

Herps in the wind: The ecology of herpetofauna in windfarms

by

Cory Trowbridge

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science (MSc) in Biology

The Faculty of Graduate Studies
Laurentian University
Sudbury, Ontario, Canada

© Cory Trowbridge, 2020

THESIS DEFENCE COMMITTEE/COMITÉ DE SOUTENANCE DE THÈSE
Laurentian Université/Université Laurentienne
Faculty of Graduate Studies/Faculté des études supérieures

Title of Thesis Titre de la thèse	Herps in the wind: The ecology of herpetofauna in windfarms		
Name of Candidate Nom du candidat	Trowbridge, Cory		
Degree Diplôme	Master of Science		
Department/Program Département/Programme	Biology	Date of Defence Date de la soutenance	June 02, 2020

APPROVED/APPROUVÉ

Thesis Examiners/Examineurs de thèse:

Dr. Jackie Litzgus
(Supervisor/Directeur(trice) de thèse)

Dr. Nate Basiliko
(Committee member/Membre du comité)

Dr. Kelly Withers
(Committee member/Membre du comité)

Dr. Glenn Cunningham
(External Examiner/Examineur externe)

Approved for the Faculty of Graduate Studies
Approuvé pour la Faculté des études supérieures
Dr. David Lesbarrères
Monsieur David Lesbarrères
Dean, Faculty of Graduate Studies
Doyen, Faculté des études supérieures

ACCESSIBILITY CLAUSE AND PERMISSION TO USE

I, **Cory Trowbridge**, hereby grant to Laurentian University and/or its agents the non-exclusive license to archive and make accessible my thesis, dissertation, or project report in whole or in part in all forms of media, now or for the duration of my copyright ownership. I retain all other ownership rights to the copyright of the thesis, dissertation or project report. I also reserve the right to use in future works (such as articles or books) all or part of this thesis, dissertation, or project report. I further agree that permission for copying of this thesis in any manner, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised my thesis work or, in their absence, by the Head of the Department in which my thesis work was done. It is understood that any copying or publication or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that this copy is being made available in this form by the authority of the copyright owner solely for the purpose of private study and research and may not be copied or reproduced except as permitted by the copyright laws without written authority from the copyright owner.

Abstract

Windfarms are reducing reliance on fossil fuels but they may present threats to wildlife. I studied the ecology of herpetofauna living in Prince Windfarm (Sault Ste Marie, Ontario) in 4 wetlands located close to wind turbines (<500 m, Turbine sites), and 4 wetlands far from wind turbines (>1.5 km, Control sites). I measured amphibian biodiversity using transect surveys and acoustic recordings of frog calls. I found lower biodiversity and richness within frog choruses in Turbine sites, and some evidence that frogs in windfarms adjust their calls similar to frogs near roads. I also investigated whether the spatial ecology of Painted Turtles (*Chrysemys picta*) was impacted by the windfarm. Turtles within the windfarm had shorter movements and marginally smaller home ranges than turtles in Control sites, and appeared to avoid service roads and turbines. Future research should investigate acoustic masking of low frequency calling amphibians and infrastructure avoidance behaviours by turtles.

Keywords

Conservation, Spatial Ecology, Bioacoustics, Diversity, Acoustic Masking, Amphibians, Turtles, Wind Energy

Acknowledgments

I first must thank my supervisor Dr. Jacqueline Litzgus for your support throughout not only this thesis but also the paths I've taken in academia. When I was still in my undergraduate years your herpetology course ignited my passion for herpetofauna and for research when we began this topic as an undergraduate thesis. You supported my pursuit to take the topic to the graduate level, without that support I'm not sure where I would be today. Thank you for the hours of work you put in to help answer my questions, offering guidance, and of course creating our friendship. I also must thank my committee members, Dr. Nathan Basiliko and Kelly Withers, for your feedback and support in this project.

I also need to thank all of those volunteers who helped in the field, whether it was walking transects or wading through wetlands, your help was invaluable. Along with these people I must thank Vanessa Brouse, my field assistant for both years. You fought through the bugs, rain, and heat to help me, and even put up with my near workaholic eagerness in the field. I also thank the Evolgen for its support through training, staff alerting me to turtles and providing me access to Prince Wind. This support towards research should be encouraged and praised in the industry.

I wish to also thank all my lab mates who helped me with specific problems, supported me during conferences, or put up with me bouncing ideas off them. I'm sure our friendships will continue throughout our growing careers. Finally, I must also thank my family for their support, specifically, I must thank my parents for not only their emotional support but also by lending me their camping trailer to be used as my home away from home during the summer field season. This not only helped the project by reducing costs, but also helped me by having a place I could be comfortable in after a long day of work.

Table of Contents

Thesis Defense Committee	i
Abstract.....	ii
Acknowledgments	iii
Table of Contents	1
Table of Tables	2
Table of Figures.....	4
General Introduction	8
Chapter I.....	11
The spatial ecology of Midland Painted Turtles (<i>Chrysemys picta</i>) in an established windfarm.....	11
Chapter II	23
Do windfarms impact amphibian ecology? An investigation of amphibian community ecology and anuran acoustics within a Northern Ontario windfarm	23
General Discussion.....	41
References.....	45
Tables	58
Figures.....	68

Table of Tables

Table I. Local reptiles and amphibians found in the study area during environmental assessment by Natural Resource Solutions Inc (NRSI) in 2002-2004. Herpetofauna listings known in the study area in 2004 described in the Ontario Herpetofauna Summary Database from the Natural Heritage Information Center (NHIC). Information in the table was retrieved and modified from the environmental impact statement (EIS 2005) preformed prior to windfarm construction and supplemented with current observations made during my study.....	58
Table 1.1. Dunn’s post hoc pairwise test of turtle home range sizes in two Turbine sites (T2 and TW) compared to a Control Site (C). A 90% Minimum Convex Polygon (MCP) was used to represent home range and 50% least square kernel density to represent core activity areas. Z represents the Z test statistic for each comparison. Both adjusted p-values using Bonferroni correction and unadjusted p-values are reported	59
Table 2.1. Call characteristics measured in anuran choruses.....	60
Table 2.2. Call characteristics measured for individual frog calls of <i>Lithobates clamitans</i> and <i>Pseudacris crucifer</i> . Call characteristics were measured using Kaleidoscope Pro v5.1.9g software	61
Table 2.3. Kaleidoscope Pro settings used for cluster analysis of chorus and individual anuran calls. All cluster analyses were conducted by a single observer. Signal parameters were adjusted to best detect the species in the study area while reducing the detection of birds. Cluster analysis settings were adjusted to reduce false identifications. Max distance from cluster center was reduced to 0.7 from the recommended 1.0 to reduce the number of false identifications without removing too many correct calls. Some settings were kept at software recommended values (*).	62
Table 2.4. Cumulative sightings of amphibian species across study sites during transect surveys between May and September of 2018 and 2019. The number of species and number of individuals per site are listed next to each site, along with the means for both treatments. Turbine sites (T) are < 500 m from a wind turbine and Control sites (C) are > 1.5 km from a wind turbine.	63

Table 2.5. Average number of anuran calls recorded in Control sites (>1.5 km from wind turbines) and Turbine sites (<500 m from a wind turbine) and total number of calls by that species indicated in parentheses. Pseudo-random paired recordings in Control and Turbine sites were conducted nightly from April to July of 2018 and 2019. Anuran calls were extracted using Kaleidoscope Pro (Wildlife Acoustics, v5.1.9g). **64**

Table 2.6. Summary of model ranking for Chorus Diversity (H) and Chorus Evenness (E). Models were created by the diversity of calling frogs recorded during the spring and summer of 2018 and 2019. k is the number of estimated parameters for each model, Δ AIC is the change in AIC between each step of model creation **65**

Table 2.7. Individual mean call characteristic for *Lithobates clamitans* and *Pseudacris crucifer* by site, nested within treatment (Control; >1.5 km from a turbine, Turbine; <500 m from a turbine). Results of an analysis of deviation, testing for differences between treatments for each call characteristic in a linear mixed effect model are included (see text for results of other predictor variables). **66**

Table 2.8. Linear mixed model summary of predictor variables excluding treatment for individual call characteristics of *Lithobates clamitans* and *Pseudacris crucifer* and analysis of deviation results. Date and Time variable was analyzed as serial date and time. Site represents the nested in treatment effect and was analyzed as a random effect variable. Coefficient estimate of the variable indicates a positive or negative influence on the call characteristic with a change in the predictor variable. Significance of the site variable was analyzed using an ANOVA-like table for random effects (see text), where LRT is the likelihood ratio test statistic. Degrees of freedom is equal to 1 for all variable analyses..... **67**

Table of Figures

Figure I. Map depiction of site area used to determine differences in reptile and amphibian ecology and behavior. Control sites (C1, C2, C3, C4 and CW) were located over 1.5 km from any wind turbines and Turbine sites (T1, T2, T3, T4, and TW) were located within 500 m of a wind turbine. Sites ending in a number (1, 2, 3, and 4) were used for transect surveys. Turtle spatial ecology data was collected in Control sites C3 and CW and Turbine sites T2 and TW. Maps were created in ArcGIS v10.7.1, all maps shown have the same scale and orientation..... **68**

Figure 1.1. Map depiction of turbine avoidance in a Turbine site (TW; left) and road avoidance in a Control site (right). White dots represent actual turtle locations within wetlands (blue hatched polygons), grey dots represent randomly generated points. Yellow polygon represents 100% MCP turtle population range. Black dashed lines show distance from turbine (circles with Xs) measurements, one turbine is missing from map depiction. Distance from all turbines within 500 m of the Turbine MCP population range was measured for all points, such that all points have a measured distance from each turbine. Grey line shows nearest road measurement, which was taken as the closest distance to a road. Maps and measurements generated in ArcGIS v10.7.1, scale bars are unique to each map..... **69**

Figure 1.2. Distance of turtle (dark grey) and random point (light grey) locations from turbines in 2 Turbine sites (T2 and TW). Distances of each point from every turbine within 500 m of population range was measured to determine if turtles are avoiding turbines. Random point locations were confined to wetlands to simulate normal activity. Two-Way ANOVA found no difference in turtle distance from random point distance in site T2 (Left, N=894), suggesting no avoidance. A significant difference in distance was found between turtle and random in site TW (Right, N=570), suggesting an avoidance behaviour towards turbines by turtles. Horizontal black bar represents median distance. **70**

Figure 1.3. Distance of turtle (dark grey) and random point (light grey) locations from roads in one Control site (>1.5 km from turbines, left) and one Turbine site (TW, <500m from turbines, right). Distance was measured as closest distance of each point to either windfarm road or public road. Only sites with population ranges that overlapped roads were considered for this comparison. Random point locations were confined to wetlands to simulate normal activity.

Results of a t-test found no difference between turtle and random distances from roads in Control, suggesting no avoidance of roads. A significant difference between turtle distances from random was found in the Turbine site, suggesting avoidance behaviour. Horizontal black bars represent median distance, and dots represent outliers..... 71

Figure 1.4. Map representation of possible wetlands turtles could disperse to within a 1 km radius around Turbine study sites. Buffer range (yellow) was determined as a conservative estimate based on previous research (Bowne 2008; Rowe 2003). White shapes highlight wetlands that were occupied by the tracked turtles during the active season. Blue shapes highlight wetlands that fall within the 1 km buffer, but were not occupied by the tracked turtles during the active season. Map was generated using ArcGIS..... 72

Figure 2.1. Transects (A) were created at 4 Turbine (B, <500 m from a wind turbine, picture taken in summer) and 4 Control (C, >1.5 km from a wind turbine, picture taken in fall) study sites. 100 m-long transects were created in east or west directions with handheld GPS units and flagging tape to mark the center, and cover boards placed at 10, 50, and 100 m. Transects were walked first on one side of the center, then rounded at the end and walked in reverse on the other side, always staying within 5 m of the transect center..... 73

Figure 2.2. Cumulative Diversity (H') of amphibians found during transect surveys in four Control sites (>1.5 km from turbines, light grey) and four Turbine sites (<500 m from a turbine, dark grey) were not significantly different. Surveys of 100 m transects extending perpendicular from wetland edge were performed from May to September of 2018 and 2019. Surveys were conducted at the same site twice per visit, first at 08:30 then at 17:30. A Shannon-Weiner index was calculated for the cumulative counts of species found at each site over the 2018 and 2019 seasons and standardized by dividing H' by the number of surveys. A two-sample t-test revealed no significant difference between treatments 74

Figure 2.3. Generalized additive model for Menhinick's Richness (D , log scale) of amphibian species found during transect surveys when at least one amphibian was found. Surveys were conducted on 100 m transects extending perpendicular from wetland edges in four Control sites (>1.5 km from turbines, light grey) and four Turbine sites (<500 m from a turbine, black). Sites were visited randomly between May and September of 2018 and 2019, with each visit consisting

of a morning survey at 08:30 and evening surveys at 17:30. The model was fit with site and smoothing factor Julian date as significant predictors of D. Treatment (Control or Turbine) did not predict D. 75

Figure 2.4. Chorus Diversity (H') of anurans in Control sites (>1.5 km from turbines, light grey) was higher than in Turbine sites (<500 m from a turbine, dark grey). Pseudo-random paired recordings in Control and Turbine sites were conducted nightly from April to July of 2018 and 2019. Calls of 6 different anuran species were identified using Kaleidoscope Pro (Wildlife Acoustics, v5.1.9g) and a Shannon-Weiner Diversity index was calculated for each recording night. An analysis of deviation on a linear mixed effects model accounting for repeated measures found diversity was significantly lower in Turbine sites and there were no differences among sites within treatments. Boxplot horizontal lines represent median and dots represent outliers .. 76

Figure 2.5. Spectrograms representations of average *Lithobates clamitans* (Left) and *Pseudacris crucifer* (Right) calls in Control sites (C, >1.5 km from turbines) and Turbine sites (T, <500 m from a turbine). Calls that best represented the average mean frequency, minimum frequency and duration (Table 2.6) for each treatment and species are displayed. Darker blacks represent amplitude or loudness of the sound, a characteristic that was not analyzed. Harmonics of Spring Peepers (as seen in the Control site call) were not included in the call characteristics measurements. Spectrograms were created with Kaleidoscope Pro software with the same brightness and contrast settings, then screen captures were cropped and overlaid to be represented on the same axis..... 77

Figure 2.6. Site-specific variation in call characteristics of *Lithobates clamitans* in Control sites (located >1.5 km from turbines; C1, C2, C3, and C4) and Turbine sites (located <500 m from a turbine; T1, T2, T3, and T4). Mean call frequency (A), minimum call frequency (B), and call duration (C). Lines represent the full range of data and dots represent the site mean. Shared letters indicate similarity between sites (i.e., groupings) within each call characteristic based on a Tukey HSD test ($p > 0.05$). Sites are arranged in descending order of means to best represent groupings..... 78

Figure 2.7. Site-specific variation in call characteristics of *Pseudacris crucifer* in Control sites (located >1.5 km from turbines; C1, C2, C3, and C4) and Turbine sites (located <500 m from a

turbine; T1, T2, T3, and T4). Mean call frequency (A), minimum call frequency (B), and call duration (C). Lines represent the full range of data and dots represent the site mean. Shared letters indicate similarity between sites (i.e., groupings) within each call characteristic based on a Tukey HSD test ($p > 0.05$). Sites are arranged in descending order of means to best represent groupings..... **79**

General Introduction

The demand for alternative green energy sources is increasing each year as governments across the globe attempt to curb climate change by relying on renewable energy (GWEC 2018, Pecl et al 2017). Nations have invested large amounts of money into the production of wind and solar power (GWEC 2018). Canada, one of the top ten nations for wind energy capacity, currently has an installed capacity of over 12,000 MW (Mega Watts), with more installations expected in the coming years (GWEC 2018). The surge in wind energy development and its potential impacts on wildlife have raised concerns in scientists and the general public, as green energy initiatives should try to minimize negative impacts on the environment.

