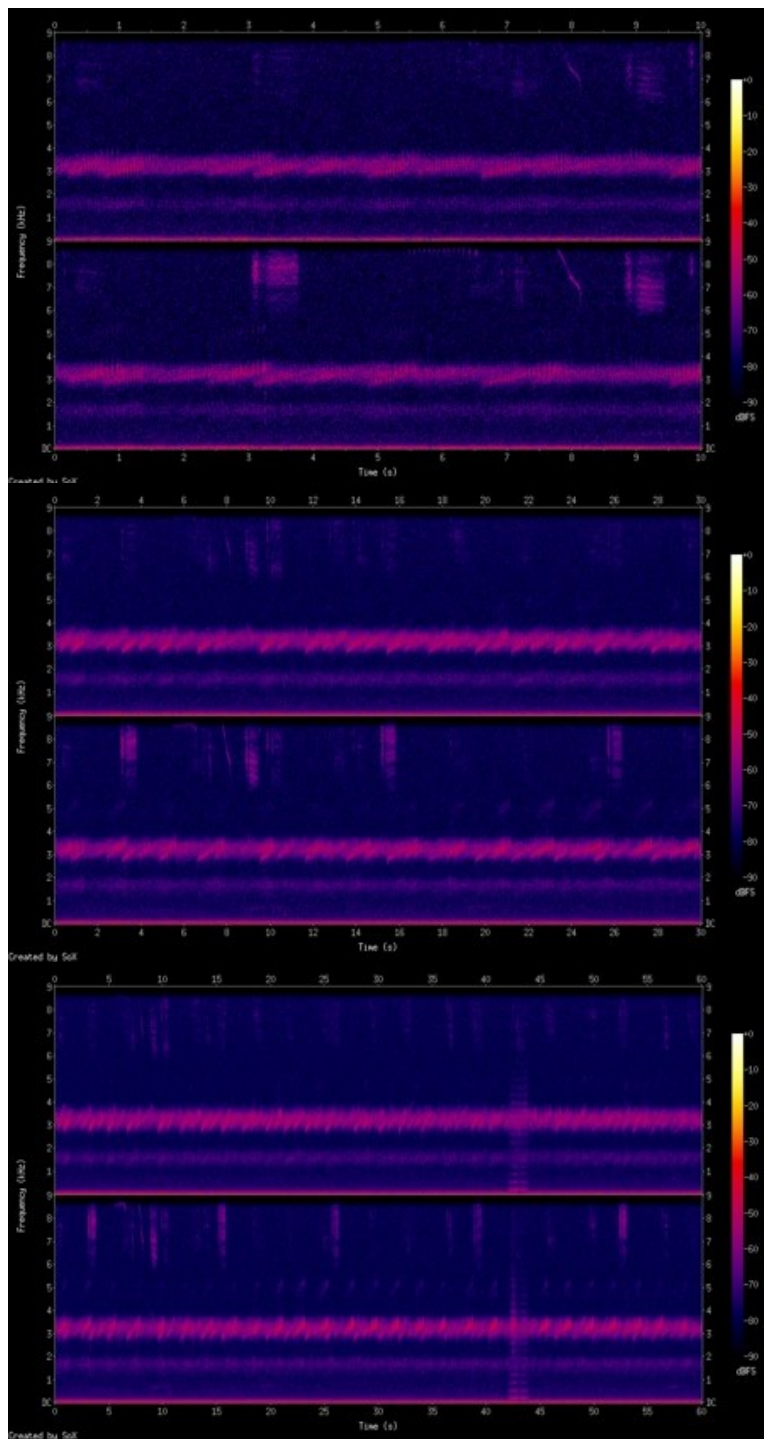
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Appendices



Appendix 2.1. Spectrogram images showing the difference in default settings (top) and with adjusted settings (bottom). The contrast was adjusted from the default 120 dB to 90 dB using the -z option in the Sound eXchange program to improve the signal to noise ratio. The x-axis is time in seconds: the y-axis is the frequency in kHz and the dBFS scale, or colour scale, indicating the amplitude of the sound. Spectrograms are of boreal chorus frog (*Pseudacris maculata*) vocalizations at the 3 kHz frequency.



Appendix 2.2. Spectrogram images illustrating different frame lengths (10, 30 and 60 seconds, top to bottom) for the same recording. The x-axis is time in seconds; the y-axis is the frequency in kHz and the dBFS scale indicating amplitude of the sounds. Spectrograms are of boreal chorus frog (*Pseudacris maculata*) vocalizations at the 3 kHz frequency.

Appendix 2.3. The hierarchical model for the combination model using a Bayesian framework. Human listening data was included at a subset of stations in addition to visual scanning data. The first section of code describes the model in WinBugs and the second section is in R that links to WinBugs to run the model.

```
#WinBugs code for combination models
```

```
model{
  # Priors
  psi ~ dunif(0, 1)
  tau ~ dunif(0, 1)
  p ~ dunif(0, 1)

  # Likelihood
  for(i in 1:I){
    z1[i] ~ dbern(psi)
    for(t in 1:T){
      tau.eff[i, t] <- tau*z1[i]
      delta[i, t] ~ dbern(tau.eff[i, t])
      p.eff[i, t] <- p*delta[i, t]
      y[i, t] ~ dbern(p.eff[i, t])
    }
  }

  for(j in 1:J){
    z2[j] ~ dbern(psi)
    for(s in 1:S){
      pi.eff[j, s] <- tau*z2[j]
      v[j, s] ~ dbern(pi.eff[j, s])
    }
  }
  effective.p <- p*tau
}
```

```
#R code for combination models
```

```
R <- 346 #The number of sampled sites
T <- 4
J <- 50 #The number of subsamples from the confirmatory data
S <- 4

y <- as.matrix(df2)

s <- sort(sample(seq(1:R), J, replace = FALSE))
v <- pi[s, ]
```

```
zinit <- apply(y, 1, max)
inits <- function(){list(z = zinit)}

nc <- 3
nb <- 100
ni <- 1000
nt <- 2

data.2 <- list(I = R, T = T, J = J, S = S, y = y, v = v)

params.2 <- c("psi", "p", "tau", "effective.p")

zinit.2 <- apply(v, 1, max)
inits.2 <- function(){list(z1 = zinit, z2 = zinit.2)}

library(R2WinBUGS)
bugmdir <- "C:\\Users\\Jillian Cameron\\Documents\\WinBUGS14\\"

out.2 <- bugs(data = data.2, inits = inits.2, parameters.to.save = params.2, model.file =
"occ_tau2.txt", n.chains=nc, n.iter=ni, n.burn=nb,
n.thin=nt, bugs.directory = bugmdir, debug = TRUE)

print(out.2, dig = 2)
```