Over the past several years there have been many studies concerning the impacts of wind energy development on wildlife (Gibson, Wilman, and Laurance 2017). Birds and bats are typically the most-studied, due to their risk of direct mortality resulting from collisions with wind turbines during flight (Smallwood 2013). Although direct mortality is one of the larger concerns, behavioural impacts, such as avoidance, have also been observed (Masden et al 2009). Non-flying mammals are the next most-studied animals around onshore wind turbines, and these species show varying degrees of turbine avoidance and changes in predator avoidance behaviour in response to turbines (Skarin and Alam 2017; Rabin, Coss, and Owings 2006). In contrast, reptile and amphibian interactions with windfarms have been largely overlooked, with only a few studies on even fewer species (Agha et al 2015; Keehn and Feldman 2018). These studies have found evidence of habitat use differences in Dessert Tortoises (*Gopherus agassizii*; Lovich et al 2011b) and altered predator avoidance behaviour in Side-Blotched lizards (*Uta stansburiana*; Keehn and Feldman 2018) in response to wind turbines.

This lack of research is not due to a lack of concern for reptiles and amphibians. Reptiles and amphibians are declining on a global scale (Bohm et al 2013; Gibbons et al 2000), with their extinction risks over four times the background rate (Alroy et al 2015). In most cases, habitat degradation is the main threat to populations (Cushman 2006; Lesbarrères et al 2014).

Communities in Ontario have voiced their concerns about reptiles around wind turbines, resulting in legal battles over the installation of a windfarm near vulnerable habitats (Coyle 2016). However, these concerns for reptiles and amphibians have been raised with little empirical evidence to support them. The purpose of my study is to fill knowledge gaps about the effects of windfarm proximity and operation on reptile and amphibian ecology and behaviour. I focused on two topics, community ecology and mating calls of amphibians, and turtle spatial ecology, within windfarms.

Study Sites

Study sites are located north of Sault Ste Marie, Ontario (46°36'43.4"N 84°28'49.1"W), a region composed of Great Lakes-St. Lawrence forests (Uhlir et al 2001). Eight sites of similar size and habitat composition were chosen. Each study site contains a large wetland complex, often created by beaver dams, surrounded by forests. Four sites (C1, C2, C3, and C4), located over 1.5 km from a wind turbine, were chosen as Control sites (Figure I). Four sites (T1, T2, T3, and T4), located within 500 m of at least one wind turbine, were chosen as Turbine sites. In order to supplement the chances of finding turtles, additional sites were created in Control and Turbine areas (CW and TW). The only major difference between study site treatments is presence or absence of wind turbines.

Turbine sites were located within Prince Wind, owned and operated by Evolugen (subsidiary to Brookfield Renewable). Prince Wind includes 126 turbines, giving an installed capacity of 189 MW. Construction of the facility, installed as 2 projects (Prince 1 and Prince 2), began in October 2005, and became operational in August 2006 (Prince Power Project Environmental impact statement EIS 2005). Prior to construction, an extensive environmental impact assessment was conducted that identified local flora and fauna in the area, including 9 species of reptiles and amphibians (Table I; Prince Power Project EIS 2005). An extensive gated service road network, composed of over 80 km of gravel roads, provides access to each turbine for workers while limiting public access to only the private landowners who lease their land to the windfarm (Prince Power Project EIS 2005). Access to Prince Wind for my data collection was provided through a partnership between Evolugen and Laurentian University.

Chapter I

The spatial ecology of Midland Painted Turtles (*Chrysemys picta*) in an established windfarm

Introduction

Direct mortality of flying animals (ie. birds and bats), from collisions with wind turbines is the most commonly-studied aspect of the impacts of windfarms on wildlife (Kuvlesky et al 2007). However, wind energy production can interfere with wildlife through more than just direct impacts with turbines; morphology, behaviour, distribution, and diversity can also be affected (Rabin, Coss, and Owings 2006; Santos et al 2010, Shaffer and Buhl 2016). The infrastructure of windfarms, including the access roads, may be a larger concern for terrestrial animals as they can cause habitat fragmentation (Chen and Koprowski 2016; Fahrig and Rytwinski 2009) and avoidance behaviours due to increased human interactions (Lovich and Ennen 2013a). In some cases, the impacts on one taxon or species can cascade and cause multiple impacts across the environment, including changes to communities, populations, individual behaviours and morphology, and ultimately the fitness of other species (Thaker, Zambre and Bhosale 2018).

Wind turbines can lead to changes in animal behaviour. Multiple taxa, from birds to large mammals and predators (Costa et al 2017; Shaffer and Buhl 2016; Tsegaye et al 2017), display avoidance behaviours. While some avoidance behaviours are short-term responses to construction (Tsegaye et al 2017), others include long-term avoidance and displacement (Costa et al 2017, Shaffer and Buhl 2015), and even the evolution of new behaviours, including fearlessness (Thaker, Zambre and Bhosale 2018). Impacts on wildlife can also occur through the construction phase itself (Lovich and Ennen 2013a). A model created by Santos et al (2010) for a windfarm installation predicted overall vertebrate species richness declines due mostly to

construction, disturbance, and habitat changes. In contrast, Agha et al (2015) found that the spatial ecology of Agassiz's desert tortoises (*Gopherus agassizii*) did not differ between windfarm and wildlife areas, and that tortoises within the windfarm showed slightly higher survivorship.

The spatial ecology of turtles within windfarms represents a large gap in knowledge. I examined the spatial ecology of turtles in wetlands within an established windfarm (<500 m to a turbine) compared to those in wetlands outside of the windfarm (>1.5 km from a turbine). I hypothesized that if turtles are negatively affected by wind turbine presence, then turtles within the windfarm would show avoidance of suitable habitats close to turbines and roads, smaller home ranges and daily distances moved (DDM) than turtles outside of the windfarm.

Materials and Methods

I used Midland Painted Turtles (*Chrysemys picta marginata*) to examine spatial ecology in response to windfarms because they are common in the study area and have been studied elsewhere (Lovich and Ennen 2013b), thus providing other studies for comparison. To increase the chances of finding turtles, additional sites (on top of the 4 Control and 4 Turbine sites noted above) were included in Control and Turbine areas (CW and TW, respectively). Turtles were captured by hand using 30-minute timed searches in 2 Control (CW and C3) and 2 Turbine (T2 and TW) wetlands. However, because radio-tagged turtles (see below) moved between Control sites, the 2 Control sites were merged into one site (C). In 2018, 9 turtles were outfitted with VHF radio transmitters (ATS model R1860), 5 in Control and 4 in Turbine wetlands; however, 7 transmitters were lost with scute shedding throughout the season. In 2019, I fitted 19 turtles with radio transmitters (ATS model R1680), 9 in Control and 10 in Turbine wetlands, 6 of which were

recaptures from 2018, for a total of 21 turtles tracked. In 2019, only 3 transmitters were lost, 1 during the summer and 2 during overwintering. Transmitters were attached with epoxy in 2018, and with a combination of epoxy and wired attachment through the marginal scutes of the carapace to avoid transmitter loss in 2019. Adults of both sexes were used for radio telemetry to meet the animal care requirement that the transmitter is less than 6% of the turtle's body mass. Nearly equal sex ratios of turtles were used (Control 3 males:4 females; Turbine 4 males:4 females). As painted turtles of both sexes have similar movements, sex was not included as a factor in the statistical analyses (Rowe and Dalgarn 2010). Turtles were radio-located twice per week during the active season (May to September). Upon locating the turtles, air and water temperatures (°C), GPS coordinates (UTM) and weather conditions were recorded. Tracking efforts were adjusted with reductions in turtle activity: turtles were located weekly during September and twice per month during October and November, until turtles were no longer moving between tracking dates, suggesting hibernation had begun.

Home range sizes and daily distances moved (DDMs) were calculated in R using the *adehabitatR* package (Calenge 2006). Due to the loss of transmitters between years, resulting in most turtles having less than a full active season of tracking, I used conservative estimates for home ranges. Home range sizes were estimated with 90% Minimum Convex Polygons (MCPs) to allow comparisons with other studies (Row and Blouin-Demers 2006; Rowe and Dalgarn 2010). Least squares kernels were used to estimate sizes of core activity areas (50% kernels; Row and Blouin-Demers 2006). Only turtles with at least 16 radio locations or for whom a stable home range size was reached (i.e., asymptote reached when plotting *mcp* function from *adehabitatR* package; Calenge 2006) were used in home range size analyses. DDMs were

determined as the distance between pairs of consecutive radio location points divided by the number of days between locations (Lavery, Korol, and Litzgus 2016); all 21 radio-tagged turtles were included in DDM calculations.

Because I combined two Control sites into one, and due to low sample size of turtles that met requirements for estimating home range sizes, differences in MCP and 50% kernels were examined only between sites using a Kruskal-Wallis test by ranks, followed by a Dunn's post hoc test with a Bonferroni p-value correction (Dinno 2015; Ogle, Wheeler, and Dinno 2020). The treatment effect was thus interpreted through pair-wise comparisons of the Control site to each of the two Turbine sites. Differences in DDMs were examined using a linear mixed effects model with treatment (Control and Turbine) as a fixed effect, and sites (C, T2, and TW), and turtle ID nested as random effects. An analysis of deviance table was used to determine if treatment had a significant effect on DDM and an ANOVA-like table for random effects function, *ranova*, in the R *lmerTest* package (Kuznetova, Brockhoff, Christensen 2017) was used to compare between models with and without the random effects of site and turtle ID.

I used ArcGIS (v10.7.1, ERSI, Redlands, CA) to examine whether turtles avoided turbines by comparing turtle radio location point distances from turbines to random point distances from turbines within the population range. The population range was a 100% MCP for all turtle points in each Turbine site, created with the Minimum Bounding Geometry tool in ArcGIS. I generated a number of random points equal to the number of turtle location points used to calculate the population range. Random points were confined to wetlands as turtles were almost always located in water when tracked (following a modified method of Lavery, Korol, and Litzgus 2016). I measured the distances from random and turtle points to all turbines within 500 m of the

MCP population range boundary for a given site (see Figure 1.1 for an example of these measurements). Site T2 has 3 turbines within 500 m of the MCP population range boundary and none inside, whereas site TW has 1 turbine within the MCP and 4 outside but within 500 m. The random and turtle distance-to-turbines measurements were then compared using a two-way repeated measures ANOVA for each Turbine site; avoidance behaviour was concluded if turtle locations were significantly further from turbines than random points.

Using the same methods outlined for Turbine sites, a population range for Control turtles was created, and a number of random points equal to the number of turtle locations were generated within the wetlands. I measured the distance from the turtle location or random point to the closest road for both Control (public road) and Turbine (windfarm service roads) turtles.

Avoidance behaviour was concluded if turtle locations were significantly further from roads than random points by using a two-sample t-test for Control and Turbine sites separately. An example of these avoidance measurements is shown in Figure 1.1.

Results

A total of 15 turtles, 7 Control turtles and 8 Turbine turtles (6 in Site T2 and 2 in Site TW), satisfied the criteria of a stable home range or at least 16 radio locations, for use in home range analyses. Control turtles had a median 90% MCP home range of 0.77 ha and a median 50% kernel core active area of 0.35 ha. Turbine turtles had a median 90% MCP home range of 0.30 ha and a median 50% kernel core active area of 0.19 ha. Due to the merging two control sites into one, and the low sample size of turtles, home ranges were only compared among sites (C, T2, and TW) and not between treatments. MCP home range sizes differed marginally between sites ($\chi^2=5.89$, $df=2$, $p=0.053$); in contrast, 50% kernel core areas did not differ ($\chi^2=3.89$, $df=2$,

$p > 0.05$). No significant differences in MCP home ranges between site pairs was found using the Dunn's pairwise test; however, due to the strictness of the Bonferroni correction and the small sample size of turtles increasing the probability of a type II error, both adjusted and unadjusted p-values are reported (Table 1.1).

All 21 turtles were used in the analysis of Daily Distance Moved (DDM). Mean DDM of Turbine turtles was 13.95 ± 13 m (SD) ($n=195$ total Turbine radio locations). Mean DDM of Control turtles was 22.78 ± 32.02 ($n=205$ total Control radio locations). Due to positive skewness in DDM, distance values were square root transformed for further analysis. The initial linear mixed effects model showed little variance between sites (C, T2, and TW), indicating site had no effect on the model, resulting in the removal of the site variable from the model. The resulting accepted model had treatment (Control and Turbine) as a fixed effect and turtle ID as a random effect. The analysis of deviance found treatment to be a significant predictor of DDM ($\chi^2=6.60$, $df=1$, $p < 0.05$). Turtle ID was not a significant predictor of DDM (Likelihood Ratio Test (LRT)=1.67, $df=1$, $p > 0.05$).

I examined turtle avoidance of turbines in two turbine sites, T2 and TW ($n=447$ and $n=285$ location points, respectively). In T2, turtles were 392.99 ± 85.3 m (mean \pm SD) from turbines, and random locations were 393.67 ± 84.7 m from turbines; these distances were not significantly different (Figure 1.2; $F(1,891)=0.015$, $p > 0.05$). In contrast, turtles in site TW (mean = 500.02 ± 220.23) were significantly further from turbines than random points (mean = 420.11 ± 187.59 ; $F(1,567)=10.77$, $p < 0.01$). As turbines are located at different distances from the boundary of the population range MCP, it is expected that a difference in distance from points among turbines will occur.

I examined turtle avoidance of roads in Turbine site TW (n=57 locations) and Control site (n=216 locations). Because turtles in turbine site T2 were never observed interacting with the road (i.e., crossing or nesting), and the population range did not overlap with any roads, T2 was not used in the road avoidance analysis. Turtles in TW were 170.84 ± 32.47 m from the road, whereas random points (mean 128.14 ± 60.2) were significantly closer to the road (Figure 1.3; $t = -4.71$, $df = 86.04$, $p < 0.0001$). In contrast, turtles in the Control site were 168.42 ± 105.35 m from the road, which was not different from the mean distance of random locations from the road (mean $= 157.55 \pm 95.95$; $t = -1.12$, $df = 426.3$, $p > 0.05$).

Discussion

The effects of wind energy on reptiles are still not fully understood, and few studies have examined spatial ecology and behaviour (Agha et al 2015; Thaker, Zambre, Bhosale 2018; Kheen and Feldman 2018). The goal of my study was to examine the Midland Painted Turtle's spatial ecology within a wind facility and determine if avoidance behaviour was being shown. I found marginal differences in home range size among study sites (C, T2, and TW). Home ranges appeared to be larger in the Control site than in either of the Turbine sites, although no statistical differences were found in the pairwise comparisons, likely as a result of low sample sizes. While there was no difference in the size of core activity areas among sites, turtle's activity, measured as DDM, was lower in Turbine sites than in Control, which could indicate that Control turtles are utilizing more of their core areas than Turbine turtles. As only a subset of turtles met the requirements to be used in home range analysis, but all turtles were used in DDM measurements, this difference could suggest that both parts of the home range analysis are suffering from type II error. The sex ratios were similar between treatments (Control 3 males:4 females; Turbine 4

males:4 females), so the difference is likely not attributable to sex, which is supported by other studies of painted turtles that showed no difference in daily movements between sexes or among age classes (Rowe and Dalgarn 2010). These differences in both DDM and home ranges could also be due to differences in habitat between sites, even though I tried to choose sites with similar habitat features.

Turtles in the Control site may have access to more habitat than those in Turbine sites (Figure 1.1). Some Control site turtles utilized a large interconnected wetland complex, which was made apparent when 2 turtles moved from their original Control site to the other Control site, resulting in the need to combine the two sites. Therefore, the smaller home ranges in Turbine sites might be due to a lack of interconnected waterbodies; however, this only applies to one Turbine site (T2), from which no turtles emigrated. Two turtles emigrated from the other Turbine site (TW), likely over land as painted turtle display relatively straight-line dispersal (Bowne and White 2004). This is consistent with what has been found in other populations of painted turtles and other species of turtles, in which some individuals disperse over 1 km across land to move between wetlands (Bowne 2008; Edge et al 2010). A minimum buffer of 1 km around both Turbine sites, a conservative distance based on movements reported in other studies (Bowne 2008; Rowe 2003), means other wetlands become available for the T2 turtles (Figure 1.4). So there appears to be accessible habitat; other factors might be encouraging them to stay. However, comparisons to other studies on home ranges suggest the home range sizes of turtles in both Control and Turbine sites were much smaller than those in other populations at a similar latitude (Rowe 2003).

It is possible that resources are plentiful and population density is low enough such that turtles in turbine sites have no need to disperse from current wetlands. It is difficult to quantify availability of food in these sites due to the omnivorous, generalist, and highly variable diet of painted turtles (Cooley et al 2003; Ernst and Lovich 2009). Although the data is not extensive enough for an accurate population density estimate, site T2 likely has a small population as all turtles caught in 2018 were recaptured in 2019 using only the timed wetland surveys. Based on the few sightings of adults in TW, it also likely has a low population density; however, emigration from this site occurred despite low density. Occupation of a habitat is often related to quality, connectivity, and size of wetlands (Cosentino, Schooley, and Philips 2010); connectivity and size both appear to be low in Turbine sites. It should therefore be expected that some turtles in both of the Turbine sites would attempt to disperse, unless there is another factor limiting their movements.

An external abiotic factor may be limiting the movement of turtles within the windfarm. I examined whether painted turtles avoided wind turbines and roads. Avoidance behaviour was detected in Turbine site TW, with the average turtle distance being nearly 80 m further from turbines than the average random location. Turtles in site T2 did not appear to avoid turbines, perhaps because the wetland was far enough from wind turbines to be unaffected, or because they had limited area to which to escape the turbines. Road avoidance behaviour was also noted in Turbine site TW, where the turtles were on average about 40 m further from the road than random points. In contrast, turtles in Control sites did not avoid the adjacent municipal road. Field observations supports this as a total of 13 turtles were found on roads, 10 on municipal roads (4 road mortalities) and only 3 on windfarm service roads (no road mortalities).

The avoidance of windfarm roads but not public roads could be due to a variety of factors. Both windfarm roads and the public road are unpaved, gravel roads of relatively similar construction. Extensive road maintenance in the windfarm is unlikely to be the cause, as under my recommendation, to protect turtles and nests, road maintenance in the windfarm is minimal from nesting season through the gestation period (June-September); whereas public road work continues. We should then expect road maintenance to only be a factor for Control turtles, unless this road avoidance behaviour in Turbine turtles is continuing after the maintenance change. Although not measured, traffic volume on service roads appeared much lower than on the municipal road, probably as a result of the gated access to the windfarm, suggesting that high traffic volume is unlikely to be the cause of avoidance. The windfarm roads appear to have better drainage and are dryer than control roads, which could lead to more sedimentation in the control wetlands due to dust created by traffic. Future studies should measure and compare water quality in wetlands near the roads in both Turbine and Control sites. Although painted turtles are more tolerant of habitats near roads, other species are not as tolerant and should also be considered in future studies concerning road effects on turtle populations (DeCatanzaro and Chow-Fraser 2010).

The extent of infrastructure avoidance is likely not complete avoidance, as the dispersing turtles in site TW likely encountered either road, turbine, or both, if we assume relatively straight-line movements (Bowne 2008; Bowne and White 2004). Female turtles in site TW nested on the windfarm road, so it is clear that the road avoidance is not strong enough to cause complete lack of interaction. It is possible that the habitat close to the roads or turbines is not suitable for long term occupancy. Although a previous study found that roads did not affect stress levels in painted turtles (Baxter-Gilbert et al 2014), it is possible that proximity to roads or turbines cause

physiological stress in turtles. If turtles experience elevated stress hormones as a response to encountering these new stressors, there could be impacts on reproduction (Moore and Jessop 2003).

Despite the apparent differences in turtle movements and avoidance behaviour detected in my study, the possibility that these results were caused by natural environmental differences, not the windfarm, should be considered. Agassiz's desert tortoises living in a wind energy facility not only had similarly-sized activity areas as those in wilderness areas, they also had higher apparent survival (Agha et al 2015). The large publicly-restricted area of a windfarm may be beneficial to some species; however, this is only relevant if the facility itself has no negative impacts.

The goal of my study was to determine if the windfarm negatively impacted painted turtle spatial ecology and behaviour. The results of my study show partial support for my hypotheses as daily movements of turtles in the windfarm appeared smaller, and some avoidance behaviour of windfarm infrastructure was observed, but more studies on turtle spatial ecology around windfarms is needed to make broader conclusions about impacts on home range sizes. As painted turtles, and many species of semi-aquatic turtles, can occupy large and small-scale areas (Marchand, Somers, and Poulin 2018), future studies should consider populations at both scales through long term monitoring. The effects of the windfarm on the habitat in terms of noise, vibration, and pollution, should also be examined. The impacts on fitness of individuals and the population should also be examined. This should include examination of stress responses and body conditions of turtles in the windfarm compared to those in control populations. As turtle nesting was observed on windfarm service roads, the survivorship of these nests and the fitness of hatchlings should be examined. Previous research has found lower reproductive success and

lower genetic diversity due to roads (Laporte, Silva Beaudry and Angers 2012), and it would be crucial to determine if this applies to windfarm roads as well. As reptile and amphibian populations decline across the globe (Bohm et al 2013; Gibbons et al 2000), and climate change becomes an increasing concern for conservation (Pecl et al 2017), the goal of future research should be to support conservation of at-risk animals without dismissing the advantage of green energy alternatives.

Chapter II

Do windfarms impact amphibian ecology? An investigation of amphibian community ecology and anuran acoustics within a Northern Ontario windfarm

Introduction

Noise pollution is a growing concern in the developing world as a non-physical threat to wildlife, which can come from various sources and has a range of negative impacts (Blickley and Patricelli 2010; Francis and Barber 2013). While acute extreme noise can cause physical and developmental damage to wildlife (Kight and Swaddle 2011), most studies are concerned more with chronic background noises that mask natural noise and acoustic communication, which can have negative impacts on populations (Blickley and Patricelli 2010). Studies on birds impacted by traffic noise have found decreases in species diversity and evenness, increased song frequencies (Rheindt 2003), and even reduced reproductive success (Halfwerk et al 2011). Similar to birds, anurans can be very sensitive to anthropogenic noises, showing altered calling behaviours in noisy environments such as near roads (Cunnington and Fahrig 2010). Anurans exposed to traffic noise have shown varied responses, including changing call rates (Sun and Narins 2005) and increased call frequency (Parris, Velik-Lord and North 2009). Plasticity within anuran calls during traffic noise playback has also been observed, showing a direct response to noise pollution (Cunnington and Fahrig 2010).

Globally, amphibian populations are declining rapidly as a result of many threats including climate change, habitat destruction, pollution, and disease (Gibbons et al 2000). In recent years, the wind energy industry has been expanding, creating a new possible threat to these populations in the form of noise pollution (Lovich and Ennen 2013a). Turbine noise has been linked to

changes in the calls of Greater-Prairie Chickens (*Tympanuchus cupido pinnatus*) in populations living close to wind turbines (Whalen et al 2018), although some other avian species appear to be unaffected by turbine noise (Raynor et al 2017). Changes in characteristics of advertisement calls could alter the fitness of these animals by altering mating success (Patricelli and Blickley 2006; Tennessen, Parks, and Langkilde 2014). The presence of chronic noise can also induce physiological stress on female frogs, delaying their arrival at breeding aggregations (Tennessen, Parks, and Langkilde 2014). Despite this possible new threat to amphibians, there are no published studies concerning amphibians or their acoustics near wind turbines.

As wind energy becomes increasingly popular, this gap in knowledge becomes a larger concern for amphibian populations, which are already facing numerous threats (Gibbons et al 2000). Therefore, to help fill this gap in knowledge, I examined the effects of wind turbine proximity on amphibian populations and anuran acoustics. Through transect surveys I compared amphibian population ecology within a windfarm to populations outside the windfarm. I also examined the effects of turbine noise on anuran calls by analyzing both chorus and individual call characteristics within a windfarm and compared these call properties to those of frogs living outside of the windfarm. I hypothesized that if anuran acoustics are negatively affected by turbine noise, then anuran populations and choruses within windfarms would show lower richness and diversity of species than those outside the windfarm. I also hypothesized that individuals within windfarms would have shorter and higher pitched calls than individuals outside the windfarm, as a way to compete with the low frequency wind turbine noise.

Materials and Methods

Transect surveys

Data collection took place between May and September of 2018 and 2019. At each site, 100 m long transects were created perpendicular to wetlands in an east-west direction with markers placed at 10 m intervals (Figure 2.1). At the end of the 2018 field season, new transects were created in new areas at each site and 30 cm² cover boards were added to each transect at 10, 50, and 100 m, and surveyed in the 2019 field season. Each site was randomly visited during the study period and each visit consisted of 2 transect walks, a morning walk at 08:30 and an evening walk at 17:30. Before each walk, researchers recorded air and ground temperatures (°C; Durac Digital Thermometer model 1502A) adjacent to the wetland and basic weather conditions (clear, cloudy, raining, etc.). Each walk consisted of 2-3 researchers walking one side of the transect at a steady pace searching for amphibians, including under natural cover objects and cover boards, not straying more than 5 m from the transect center. At the end of the 100 m transect, researchers walked the transect back to the starting point on the opposite side. Any amphibians found during the transect survey were captured for species identification, and sex determination for species that show sexually dimorphic characteristics identifiable in the field. During the first half of the walk, locations of individuals were noted to prevent double counting the same individuals on the return walk to the starting spot. For each capture, air temperature (°C), ground or water temperature (°C), GPS coordinates, and weather conditions were also recorded.

The transect survey data were used to calculate a species diversity index for each study site using the Shannon-Weiner diversity index (H' ; Equation 1, Spellerberg and Fedor 2003):

$$(1) \quad H' = -\sum_{i=1}^n p_i \ln p_i$$

where p_i is the frequency of species (i) in a community for a number of species (n , Spellerberg and Fedor 2003). Only common species that were observed multiple times or in majority of sites over the study period were used in calculating species diversity to avoid counting rare or cryptic species that might be present in all sites but only recorded in some sites.

H' at each site will then be used to calculate Pielou's evenness (E) for each site (Equation 2, Beisel et al. 2003):

$$(2) \quad E = \frac{H'}{H'_{max}} = \frac{H'}{\ln i}$$

Pielou's evenness creates a ratio for each site using the site's H' and the maximum possible diversity for each site (H'_{max}) determined by the natural logarithm of i species in that site (Beisel et al. 2003). A maximum ratio value of 1 indicates a completely even community of species, such that diversity is evenly spread across all species present in the community (Beisel et al. 2003). Both H' and E were determined using the cumulative counts of each species found during transect surveys at each site. H' and E were then standardized for the number of surveys at each site by dividing each site's indices by the number of surveys conducted. A Shapiro-Wilk normality test was conducted for the standardized H' and E , followed by a Student's t-test to determine if the means differed between treatments.

Species richness was calculated for each survey conducted to determine if there was a difference in the number of species encountered during each visit at control and turbine sites. Menhinik's

Richness index (D) was used to measure species richness as it accounts for both the number of species and the total number of individuals (Equation 3; Magurran 1988).

$$(3) D = \frac{S}{\sqrt{N}}$$

Where S is the number of species within a sample or survey and N is the total number of individuals within the sample (Magurran 1988).

Due to zero inflation of D , caused by surveys that yielded no individuals, a gamma-hurdle approach was used (Zuur et al. 2007). The gamma-hurdle is a two-step analysis, where the first step applies a model with a gamma distribution to the data to analyze the non-zero data, and the second step applies a binomial model to analyze the presence or absence of zeros (Zuur et al. 2007). A Generalized Additive Model (GAM) was used to model both steps of the hurdle, with treatment (Control and Turbine) and sites (4 replicates of each treatment) as factors and Julian date for each survey applied as an un-fixed smoothing function with a cubic spline (Zuur et al. 2007). Models were created and tested using a backwards elimination of fixed effects to analyze the data with a repeated measures ANOVA (Zuur et al. 2007). To determine the differences in D among sites, deviation contrasts of site levels were compared to the overall mean with a Bonferroni corrected p-value ($p=0.00625$), using the *emmeans* package in R (R Core Team 2019; Lenth 2019).

Chorus and Individual Call Characteristics

Anuran call data were collected in 2018 and 2019 between the months of April and July. Pseudo-random paired recordings were conducted in Control and Turbine sites using SM4 Song Meters (Wildlife Acoustics) to capture choruses of anuran calls during their breeding seasons. SM4s

were placed on trees within 5 m of wetlands containing calling anurans, and were scheduled to record chorus activity for 1 hour at sunset and sunrise, and for 15 mins every hour between sunset and sunrise, from mid-April through July, to capture the peak calling periods of all local species (Table I). Ambient air temperature was measured using the SM4 internal thermometer. Chorus richness, diversity, and evenness (Table 2.1) were measured for each night at each site. To account for repeated measures, chorus diversity (H') and evenness (E) were compared between Turbine and Control sites using a forward step method in R to create the best fitting linear mixed effects model, based on the greatest decrease in AIC values for each addition of variables (R Core Team 2019; Kuznetova, Brockhoff, Christensen 2017). Model variables included treatment (Turbine or Control), site (4 replicates of each treatment, nested within treatment, as a random effect), and date (serial date, where January 1, 2018 is equal to 43,101). An analysis of deviance was used on the resulting model to determine if fitted variables had a significant effect on predicting diversity or evenness.