Appendix 3.1. Terms used in Scopus and Web of Science for the literature search. A complete list of anuran genera (452 genera; amphibiaweb 2018) was used in the terms under the anuran subject.

Subject	Terms
Anuran	(amphibia* OR anura* OR frog OR toad OR <i>list of anuran genera</i>) AND
Artificial light	("light pollution" OR "artificial light" OR "anthropogenic light" OR "light at night" OR "sky glow" OR "skyglow" OR "ecological light pollution" OR "artificial night light*" OR
Lunar phase	moonlight OR "lunar phase" OR "lunar cycle" OR "moon phase" OR "lunar illumination")

Appendix 3.2. The final papers used in the literature review.

Title	Author	Year	Species	Location
The Role of Synchronized Calling, Ambient Light, and Ambient Noise, in Anti-Bat-Predator Behavior of a Treefrog	Tuttle, MD; Ryan, MJ	1982	1	Panama
Vocalizations of treefrogs (<i>Smilisca sila</i>) in response to bat predation	Da Silva Nunes, V.	1988	1	Barro Colorado Island, Panama
Environmental and social factors influence chorusing behaviour in a tropical frog: examining various temporal and spatial scales	Brooke, PN; Alford, RA; Schwarzkopf, L	2000	1	Queensland, Australia
Effects of season and weather on calling in the Australian microhylid frogs <i>Austrochaperina robusta</i> and <i>Cophixalus ornatus</i>	Hauselberger, KF; Alford, RA	2005	2	Queensland, Australia
Modeling anuran detection and site occupancy on North American Amphibian Monitoring Program (NAAMP) routes in Maryland	Weir, LA; Royle, JA; Nanjappa, P; Jung, RE	2005	10	Maryland, USA
The effect of artificial light on male breeding-season behaviour in green frogs, <i>Rana clamitans melanota</i>	Baker, BJ; Richardson, JML	2006	1	Ontario, Canada
Effects of disturbance, position of observer, and moonlight on efficiency of anuran call surveys	Granda, JR; Pena, RM; Pierce, BA	2008	9	Texas, USA
The lunar cycle: a cue for amphibian reproductive phenology?	Grant, RA; Chadwick, EA; Halliday, T	2009	8	UK, Italy
Spawning and non-breeding activity of adult giant bullfrogs (<i>Pyxicephalus adspersus</i>)	Yetman, CA; Ferguson, JWH	2011	1	South Africa
Factors influencing the timing of spring migration in common toads (<i>Bufo bufo</i>)	Arnfield, H; Grant, R; Monk, C; Uller, T	2012	1	UK
Contrasted influences of moon phases on the reproduction and movement patterns of four amphibian species inhabiting different habitats in central Italy	Vignoli, L; D'Amen, M; Della Rocca, F; Bologna, MA; Luiselli, L	2014	3	Italy
Factors Affecting the Breeding Activity of the Japanese Common Toad, <i>Bufo japonicus formosus</i> (Amphibia: Bufonidae) with Special Reference to the Lunar Cycle	Kusano, T; Miura, T; Terui, S; Maruyama, K	2015	1	Japan
Acute Artificial Light Diminishes Central Texas Anuran Calling Behavior	Hall, AS	2016	6	Texas, USA
Springtime Emergence of Overwintering Toads, <i>Anaxyrus fowleri</i> , in Relation to Environmental Factors	Green, T; Das, E; Green, DM	2016	1	Ontario, Canada

Better in the dark: Two Mediterranean amphibians synchronize reproduction with moonlit nights	Vignoli L.; Luiselli L.	2017	2	Italy
Light and noise pollution interact to disrupt interspecific interactions	McMahon, TA; Rohr, JR; Bernal, XE	2017	1	Panama City
The darker the night, the brighter the stars: consequences of nocturnal brightness on amphibian reproduction	Onorati, M; Vignoli, L	2017	1	Italy
Moonlighting? - Consequences of lunar cues on anuran reproductive activity	Underhill, VA; Hobel, G	2018	1	Wisconsin, USA

Appendix 3.3. Results of the importance function for each species, the boreal chorus frog and the wood frog. The importance function takes the sums Akaike weights from the models' sub-set from the global models and outputs the relative importance of each variable. Each variable is ranked in relative importance and is assigned a value based on the number of models that contain it. The variables are listed in order of importance for each species.

<i>Boreal chorus frog</i>	Relative humidity	Day of year, scaled	Light pollution	Lunar phase	Light pollution*Lunar phase
Importance	1	1	0.69	0.45	0.24
Models containing variable	10	10	12	12	4
<i>Wood frog</i>	Day of year, scaled	Lunar phase	Relative humidity	Light pollution	Light pollution*Lunar phase
Importance	1	1	0.92	0.85	0.79
Models containing variable	10	12	10	12	4