Individual calls of Spring Peepers (*Pseudacris crucifer*) and Green Frogs (*Lithobates clamitans*) were extracted from chorus recordings using a separate classifier created with Kaleidoscope Pro v5.1.9g (Wildlife Acoustics; see below), and 3 call characteristics (Table 2.2) were measured: mean frequency, minimum frequency, and duration. To examine differences in call characteristics between treatments and among sites, a linear mixed effects model was used for each call characteristic (R Core Team 2019; Kuznetova, Brockhoff, Christensen 2017).

Treatment, site (nested within treatment as a random effect), serial date and time (where January 1, 2018 00:00 is equal to 43,101.00), and temperature (recorded by the SM4's internal thermometer) were used as predictor variables. An analysis of deviance was used on the resulting model to determine if fitted variables had a significant effect on predicting call characteristics.

To further examine the effect of site on call characteristics, I used the ANOVA-like table for random effects function, *ranova*, in the R *lmerTest* package (Kuznetova, Brockhoff, Christensen 2017) to compare between models with and without the random effect of site. Differences between sites were examined with a one-way ANOVA followed by a Tukey HSD test.

Kaleidoscope Pro Software Classifiers

Kaleidoscope Pro's Cluster Analysis function was used to count and extract anuran calls from the recordings. The Cluster Analysis works by using pattern recognition to group detected sounds (referred to as detections) with similar sound properties and spectrogram patterns into groups called 'clusters' (Wildlife Acoustics). Kaleidoscope Pro cannot identify what species the resulting clusters are and instead relies on the user to manually identify the source of the sound and rename the clusters. Simple Classifiers are created by identifying and renaming clusters of interest, in this case anuran calls, creating a separate file that can be used to identify sounds in future recordings. Classifiers can then be tuned to increase accuracy of detection and reduce error by manually correcting incorrect detections within each cluster and using these corrections to tune the classifier; this is referred to as an advanced classifier. Classifiers for Turbine and Control recordings were created separately using the same methods to ensure species and call properties that are unique to one treatment were not masked by the other treatment. Separate Kaleidoscope cluster analyses were conducted for chorus and individual recordings, but the same settings were used for both Turbine and Control treatments (Table 2.3). Classifier creation and manual verifications of calls, for both treatments were conducted by the same observer.

I used clips extracted from recordings made during the 2018 field season to create a simple chorus classifier. All detections of anuran calls, regardless of the clusters they were grouped into,

were manually identified to species to create an initial classifier. Using this initial classifier to cluster anuran calls by species, the entire 2018 recording data set was used to create a final advanced classifier by manually correcting any incorrect identifications within clusters. This final advanced classifier was then used in a final cluster analysis containing both 2018 and 2019 recordings, providing the final clusters of each species. The number of detections in these final clusters were used as estimated call abundances of each species for the calculations of chorus diversity and evenness.

To test the accuracy of the final classifier, 1000 identified calls were sampled from the final cluster analysis and determined to be correct, containing at least one vocalization of the identified species, or incorrect, containing no vocalizations of the identified species. To take the sample of identified calls, the final cluster analysis file was first sorted by species then by distance from cluster center. Distance from cluster center is a measurement used by Kaleidoscope to quantify how similar a detected sound is to known clusters within a classifier, where a detection with a distance of 0 is a perfect representation of that cluster. I then sub-sampled 250 calls from the beginning (first quartile), 250 calls from the second, third and final quartiles of all the calls in the file. Classifier accuracy was then determined by the ratio of correct identifications out the 1000 calls to the number of incorrect identifications.

The individual classifier was created to extract both Spring Peeper and Green Frog calls at the same time. Similar to the chorus classifier, individual classifiers for Turbine and Control recordings were created separately using the same methods to ensure call properties that are unique to one treatment were not masked by the other treatment. Using 2018 recordings, an initial classifier for only Spring Peepers and Green Frogs was created by labeling detections that

contained only single calls of these species with no other calls (interspecific or intraspecific) or background noise. Spring Peeper calls without harmonics were selected to create the initial classifier to prevent the software from including harmonics in the mean and minimum frequency measurements. This classifier was then used in a cluster analysis of all 2018 and 2019 recordings and individual calls of each species were then manually labelled for analysis if they contained only one clear call of the target species with no other anuran calls or background noise such as rain or excessive wind. Measurements of call characteristics used in the analyses were taken from the cluster analysis results output.

Results

Transect surveys

A total of 8 species of amphibians were found between Control and Turbine sites, with an average of 5 or more species per site (Table 2.4). While most species were found during transect surveys, some species, such as Eastern Newts (*Notophthalmus viridescens*), were also confirmed in sites opportunistically outside of scheduled surveys or by recordings of their calls in the case of anurans. Both cumulative H' and E data were normally distributed ($W=0.904$, $p>0.05$ and $W=0.904$ $p>0.05$; respectively). Turbine sites had a mean cumulative H' of 0.02325 ± 0.00675 (SD) and E of 0.0118 ± 0.00325 . The Control mean H' (0.02053 ± 0.00859) was not significantly different from the Turbine mean ($t=-0.50$, $df=5.68$, $p>0.05$). Neither did the Control mean E (0.00987 ± 0.00413) differ significantly from Turbine sites ($t=-0.50$, $df=5.68$, $p>0.05$).

The first step of the gamma-hurdle, the generalized additive model (GAM) with a gamma distribution for non-zero data, resulted in the removal of the treatment factor (Control or Turbine), from the GAM model as a non-significant predictor of species richness D

($F(1,195.9)=0.23$, $p>0.05$). The two significant predictors that remained in the final accepted model were site ($F(7,195.9)=4.46$, $p<0.01$) and the smoothing factor of Julian date ($F(1.103,195.9)=4.96$, $p<0.05$; Figure 2.3). Sites C3 ($t=-4.014$, $df=196$, $p<0.00625$), C4 ($t=-3.52$, $df=196$, $p<0.00625$), and T4 ($t=-3.16$, $df=196$, $p<0.00625$) were significantly different from the overall site mean. The second step of the hurdle, binomial model for zero data, found treatment ($\chi^2(1,322.2)=5.75$, $p<0.05$), site ($\chi^2(7,322.2)=22.086$, $p<0.01$), and the smoothing factor of Julian date ($\chi^2(7.827,322.2)=43.23$, $p<0.001$) to be significant predictors of D. The likelihood of finding an individual amphibian was higher in Turbine sites than in Control sites, and increased with Julian date. Site T2 was marginally different from the overall mean ($t=2.73$, $df=322$, $p=0.00671$).

Chorus Characteristics

Calls of 6 anuran species were recorded during the 2018 and 2019 field seasons (Table 2.5).

Green Frogs, Spring Peepers, and American Toads (*Anaxyrus americanus*) were recorded in both Control and Turbine sites. However, American Bullfrogs (*Lithobates catesbeianus*), Wood Frogs (*Lithobates sylvaticus*), and Gray Treefrogs (*Hyla versicolor*) were recorded only in Control sites. The Kaleidoscope Pro software has limited ability to differentiate rare species, so some caution should be taken for Gray Treefrog and Wood Frog abundance, as the classifier was less accurate for these rare species. For example, although Gray Treefrogs were identified by the software in all 4 Control sites, field observations confirmed their presence only in Control Site 1. American Bullfrogs were unique to Control sites in both recordings and field observations. Despite these limitations, the software correctly identified species 95% and 92% of the time for Control and Turbine sites, respectively.

The final mixed linear effects model with the best AIC values was accepted after two forward steps following the null model (Table 2.6). The final accepted model contained only Treatment (Control or Turbine) and Date as predictor variables for both diversity (H') and evenness (E). H' and E differed significantly between Treatments (H': $\chi^2=23.42$, $df=1$, $p<0.001$, Figure 2.4; E: $\chi^2=23.42$, $df=1$, $p<0.001$), and differed marginally among Dates (H': $\chi^2=3.18$, $df=1$, $p=0.075$; E: $\chi^2=3.18$, $df=1$, $p=0.075$).

Individual Call Characteristics

A total of 1648 Green Frog calls were analyzed for individual call characteristics, 626 calls from Control sites and 1058 calls from Turbine sites (Figure 2.5; Table 2.7). Mean frequency, minimum frequency, and durations of Green Frog calls did not differ between treatments (Table 2.7). Date and time were significant predictors for call duration in Green Frogs, with a slight increase in call duration later in the season (Table 2.8). Temperature was a significant predictor for all Green Frog call characteristics, with a negative trend on mean frequency, minimum frequency and duration with a decrease in frequency and duration when temperature increases (Table 2.8). Site had a significant effect on the models for all 3 Green Frog call characteristics (Table 2.8).

A total of 1576 Spring Peeper calls were analyzed for individual call characteristics, 798 calls from Control sites and 778 calls from Turbine sites (Figure 2.5; Table 2.7). Mean frequency, minimum frequency, and durations of Spring Peeper calls did not differ between treatments (Table 2.7). Date and time, temperature, and site were significant predictors of all 3 call characteristics of Spring Peepers (Table 2.8).

Because site had a significant effect on call characteristics, I performed Tukey's HSD post hoc analyses to detect groupings of sites for each call characteristic for Green Frogs (Figure 2.6) and Spring Peepers (Figure 2.7). I found significant differences among Control sites in mean call frequency ($F(3,622)=30.73, p<0.001$), minimum call frequency ($F(3,622)=9.28, p<0.001$), and call duration ($F(3,622)=89.93, p<0.001$) for Green Frogs. Green Frog call characteristics in C1 and C4 never differed, whereas characteristics in C2 and C3 often differed from the other control sites. I found significant differences among Turbine sites in mean call frequency ($F(3,1054)=10.62, p<0.001$) and call duration ($F(3,1054)=3.96, p<0.01$), but not minimum call frequency ($F(3,1054)=1.021, p>0.05$) for Green Frogs. Mean call frequency of Green Frog calls in T1 were significantly different than mean frequencies in all other Turbine sites.

I found significant differences among Control sites in mean call frequency ($F(3,794)=6.27, p<0.001$), minimum call frequency ($F(3,794)=63.15, p<0.001$), and call duration ($F(3,794)=68.08, p<0.001$) for Spring Peepers. Similar to Green Frogs, Spring Peeper calls did not differ between C1 and C4 whereas C2 and C3 again differed from the other control sites. I found significant differences among Turbine sites in mean call frequency ($F(3,774)=15.16, p<0.001$), minimum call frequency ($F(3,774)=21.41, p<0.001$), and call duration ($F(3,774)=55.19, p<0.001$) for Spring Peepers. Spring Peeper calls in T1 often differed from the other turbine sites.

Discussion

Amphibian populations are declining globally (Gibbons et al 2000), yet they are largely overlooked in research investigating the impacts of windfarms. I found evidence of negative impacts of wind turbines on amphibian communities in terms of species richness. While field

transect surveys found similar numbers of species in Control and Turbines sites, acoustic recordings revealed a chorus richness of only 3 anuran species in wetlands located close to wind turbines, in contrast to Control site wetlands that had 6 species, suggesting that the presence of wind turbines could be reducing anuran chorus richness. This difference in chorus richness translated into differences in both chorus diversity and chorus evenness between Turbine sites and Control sites. This suggests that wind turbines could have an impact on the calling environment for anurans. However, transect surveys found similar species richness, diversity and evenness between treatments.

The differences in call diversity and evenness could be caused by a variety of factors, including infrastructure, construction and habitat alteration associated with windfarms. Windfarm service roads could increase road density around windfarm wetlands, leading to road mortality of frogs during migrations (Glista, DeVault, and DeWoody 2008; Heigl et al 2008), and to reduced habitat quality in adjacent wetlands from pollution run-off (Hall et al 2017). However, the low traffic volume on the windfarm service road might not be high enough to cause a significant impact through road mortality. Increased road density is known to negatively impact frog species richness due to mortality of species sensitive to forest loss in wetlands near roads (Findlay, Lenton, and Zheng 2001). One of these sensitive species are wood frogs (Findlay, Lenton, and Zheng 2001), a species whose calls I did not record within the windfarm but that I observed during transect surveys. The combined effect of these known impacts on frog populations could be the reasons for lower chorus richness in the windfarm sites.

Habitat suitability could have caused the differences in anuran chorus richness. Despite our best efforts to match the environments in Control and Turbine sites, the difference in species richness

could be due to a lack of habitat for more sensitive species. Bullfrogs for example were recorded exclusively in Control sites; I did not record their calls or observe them in Turbine sites, and it is not possible to determine whether their absence was due to the impact of the windfarm or simply due to unsuitable habitat. Bullfrogs in northern ranges typically breed in permanent waterbodies that do not freeze to the substrate (Durham and Bennett 1963; Nie, Crim, and Ultsch 1999), meaning our largely beaver-created wetlands may not be ideal habitat. However, these waterbodies are not seasonal and, in most cases, have existed since at least the construction of the windfarm in 2006. Also, Eastern Newts, a mostly aquatic species that often remain in wetlands year-round as adults (Grayson, Bailey, and Wilbur 2011; Roe and Grayson 2008), were found in all Turbine wetlands, suggesting these waterbodies do not freeze to the substrate.

Bullfrogs being present in Control sites shows they are in the region, just possibly not in the windfarm. Although Bullfrogs were not found in the initial Environmental impact statement (EIS) in 2002-2004, prior to windfarm construction, their previous absence could be due to the survey methods for amphibians and reptiles, which were described as opportunistic with auditory surveys in the spring and fall, possibly outside Bullfrog breeding season (Prince Power Project EIS 2005). Bullfrogs are considered excellent dispersers, often using semi-permanent wetlands for dispersal, and are often one of the first species to colonize new wetlands (Gahl, Calhoun, and Graves 2009). It is for this reason they have invaded many areas and countries and are considered one of the most invasive species in the world (Ficetola, Thuiller, and Miaud 2007). We should therefore expect to see Bullfrogs in the windfarm wetlands if the species is present in the area, even if the wetlands are not used for calling and breeding.

Considering the open spaces created by the service roads, as well as the open areas directly under the wind turbines, the infrastructure of a windfarm creates large areas of low canopy cover. As a result, most of a windfarm's footprint is not the turbines themselves, which only contribute 1-10% of the footprint, it is the infrastructure (Lovich and Ennen 2013a). In many cases, distances and success of frog dispersal, as well as species richness of amphibians surrounding wetlands, are positively correlated with increased canopy cover (Patrick, Hunter, and Calhoun 2006; Crosby, Licht, and Fu 2008; Houlihan and Findlay 2003). These low canopy areas could be fragmenting the habitat, impeding dispersal of frogs within the windfarm. Although noise pollution alone may not impact dispersal, the combination of road noise and reduced canopy cover can alter dispersal for some species (Nakano et al 2018). However, the results of the transect surveys contradicts this explanation, as not only did we find similar diversity, evenness, and richness between Control and Turbine sites; when amphibians were found, they were more often in Turbine sites than Control sites according to the binomial portion of the gamma hurdle analysis.

Noise pollution creates a new calling environment for species that use auditory communication (Blickley & Patricelli 2010). This has been documented for both birds and frogs, which adjust their calls around noisy roads (Cunnington & Fahrig 2010; Parris, Velik-Lord and North 2009; Rheindt 2003; Patricelli & Blickley 2006). I tested if this was the case for Green Frogs and Spring Peepers around the noise of wind turbines. I found that there were no differences in call characteristics between Control and Turbine sites in both species. Instead, the major predictors of call characteristics were date and time, temperature, or both, suggesting that turbine noise is not a significant source of noise pollution for these frog species. Although there were no differences in call characteristics between treatments, there were differences among the sites.

The differences in call characteristics among the sites regardless of treatment suggest that factors other than, or in addition to, turbine presence are impacting frog calling behaviour. In the case of mean call frequency of Green Frogs, Control sites C2 and C3 grouped with most of the Turbine sites. In contrast, Control sites C1 and C4 were distinct from most Turbine sites, apart from T1, which was often distinct from the other turbine sites. A likely explanation for the separation of C2 and C3 from the other two Control sites is the presence of roads near the wetlands in C2 and C3. In support, Cunnington & Fahrig (2010) documented that Green Frogs adjust the dominant frequency of their calls in response to road noise, calling at a higher frequency in the noisy environment, similar to what is seen in C2 and C3 with frogs calling higher than in the other Control sites. When designing and planning this study, I assumed that the low traffic volume on the surrounding roads would not impact frogs living in Control site wetlands. However, my data indicate that Green Frogs within the windfarm are calling in similar ways to Green Frogs close to roads. Future studies should include differentiation of windfarm presence (e.g., service roads, other infrastructure) from wind turbine noise. Audio playback methods of recorded noises (Cunnington & Fahrig 2010; Hanna et al 2014) could be used to test multiple treatments including turbine noise, service road noise, and turbine and service road noise combined, compared to controls.

When considering wind turbine noise, it should be noted that the turbines in my study are only 1.5 MW, smaller than newer 2-3.5 MW turbines. Although the sound pressure (or amplitude) of smaller and larger turbines can differ at higher and lower frequency ranges, frequencies between 250 – 4000 Hz, where majority of the noise is produced, is similar for both large and small turbines (Moller and Pedersen 2011). Ontario frogs also call within this frequency range, and road noise is also within this range (Cunnington and Fahrig 2010). It is therefore be expected that

frogs would adjust their calls in a similar way when near wind turbines or roads. However, what does differ between wind turbines and roads is the persistence of the noise. Wind turbines typically operate throughout day and night (Shuster, Bulling, and Koppel 2015), whereas road noise can decrease at night (Vargas-Salinas et al 2014). To compete with noise, frogs calling around roads can adjust their calling to times when traffic volume and noise are low (Vargas-Salinas et al 2014); however, this option is not available to frogs calling around turbines if operation continues through the night. As a result, frogs within windfarms would need to adjust the frequency of their calls at all times or not call until turbine noise ceases, which may not happen often or at all during their breeding period. Future studies should consider altering the operation times of wind turbines to determine if frogs will shift their calling period to when turbines are not running.

I did not find Bullfrogs in the windfarm during my study. Should windfarm noise be a source of chronic noise pollution, it is possible that Bullfrogs are falling victim to it. Bullfrogs call at a low frequency (0.2-2 kHz), compared to Green Frogs (Simmons, Simmons, and Bates 2008), directly overlapping with the low frequency noise of both roads (measured during this study: 0-3 kHz at closest range, 0.5-1.5 kHz at a distance of about 200 m) and turbine noise. This direct overlap could mean that Bullfrogs are unable to adjust their calls or compete with the turbine noise, leading to a lack of breeding, and thus eventually extirpation, explaining their absence from Turbine sites. This hypothesis could be tested with an audio playback method, by playing recordings of wind turbine noise to calling males and determining if they continue to call during and after playback, as well as determine if they adjust their calls (Cunnington & Fahrig 2010; Hanna et al 2014). In contrast to Bullfrogs, Spring Peepers call at a high frequency (Hanna et al 2014), higher than that of turbine noise. As a result, Spring Peepers do not need to adjust their

calls to compete with low frequency noise of the wind turbines. This can be seen in the post hoc results that show no obvious separation of Control sites from Turbine sites. Furthermore, the testing of this hypothesis does not explain the absence of other high frequency calling species, such as Gray Treefrogs, from Turbine sites. However, Gray Treefrogs could be a new species to the area as they were not included in the list of species in the region during the initial EIS.

Although my study was unable to provide conclusive results about whether frogs call differently in the presence of wind turbine noise, I did find evidence that the presence of a windfarm has negative impacts on amphibian biodiversity. Differences in diversity and evenness only occurring for anuran choruses, and not within the habitat based on transect surveys, suggests a possible impact on the calling environment around wind turbines. The suggested future studies should be conducted to determine the extent to which windfarms impact amphibian population persistence and individual fitness. As the wind energy industry grows, these gaps in knowledge become evermore worrisome, especially when they concern a group of animals that face multiple threats and are declining at an alarming rate (Grant et al 2019; Lesbarrères et al 2014; McCallum 2007).

General Discussion

Green energy sources are becoming increasingly popular around the globe, but the definition of 'green' needs to extend to the ecosystems as well as the emissions. Unfortunately, these rapid developments often lack quantified knowledge about their impacts on ecosystems. Wind energy has been criticized for its negative impacts on animals, most notably direct mortality of birds and bats (Frick et al 2017; Masden et al 2009; Smallwood 2013). The impacts of this industry on reptile and amphibian populations are under-investigated, despite concerns being raised (Coyle 2016; Lovich et al 2011a; Lovich and Ennen 2013a), and despite general concerns over declines in many populations of these taxa from other threats (Bohm et al 2013; Gibbons et al 2000). The purpose of my study was to help fill these gaps in knowledge about reptiles and amphibians in windfarms. My study detected some negative impacts of windfarms on populations and behaviours of both reptiles and amphibians, providing some of the first stepping stones in determining how wind energy interacts with these animals, allowing us to determine next steps in research and solutions through mitigation.

I found evidence of altered turtle spatial ecology (e.g., smaller movements) and apparent avoidance of wind turbines and service roads. Habitat fragmentation and destruction are the leading threats to both reptiles and amphibians (Lesbarrères et al 2014). This apparent avoidance behaviour of service roads and turbines could further the disturbance and fragmentation of habitats where windfarms are present. Future studies should consider long-term monitoring of turtle populations in windfarms that reside in both large- and small-scale landscapes such as lakes and small wetlands, to determine if this behaviour continues to be observed in all habitat types. Wetlands of various distances from turbines and service roads should also be considered

to determine if this behaviour is only seen in populations very close to turbines. On top of spatial ecology, physiological impacts on turtles in terms of stress, body condition, and reproductive and individual fitness, should also be examined. As previous research has found lower reproductive success around roads (Laporte, Silva Beaudry and Angers 2012), and in some cases populations shifting away from even sex ratios due road mortality of females (Gibbs and Steen 2005; Dupuis-Désormeaux et al 2017), ensuring this does not apply to windfarm service roads should be a high priority.

Current mitigation practices should focus on the semi-controllable threats of road mortality and nest destruction. This can be done by maintaining the recommended practice of minimizing road construction and maintenance during the nesting and gestation period (June-September), and applying this practice to all locations where turtles are present in the windfarm. Should serious problem areas arise, preventative structures such as exclusion fencing, could be implemented. However, research to identify turtle travel corridors should first be conducted as improper use of mitigation structures can result in increased road mortality and partial fencing is ineffective and can result in more reptiles on roads (Baxter-Gilbert et al 2015; Markle et al 2017). Although it was not a focus of my research, I did not detect any mortalities or nest destruction on service roads, while mortalities were often seen on public roads. In an act of good stewardship, advocating to raise awareness and mitigate turtle road mortality on public roads would benefit local turtle conservation.

Although direct impacts can be obvious and are a high concern, indirect or less obvious impacts can go unnoticed. My study aimed to look at the impacts of windfarms on amphibian ecology and behaviour through surveys and frog call recordings. While transect surveys did not detect

differences between Turbine and Control sites in species richness and diversity, acoustic analyses detected lower frog chorus diversity and richness in Turbine sites, suggesting a possible impact on calling environment. This difference was most obvious in the lack of a very common low frequency calling frog, Bullfrogs. Analyses of call characteristics of Green Frogs suggest that low frequency noise pollution, similar to that of road noise, is being perceived by the frogs resulting in adjusted calls. Should the sound be chronic pollution to a frog species that cannot compete or call over the sound, these frogs may choose not to call or avoid these affected areas leading to extirpation. This could lead to community impacts with reduced diversity, similar to what was found in my research, or fitness consequences if reproductive success is reduced for species that cannot call or must adjust calls to compete with anthropogenic noise pollution (Patricelli and Blickley 2006; Tennessen, Parks, and Langkilde 2014).

Future studies should aim to determine with confidence if anthropogenic noise in windfarms is impacting frog calls and the extent of the impact. Through audio play-back experiments done in other studies (Cunnington & Fahrig 2010; Hanna et al 2014), various recordings of noises (roads, wind turbines, and controls), can be played to calling frogs and responses can be measured to detect adjustments in call characteristic or refusal to call. If male frogs alter their calls, then recordings of the altered calls can then be played to females to examine if mate attraction, and thus reproductive success, is affected. Unfortunately, until this research is conducted, it is difficult to recommend mitigation methods that do not seriously affect windfarm operations. Until future studies are conducted, the best option currently available is to ensure that healthy environments for breeding amphibians are present, protected, and actively monitored.

The goal of my study was to fill the gaps in knowledge about the affects of windfarms on reptile and amphibian ecology and behaviour. Although many questions still remain unanswered, my research helps to narrow the scope of the topic for future research. Through continued research providing knowledge for mitigation and prevention of impacts, expansion in wind energy can continue while ensuring the safety and protection of at-risk species. Prince Wind provides the unique ability to study wildlife in a natural environment in the presence of a windfarm, a condition that is not often available in other windfarms. It is strongly encouraged that Evolugen and Prince Wind remain active in the pursuit of knowledge and conservation through environmental studies and active stewardship of local flora and fauna.

References

- Agha M, Lovich J, Ennen J, Augustine B, Arundel T, Murphy M, Meyer-Wilkins K, Bjurlin C, Delaney D, Briggs J, Austin M, Madrak S, and Price S. 2015. Turbines and terrestrial vertebrates: variation in tortoise survivorship between a wind energy facility and an adjacent undisturbed wildland area in the desert southwest (USA). *Envir. Mang.* **56**(2):332-341.
- Alroy J. 2015. Current extinction rates of reptiles and amphibians. *PNAS*. **112**(42):13003-13008.
- ATS. Advanced Selemetry Systems. <<https://atstrack.com/tracking-products/transmitters/product-transmitters.aspx?serie=R1800>> retrieved on 2018-10-25.
- Baxter-Gilbert J, Riley J, Lesbarrères D, and Litzgus J. 2015. Mitigating reptile road mortality: fence failures compromise ecopassage effectiveness. *PLoS One*. **10**(3):e0120537.
- Baxter-Gilbert J, Riley J, Mastro Monaco G, Litzgus J, and Lesbarrères D. 2014. A novel technique to measure chronic levels of corticosterone in turtles living around a major roadway. *Conserv. Physiol.* **2**(1):cou036.
- Beisel J, Usselglio-Polatera P, Bachmann V, and Moreteau J. 2003. A comparative analysis of evenness index sensitivity. *Internat. Rev. Hydrobiol.* **88**(1):3-15.
- Blickley J, and Patricelli G. 2010. Impacts of anthropogenic noise on wildlife: research priorities for the development of standards and mitigation. *J. Int. Wildl. Law Policy*. **13**(4):274-292.

- Böhm M. et al. 2013. The conservation status of the world's reptiles. *Biol. Conserv.* **157**:372-385.
- Bowne D. 2008. Terrestrial activity of *Chrysemys picta* in Northern Virginia. *Copeia*. **2**:306-310.
- Bowne D and White H. 2004. Searching strategy of the painted turtle *Chrysemys picta* across spatial scales. *Anim. Behav.* **68**(6):1401-1409.
- Calenge C. 2006. The package *adehabitat* for the R software: a tool for the analysis of space and habitat use by animals. *Ecol Model.* **197**:516-519.
- Chen H and Koprowski J. 2016. Barrier effects of roads on an endangered forest obligate: influences of traffic, road edges, and gaps. *Biol. Conserv.* **199**:33-40.
- Cooley C, Floyd A, Dolinger A, and Tucker P. 2003. Demography and diet of the painted turtle (*Chrysemys picta*) at high-elevation sites in Southwestern Colorado. *Southwest. Nat.* **48**(1):47-53.
- Cosentino B, Schooley R, and Phillips C. 2010. Wetland hydrology, area, and isolation influence occupancy and spatial turnover of the painted turtle, *Chrysemys picta*. *Landscape Ecol.* **25**:1589-1600.
- Coyle J. 2016. Blanding's turtle protected as turbine approval revoked. *The Toronto Star*.
<<https://www.thestar.com/news/canada/2016/06/07/blandings-turtle-protected-as-turbine-approval-revoked.html>> retrieved on 2018-10-25.

- Crosby K, Licht L, and Fu J. 2009. The effect of habitat fragmentation on finescale population structure of wood frogs (*Rana sylvatica*). *Conserv. Genet.* **10**:1707-1718.
- Cunnington G and Fahrig L. 2010. Plasticity in the vocalizations of anurans in response to traffic noise. *Acta Oecol.* **36**(5):463-470.
- Cushman S. 2006. Effects of habitat loss and fragmentation on amphibians: A review and prospectus. *Biol Conserv.* **128**(2):231-240.
- DeCatanzaro R and Chow-Fraser P. 2010. Relationship of road density and marsh condition to turtle assemblage characteristics in the Laurentian Great Lakes. *J. Great Lakes Res.* **36**(2):357-365.
- Dinno A. 2015. Nonparametric pairwise multiple comparisons in independent groups using Dunn's test. *Stata J.* **15**(1):292-300.
- Dupuis-Désormeaux M, D'Elia V, Cook C, Pearson J, Adhikari V, and MacDonald S. 2017. Remarkable male bias in a population of Midland Painted Turtles (*Chrysemys picta marginata*) in Ontario, Canada. *Herpetol. Conserv. Bio.* **12**:225-232.
- Durham L and Bennett G. 1963. Age, growth, and homing in the bullfrog. *J. Wildlife Manage.* **27**(1):107-123.
- Edge C, Steinberg B, Brooks R, and Litzgus J. 2015. Habitat selection by Blanding's turtles (*Emydoidea blandingii*) in a relatively pristine landscape. *Écoscience.* **17**(1):90-99.

- Ernst C and Lovich J. 2009. Turtles of the United States and Canada (2nd edition). The Johns Hopkins University Press, Baltimore, Maryland.
- ESRI 2019. ArcGIS Desktop: Release 10.7.1. Redlands, CA: Environmental Systems Research Institute.
- Fahrig L and Rytwinski T. 2009. Effects of roads on animal abundance: an empirical review and synthesis. *Ecol. Soc.* **14**(1):21.
- Ficetola G, Thuiller W, and Miaud C. 2007. Prediction and validation of the potential global distribution of a problematic alien invasive species – the American bullfrog. *Divers. Distrib.* **13**(4):476-485.
- Findlay S, Lenton J, and Zheng L. 2001. Land-use correlates of anuran community richness and composition in southeastern Ontario wetlands. *Écoscience.* **8**(3):336-343.
- Francis C and Barber J. 2013. A framework for understanding noise impacts on wildlife: an urgent conservation priority. *Front. Ecol. Environ.* **11**(6):305-313.
- Frick W, Baerwald E, Pollock J, Barclay R, Szymanski J, Weller T, Russell A, Loeb S, Medellin R, and McGuire L. 2017. Fatalities at wind turbines may threaten population viability of a migratory bat. *Biol. Conserv.* **209**:172-177.
- Gahl M, Calhoun A, and Graves R. 2009. Facultative use of seasonal pools by American bullfrogs (*Rana catesbeiana*). *Wetlands.* **29**(2):697-703.

Gibbons W, Scott D, Ryan T, Buhlmann K, Tuberville T, Metts B, Greene J, Mills T, Leiden P, Winne C. 2000. The global decline of reptiles, déjà vu amphibians: reptile species are declining on a global scale. Six significant threats to reptile populations are habitat loss and degradation, introduced invasive species, environmental pollution, disease, unsustainable use, and global climate change. *BioScience*. **50**(8):653-666.

Gibbs J and Steen D. 2008. Trends in sex ratios of turtles in the United States: implications of road mortality. *Biol Conserv*. **19**(2):552-556.

Gibson L, Wilman E, and Laurence W. 2017. How green is 'green' energy? *Trends. Ecol. Evol.* **32**(12):922-935.

Glista D, DeVault T, and DeWoody A. 2007. Vertebrate road mortality predominantly impacts amphibians. *Herpetol. Conserv. Bio*. **3**(1):77-87.

Grant E, Muths E, Schmidt B, and Petrovan S. 2019. Amphibian conservation in the Anthropocene. *Biol. Conserv*. **236**:543-547.

Grayson K, Bailey L, and Wilbur H. 2011. Life history benefits of residency in a partially migrating pond-breeding amphibian. *Ecology*. **92**(6):1236-1246.

GWEC. 2018. Global wind report. Global Wind Energy Council.

<<http://gwec.net/publications/global-wind-report-2/>> retrieved on 2018-10-25.

Halfwerk W, Holleman L, Lessells C, and Slabbekoorn H. 2011. Negative impact of traffic noise on avian reproductive success. *J. Appl. Ecol*. **48**(1):210-219.

- Hall E, Brady S, Mattheus N, Earley R, Diamond M, and Crespi E. 2017. Physiological consequences of exposure to salinized roadside ponds on wood frog larvae and adults. *Biol Conserv.* **209**:98-106.
- Hanna D, Wilson D, Blouin-Demers G, and Mennill D. 2014. Spring peepers *Pseudacris crucifer* modify their call structure in response to noise. *Curr. Zool.* **60**(4):438-448.
- Heigl F, Horvath K, Laaha G, and Zaller J. 2017. Amphibian and reptile road-kills on tertiary roads in relation to landscape structure: using a citizen science approach with open-access land cover data. *BMC Ecol.* **17**(24).
- Houlahan J and Findlay S. 2003. The effects of adjacent land use on wetland amphibian species richness and community composition. *Can. J. Fish. Aquat. Sci.* **60**(9):1078-1094.
- Keehn J and Feldman C. 2018. Predator attack rates and anti-predator behavior of Side-Blotched Lizards (*Uta stansburiana*) at Southern California wind farms, USA. *Herpetol. Conserv. Biol.* **13**(1):194-204.
- Kight C and Swaddle J. 2011. How and why environmental noise impacts animals: an integrative, mechanistic review. *Ecol. Lett.* **14**(10):1052-1061.
- Kuznetsova A, Brockhoff P, Christensen R. 2017. *lmerTest* Package: tests in linear mixed effects models. *J. Stat. Softw.* **82**(13):1-26.
- Kuvlesky W, Brennan L, Morrison M, Boydston K, Ballard B, and Bryant F. 2007. Wind energy development and wildlife conservation: challenges and opportunities. *J. Wildl. Manag.* **71**(8):2487-2498.

- Laporte M, Silva Beaudry, and Angers B. 2013. Effects of road proximity on genetic diversity and reproductive success of the painted turtle (*Chrysemys picta*). *Conserv Genet.* **14**:21-30.
- Lavery J, Korol B, and Litzgus J. 2016. Measuring the effects of water-based recreation on the spatial ecology of Eastern Musk Turtles (*Sternotherus odoratus*) in a provincial park in Ontario, Canada. *Copeia* **104**(2):440-447.
- Lenth R. 2019. *emmeans*: Estimated marginal means, aka least-squares means. R package version 1.4.2.
- Lesbarrères D, Ashpole S, Bishop C, Blouin-Demers G, Brooks R, Echaubard P, Govindarajulu P, Green D, Hecnar S, Herman T, Houlahan J, Litzgus J, Mazerolle M, Paszkowski C, Rutherford P, Schock D, Storey K, Loughheed S. 2014. Conservation of herpetofauna in northern landscapes: threats and challenges from a Canadian perspective. *Biol. Conserv.* **170**:48-55.
- Lovich J and Ennen J. 2013a. Assessing the state of knowledge of utility-scale wind energy development and operation on non-volant terrestrial and marine wildlife. *Appl. Energy.* **103**:52-60.
- Lovich J and Ennen J. 2013b. A quantitative analysis of the state of knowledge of turtles of the United States and Canada. *Amphibia-Reptilia.* **34**(1):11-23.

- Lovich J, Ennen J, Madrak S, and Grover B. 2011a. Turtles and culverts, and alternative energy development: an unreported but potentially significant mortality threat to the Desert Tortoise (*Gopherus agassizii*). *Chelonian Conserv. Biol.* **10**(1):124-129.
- Lovich J, Ennen, J, Madrak, S, Meyer K, Loughran, C, Bjurlin, C, Arundel T, Turner W, Jones C, and Groenendaal G. 2011b. Effects of wind energy production on growth, demography, and survivorship of a desert tortoise (*Gopherus agassizii*) population in Southern California with comparisons to natural populations. *Herpetol. Conserv. Biol.* **6**(2):161-174.
- Magurran A. 1988. Diversity indices and species abundance models. In: *Ecological diversity and its measurement*. Springer, Dordrecht.
- Marchand K, Somers C, and Poulin R. 2018. Spatial ecology and multi-scale habitat selection by Western Painted Turtles (*Chrysemys picta bellii*) in an urban area. *Can. Field-Nat.* **132**(2):108-119.
- Markle C, Gillingwater S, Levick R, Chow-Fraser P. 2017. The true cost of partial fencing: Evaluating strategies to reduce reptile road mortality. *Wildl. Soc. Bull.* **41**:342-350.
- Masden E, Haydon D, Fox A, Furness R, Bullman R, and Desholm M. 2009. Barriers to movement: impacts of wind farms on migrating birds. *ICES J. Mar. Sci.* **66**(4):746-753.
- McCallum M. 2007. Amphibian decline or extinction? Current declines dwarf background extinction rate. *J. Herpetol.* **41**(3):483-491.

Moller H and Pedersen C. 2011. Low-frequency noise from large wind turbines. *J. Acoust. Soc. Am.* **126**(6):3727-3744.

Moore I and Jessop T. 2003. Stress, reproduction, and adrenocortical modulation in amphibians and reptiles. *Horm. Behav.* **43**(1):39-47.

Nakano Y, Senzaki M, Ishiyama N, Yamanaka S, Miura K, and Nakamura F. 2018. Noise pollution alters matrix permeability for dispersing anurans: differential effects among land covers. *Glob. Ecol. Conserv.* **16**(2018):e00484

Nie M, Crim J, and Ultsch G. 1999. Dissolved oxygen, temperature, and habitat selection by Bullfrog (*Rana catesbeiana*) tadpoles. *Copeia.* **1999**(1):153-162.

Ogle D, Wheeler P, Dinno A. 2020. FSA: Fisheries Stock Analysis. R package version 0.8.30. <<https://github.com/droglenc/FSA>>

Parris K, Velik-Lord M, and North J. 2009. Frogs call at a higher pitch in traffic noise. **14**(1):25.

Patricelli G and Blickley J. 2006. Avian communication in urban noise: causes and consequences of vocal adjustment. *Auk.* **123**(3):639-649.

Patrick D, Hunter M, and Calhoun A. 2006. Effects of experimental forestry treatments on a Maine amphibian community. *For. Ecol. Manag.* **234**(1-3):323-332.

Pecl G et al. 2017. Biodiversity redistribution under climate change: impacts on ecosystems and human well-being. *Science* **355**(6332): eaai9214.

- Powell R, Conant R, and Collins J. 2016. Peterson field guide to reptiles and amphibians of eastern and central North America (4th edition). Houghton Mifflin Harcourt. Boston, MA.
- Prince Power Project Environmental Impact Statement (EIS). 2005. Unpublished data presented for Brookfield Renewable (Formerly Brascan Power).
- R Core Team. 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. < <https://www.R-project.org/> >
- Rabin L, Coss R, and Owings D. 2006. The effects of wind turbines on antipredator behavior in California ground squirrels (*Spermophilus beecheyi*). Biol. Conserv. **131**(3):410-420.
- Raynor E, Whalen C, Brown M, and Powell L. 2017. Grassland bird community and acoustic complexity appear unaffected by proximity to a wind energy facility in the Nebraska Sandhills. Condor **119**:484-496.
- Rheindt F. 2003. The impact of roads on birds: does song frequency play a role in determining susceptibility to noise pollution? J. Ornithol. **144**(3):295-306.
- Roe A and Grayson K. 2008. Terrestrial movements and habitat use of juvenile and emigrating adult Eastern Red-Spotted Newts, *Notophthalmus viridescens*. J. Herpetol. **42**(1):22-30.
- Row J and Blouin-Demers G. 2006. Kernels are not accurate estimators of home-range size for herpetofauna. Copeia **2006**(4):797-802.
- Rowe J. 2003. Activity and movements of Midland Painted Turtles (*Chrysemys picta marginata*) living in a small marsh system on Beaver Island, Michigan. J. Herpetol. **37**(2):342-353.

- Rowe J and Dalgarn S. 2010. Home range size and daily movements of Midland Painted Turtles (*Chrysemys picta marginata*) in relation to body size, sex, and weather patterns. *Herpetol. Conserv. Biol.* **5**(3):461-473.
- Santos M, Bastos R, Travassos P, Bessa R, Repas M, and Cabral J. 2010. Predicting the trends of vertebrate species richness as a response to wind farms installation in mountain ecosystems of northwest Portugal. *Ecol. Indic.* **10**(2):192-205.
- Schuster E, Bulling L, and Koppel J. 2015. Consolidating the state of knowledge: A synoptical review of wind energy's wildlife effects. *Envir. Mang.* **56**: 300-331.
- Shaffer J and Buhl D. 2016. Effects of wind-energy facilities on breeding grassland bird distributions. *Conserv. Biol.* **30**(1):59-71.
- Simmons A, Simmons J, and Bates M. 2008. Analyzing acoustic interactions in natural bullfrog choruses. *J. Comp. Psychol.* **122**(3):274-282.
- Skarin A and Alam M. 2017. Reindeer habitat use in relation to two small wind farms, during preconstruction, construction, and operation. *Ecol. Evol.* **7**(11):3870-3882.
- Smallwood K. 2013. Comparing bird and bat fatality-rate estimates among North American wind-energy projects. *Wildl. Soc. Bull.* **37**(1):19-33.
- Spellerberg I and Fedor P. 2003. A tribute to Claude Shannon (1916-2001) and a plea for more rigorous use of species richness, species diversity and the 'Shannon-Wiener' index. *Global Ecol. Biogeogr.* **12**:177-179.

- Sun J and Narins P. 2005. Anthropogenic sounds differentially affect amphibian call rate. *Biol Conserv.* **121**(3):419-427.
- Tennessen J, Parks S, and Langkilde T. 2014. Traffic noise causes physiological stress and impairs breeding migration behaviour in frogs. *Conserv. Physiol.* **2**(1):cou032.
- Thaker M, Zambre A, and Bhosale H. 2018. Wind farms have cascading impacts on ecosystems across trophic levels. *Nat. Ecol. Evol.* **2**:1854-1858.
- Tsegaye D, Colman J, Eftestol S, Flydal K, Rothe G, and Rapp K. 2017. Reindeer spatial use before, during and after construction of a wind farm. *Appl. Anim. Behav. Sci.* **195**:103-111.
- Uhlig P, Harris A, Craig G, Bowling C, Chambers B, Naylor B, and Beemer G. 2001. Old growth forest definitions for Ontario. *Ont. Min. Nat. Res.*, Queen's Printer for Ontario, Toronto, ON. 53p.
- Vargas-Salinas F, Cunnington G, Amezcuita A, and Fahrig L. 2014. Does traffic noise alter calling time in frogs and toads? A case study of anurans in Eastern Ontario, Canada. *Urban Ecosyst.* **17**:945-953.
- Whalen C, Brown M, McGee J, Powell L, and Walsh E. 2018. Male Greater Prairie-Chickens adjust their vocalization in the presence of wind turbine noise. *Condor* **120**(1):137-148.
- Wildlife Acoustics. <<https://www.wildlifeacoustics.com/>> retrieved on 2018-10-25.

Zuur A, Ieno E, and Smith G. 2007. *Analysing ecological data*. Springer Science + Business Media. New York, NY.

Tables

Table I. Local reptiles and amphibians found in the study area during environmental assessment by Natural Resource Solutions Inc (NRSI) in 2002-2004. Herpetofauna listings known in the study area in 2004 described in the Ontario Herpetofauna Summary Database from the Natural Heritage Information Center (NHIC). Information in the table was retrieved and modified from the environmental impact statement (EIS 2005) performed prior to windfarm construction and supplemented with current observations made during my study.

*Species only found in Control areas during study

Species	Herpetofauna Atlas (EIS 2005)	NRSI Observed	Current (2018-19)
Jefferson/Blue-spotted Salamander	X		X
Complex/Polyploids (<i>Ambystoma laterale</i>)			
Spotted Salamander (<i>Ambystoma maculatum</i>)	X		X
Mudpuppy (<i>Necturus maculosus</i>)	X		
Red-spotted Newt (<i>Notophthalmus viridescens</i>)	X		X
Eastern Redbacked Salamander (<i>Plethodon cinereus</i>)	X	X	X
American Toad (<i>Anaxyrus americanus</i>)	X	X	X
American Bullfrog (<i>Lithobates catesbeianus</i>)	X		X*
Green Frog (<i>Lithobates clamitans</i>)	X	X	X
Northern Leopard Frog (<i>Lithobates pipiens</i>)	X	X	
Mink Frog (<i>Lithobates septentrionalis</i>)	X		
Wood Frog (<i>Lithobates sylvaticus</i>)	X	X	X
Spring Peeper (<i>Pseudacris crucifer</i>)	X	X	X
Gray Treefrog (<i>Hyla versicolor</i>)			X
Eastern Gartersnake (<i>Thamnophis sirtalis</i>)	X	X	X
Northern Red-bellied snake (<i>Storeia occipitamaculata</i>)	X	X	X
Eastern Milksnake (<i>Lampropeltis triangulum</i>)	X		
Common Snapping Turtle (<i>Chelydra serpentina</i>)			
Midland Painted Turtle (<i>Chrysemys picta marginata</i>)	X	X	X
Blanding's Turtle (<i>Emydoidea blandingii</i>)	X		
Wood Turtle (<i>Glyptemys insculpta</i>)	X		
Northern Map Turtle (<i>Graptemys geographica</i>)	X		

Table 1.1. Dunn's post hoc pairwise test of turtle home range sizes in two Turbine sites (T2 and TW) compared to a Control Site (C). A 90% Minimum Convex Polygon (MCP) was used to represent home range and 50% least square kernel density to represent core activity areas. Z represents the Z test statistic for each comparison. Both adjusted p-values using Bonferroni correction and unadjusted p-values are reported.

Home range	Site Comparison	Z	p-value (unadjusted)
90% MCP	C - T2	1.8851	0.1782 (0.0594)
	C - TW	2.0518	0.1205 (0.0401)
	T2 - TW	0.7302	1.0000 (0.4652)
50% Kernel	C - T2	1.7799	0.2252 (0.0750)
	C - TW	1.3745	0.5078 (0.1693)
	T2 - TW	0.1369	1.0000 (0.8911)

Table 2.1. Call characteristics measured in anuran choruses.

Call	
Characteristic	Definition
Chorus Richness	Number of species calling.
Chorus Diversity (H'_c)	Modified Shannon-Weiner index, where pi_c is frequency of recognized matches of species, i , in a community for a number of species, n (Equation 1, Spellerberg and Fedor 2003). $(1) H' = - \sum_{i=1}^n pi \ln pi$
Chorus Evenness (E_c)	Modified Pielou's Evenness, a ratio created with the chorus diversity (H') and the maximum possible diversity (H'_{max}), or the natural logarithm of i species in the community (Equation 2, Beisel et al 2003). $(2) E = \frac{H'}{H'_{max}} = \frac{H'}{\ln i}$

Table 2.2. Call characteristics measured for individual frog calls of *Lithobates clamitans* and *Pseudacris crucifer*. Call characteristics were measured using Kaleidoscope Pro v5.1.9g software.

Call Characteristic	Definition
Call Duration	Duration of the detected call.
Minimum Frequency	Lowest frequency of detected call.
Mean Frequency	Overall mean frequency of detected call.

Table 2.3. Kaleidoscope Pro settings used for cluster analysis of chorus and individual anuran calls. All cluster analyses were conducted by a single observer. Signal parameters were adjusted to best detect the species in the study area while reducing the detection of birds. Cluster analysis settings were adjusted to reduce false identifications. Max distance from cluster center was reduced to 0.7 from the recommended 1.0 to reduce the number of false identifications without removing too many correct calls. Some settings were kept at software recommended values (*).

	Kaleidoscope Setting	Value
Signal Parameters	Min and Max Frequency Range	50 - 3500 Hz
	Min and Max Length of Detection	0.1 - 5 s
	Maximum inter-syllable gap	0.5 s
Cluster Analysis	Max distance from cluster center for output	0.7
	Max states	12*
	Max distance to cluster center for building clusters	0.5*
	Max clusters	500

Table 2.4. Cumulative sightings of amphibian species across study sites during transect surveys between May and September of 2018 and 2019. The number of species and number of individuals per site are listed next to each site, along with the means for both treatments. Turbine sites (T) are < 500 m from a wind turbine and Control sites (C) are > 1.5 km from a wind turbine.

Site	<i>Pseudacris crucifer</i>	<i>Plethodon cinerus</i>	<i>Lithobates clamitans</i>	<i>Lithobates sylvaticus</i>	<i>Anaxyrus americanus</i>	<i>Ambystoma maculatum</i>	<i>Ambystoma laterale</i>	<i>Notophthalmus viridescens</i>	# of Species	# of Individuals	Mean # of Species	Mean # of Individuals
T1	21	2	1	0	1	1	0	0*	5	26	5.75	68
T2	28	16	10	0	8	0	0	1	5	63		
T3	29	10	1	2	1	0	0	1	6	44		
T4	114	7	2	1	13	1	1	0*	7	139		
C1	15	6	2	0	4	0*	1	0*	5	28	5.25	72.25
C2	64	3	8	4	2	0	0	1	6	82		
C3	7	0	59	2	1	0	0	0	4	69		
C4	90	0	11	2	3	1	0	3	6	110		

*Species sighted opportunistically in site; not during a survey.

Table 2.5. Average number of anuran calls recorded in Control sites (>1.5 km from wind turbines) and Turbine sites (<500 m from a wind turbine) and total number of calls by that species indicated in parentheses. Pseudo-random paired recordings in Control and Turbine sites were conducted nightly from April to July of 2018 and 2019. Anuran calls were extracted using Kaleidoscope Pro (Wildlife Acoustics, v5.1.9g).

Site		Species					<i>Hyla versicolor</i>
		<i>Lithobates catesbeianus</i>	<i>Lithobates clamitans</i>	<i>Lithobates sylvaticus</i>	<i>Anaxyrus americanus</i>	<i>Pseudacris crucifer</i>	
Control	C1	97 (1460)	367 (4894)	33 (502)	36 (544)	1448 (21,721)	3 (52)
	C2	131 (3933)	476 (14,283)	101 (3018)	122 (3647)	1979 (59,367)	7 (207)
	C3	77 (1621)	383 (8039)	120 (2517)	120 (2525)	1741 (36,561)	7 (151)
	C4	128 (1922)	402 (6033)	136 (2037)	110 (1643)	1458 (21,866)	7 (104)
Turbine	T1	0	448 (10,305)	0	84 (1941)	1599 (36,781)	0
	T2	0	515 (10,814)	0	199 (4180)	1196 (25,125)	0
	T3	0	434 (5636)	0	148 (1928)	1057 (13,743)	0
	T4	0	506 (8598)	0	147 (2492)	1455 (24,743)	0

Table 2.6. Summary of model ranking for Chorus Diversity (H) and Chorus Evenness (E). Models were created by the diversity of calling frogs recorded during the spring and summer of 2018 and 2019. k is the number of estimated parameters for each model, ΔAIC is the change in AIC between each step of model creation.

Analysis	Model	k	AIC	ΔAIC
H	H~Treatment	5	46.09	0
	H~Treatment + Date	6	30.64	-15.45
E	E~Treatment	5	277.04	0
	E~Treatment + Date	6	260.42	-16.62

Table 2.7. Individual mean call characteristic for *Lithobates clamitans* and *Pseudacris crucifer* by site, nested within treatment (Control; >1.5 km from a turbine, Turbine; <500 m from a turbine). Results of an analysis of deviation, testing for differences between treatments for each call characteristic in a linear mixed effect model are included (see text for results of other predictor variables).

Species	Sites	n	Mean Frequency				Minimum Frequency				Duration			
			Mean (Hz)	±SE	χ^2	p	Mean (Hz)	±SE	χ^2	p	Mean (s)	±SE	χ^2	p
<i>Lithobates clamitans</i>	C1	104	366.95	6.96			254.66	2.22			0.21	0.01		
	C2	115	459.25	21.61			285.94	9.25			0.17	0.01		
	C3	70	583.26	34.83			265.51	10.88			0.38	0.02		
	C4	337	390.65	6.79			253.85	1.79			0.21	0		
	Control	626	420.85	7.21	0.136	>0.05	261.18	2.37	1.109	>0.05	0.22	0.00	2.063	>0.05
	T1	123	375.76	5.92			277.32	2.10			0.18	0		
	T2	507	523.28	12.54			277.14	3.87			0.17	0		
	T3	152	537.33	27.83			291.74	14.85			0.16	0		
	T4	276	532.10	18.48			277.17	2.58			0.18	0		
	Turbine	1058	510.45	8.82			279.28	2.92			0.17	0.00		
<i>Pseudacris crucifer</i>	C1	108	2922.93	15.32			2628.91	25.68			0.21	0.01		
	C2	477	2949.98	5.69			2347.63	16.98			0.14	0		
	C3	101	2994.64	13.37			2741.26	15.07			0.19	0.01		
	C4	112	2931.41	11.33			2618.93	24.61			0.19	0.01		
	Control	798	2949.36	4.66	0.403	>0.05	2473.60	12.70	0.664	>0.05	0.16	0.00	0.748	>0.05
	T1	317	2937.79	6.89			2719.05	7.15			0.17	0		
	T2	223	2894.55	7.06			2663.05	7.09			0.25	0.01		
	T3	105	2974.23	13.56			2660.04	13.70			0.19	0.01		
	T4	133	2893.38	9.81			2614.43	15.57			0.21	0.01		
	Turbine	778	2922.72	4.37			2677.15	4.99			0.20	0.00		

Table 2.8. Linear mixed model summary of predictor variables excluding treatment for individual call characteristics of *Lithobates clamitans* and *Pseudacris crucifer* and analysis of deviation results. Date and Time variable was analyzed as serial date and time. Site represents the nested in treatment effect and was analyzed as a random effect variable. Coefficient estimate of the variable indicates a positive or negative influence on the call characteristic with a change in the predictor variable. Significance of the site variable was analyzed using an ANOVA-like table for random effects (see text), where LRT is the likelihood ratio test statistic. Degrees of freedom is equal to 1 for all variable analyses.

Species	Call Characteristic	Date and Time			Temperature			Site	
		Coefficient estimate	χ^2	p	Coefficient estimate	χ^2	p	LRT	p
<i>Lithobates clamitans</i>	Mean Frequency	-2.31E-04	1.627	>0.05	-3.84E-03	6.020	<0.05	91.10	<0.001
	Minimum Frequency	7.50E-04	0.0004	>0.05	-1.06E+00	8.359	<0.01	8.40	<0.01
	Duration	-7.57E-05	8.655	<0.01	-1.67E-03	58.096	<0.001	53.40	<0.001
<i>Pseudacris crucifer</i>	Mean Frequency	-1.29E-05	3.908	<0.05	1.56E-03	56.602	<0.001	27.92	<0.001
	Minimum Frequency	4.80E-01	175.330	<0.001	7.64E+00	44.196	<0.001	270.21	<0.001
	Duration	4.11E-05	19.237	<0.001	-2.46E-03	68.053	<0.001	279.62	<0.001

Figures

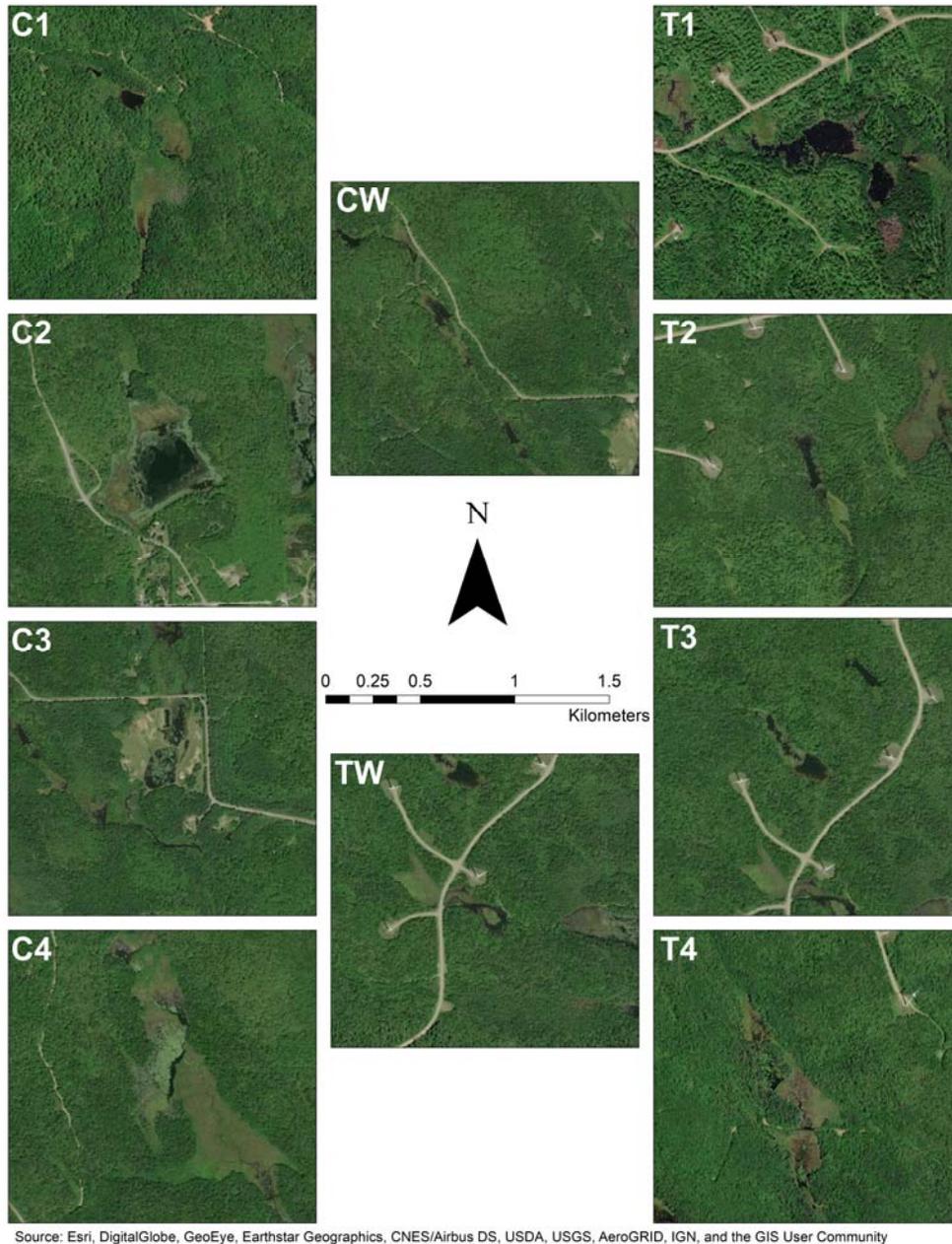


Figure I. Map depiction of study site area used to determine differences in reptile and amphibian ecology and behavior. Control sites (C1, C2, C3, C4 and CW) were located over 1.5 km from any wind turbines and Turbine sites (T1, T2, T3, T4, and TW) were located within 500 m of a wind turbine. Sites ending in a number (1, 2, 3, and 4) were used for transect surveys. Turtle spatial ecology data was collected in Control sites C3 and CW and Turbine sites T2 and TW. Maps were created in ArcGIS v10.7.1, all maps shown have the same scale and orientation

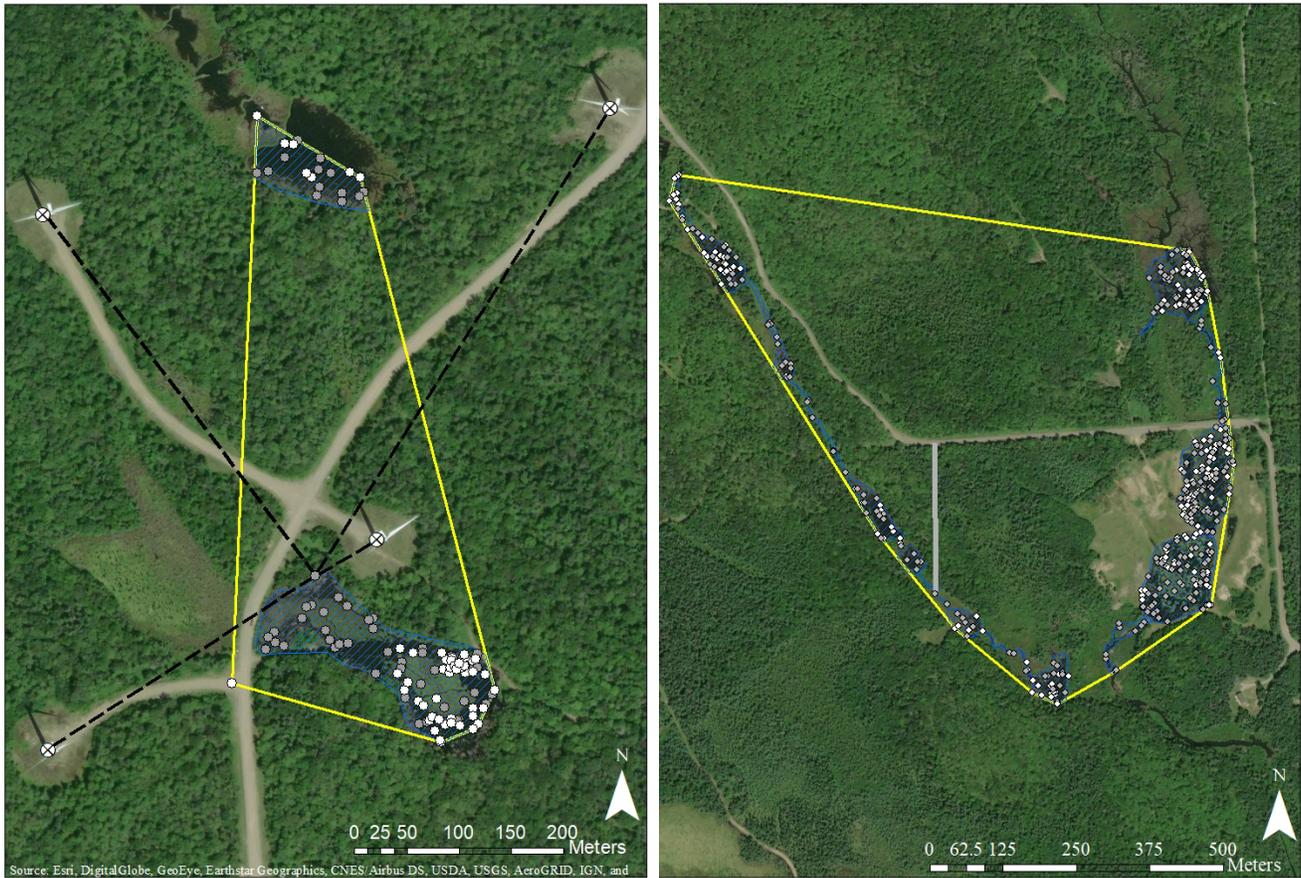


Figure 1.1. Map depiction of turbine avoidance in a Turbine site (TW; left) and road avoidance in a Control site (right). White dots represent actual turtle locations within wetlands (blue hatched polygons), grey dots represent randomly generated points. Yellow polygon represents 100% MCP turtle population range. Black dashed lines show distance from turbine (circles with Xs) measurements, one turbine is missing from map depiction. Distance from all turbines within 500 m of the Turbine MCP population range was measured for all points, such that all points have a measured distance from each turbine. Grey line shows nearest road measurement, which was taken as the closest distance to a road. Maps and measurements generated in ArcGIS v10.7.1, scale bars are unique to each map.

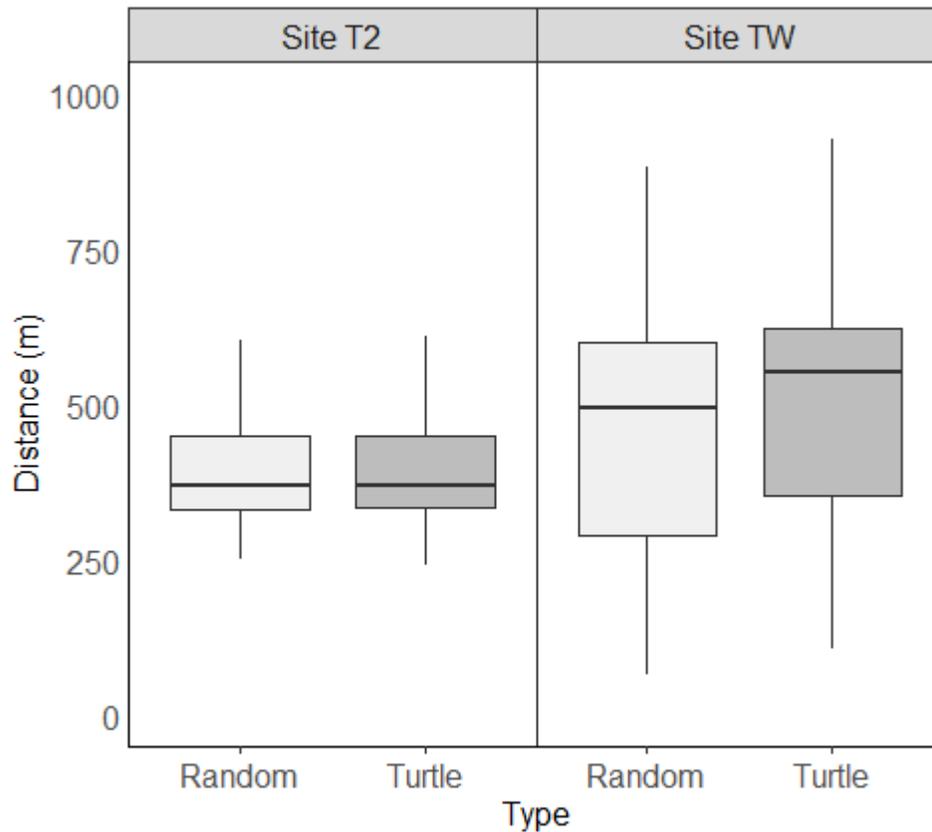


Figure 1.2. Distance of turtle (dark grey) and random point (light grey) locations from turbines in 2 Turbine sites (T2 and TW). Distances of each point from every turbine within 500 m of population range was measured to determine if turtles are avoiding turbines. Random point locations were confined to wetlands to simulate normal activity. Two-Way ANOVA found no difference in turtle distance from random point distance in site T2 (Left, N=894), suggesting no avoidance. A significant difference in distance was found between turtle and random in site TW (Right, N=570), suggesting an avoidance behaviour towards turbines by turtles. Horizontal black bar represents median distance.

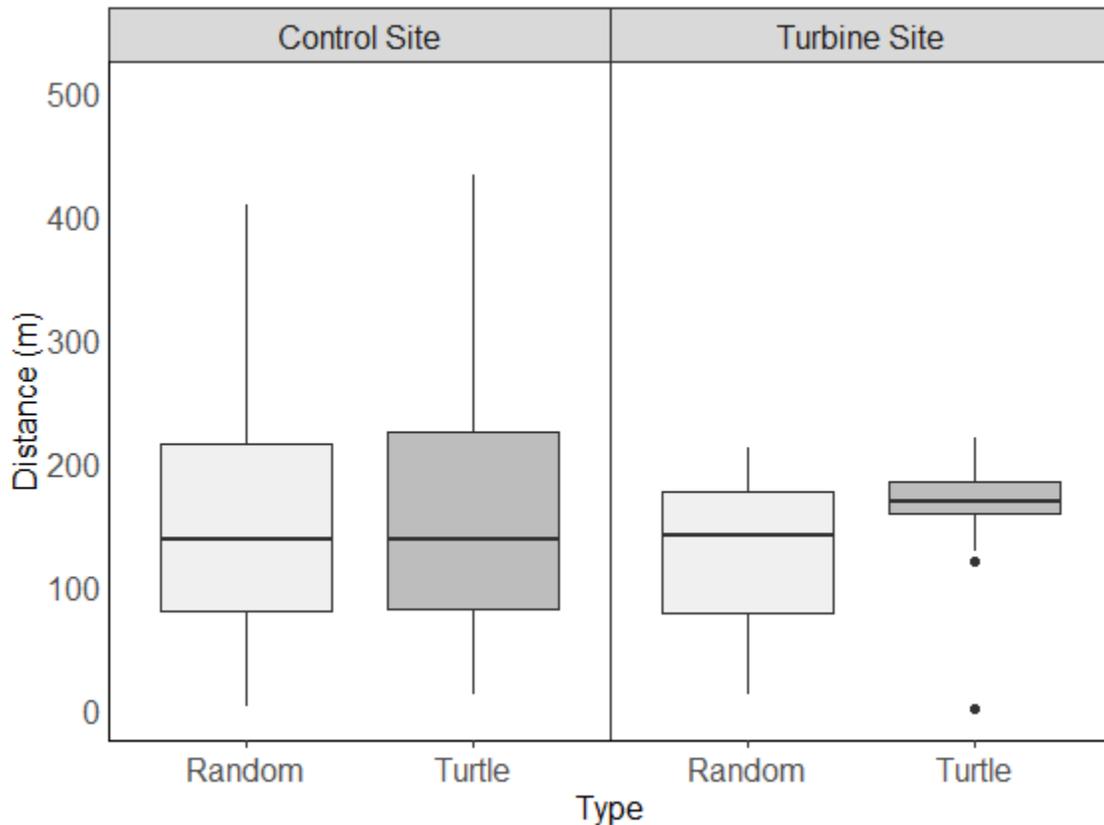


Figure 1.3. Distance of turtle (dark grey) and random point (light grey) locations from roads in one Control site (>1.5 km from turbines, left) and one Turbine site (TW, <500m from turbines, right). Distance was measured as closest distance of each point to either windfarm road or public road. Only sites with population ranges that overlapped roads were considered for this comparison. Random point locations were confined to wetlands to simulate normal activity. Results of a t-test found no difference between turtle and random distances from roads in Control, suggesting no avoidance of roads. A significant difference between turtle distances from random was found in the Turbine site, suggesting avoidance behaviour. Horizontal black bars represent median distance, and dots represent outliers.



Figure 1.4. Map representation of possible wetlands turtles could disperse to within a 1 km radius around Turbine study sites. Buffer range (yellow) was determined as a conservative estimate based on previous research (Bowne 2008; Rowe 2003). White shapes highlight wetlands that were occupied by the tracked turtles during the active season. Blue shapes highlight wetlands that fall within the 1 km buffer, but were not occupied by the tracked turtles during the active season. Map was generated using ArcGIS.

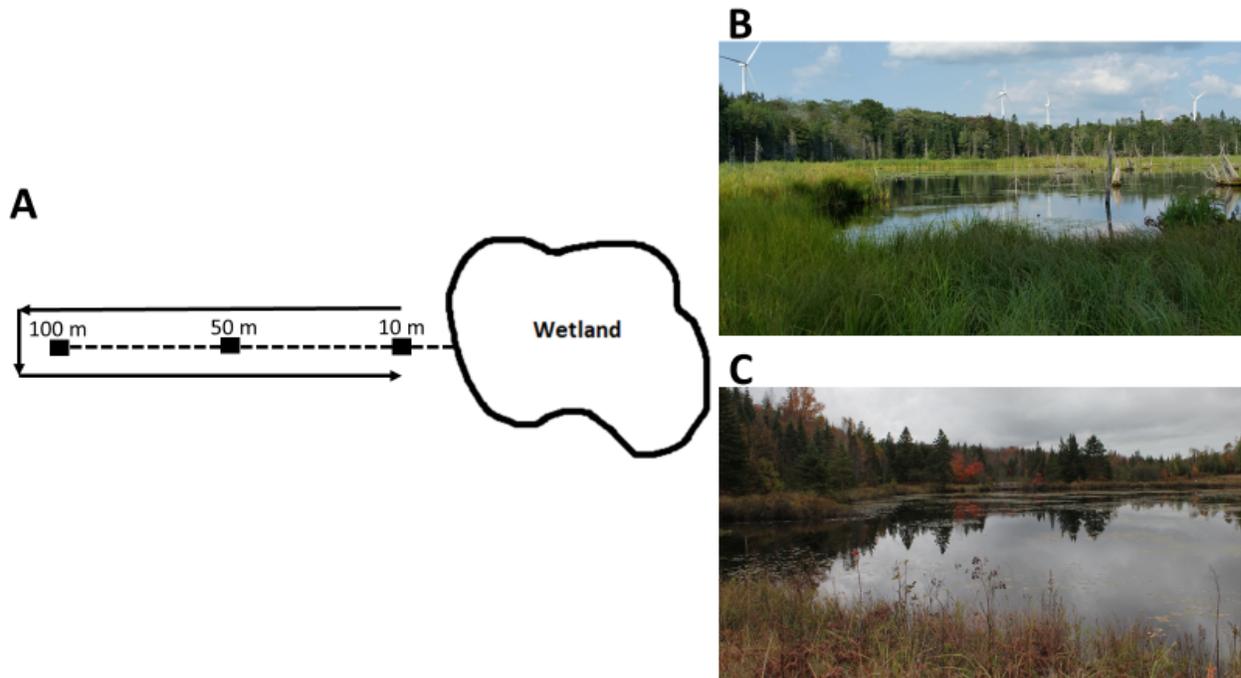


Figure 2.1. Transects (A) were created at 4 Turbine (B, <500 m from a wind turbine, picture taken in summer) and 4 Control (C, >1.5 km from a wind turbine, picture taken in fall) study sites. 100 m-long transects were created in east or west directions with handheld GPS units and flagging tape to mark the center, and cover boards placed at 10, 50, and 100 m. Transects were walked first on one side of the center, then rounded at the end and walked in reverse on the other side, always staying within 5 m of the transect center.

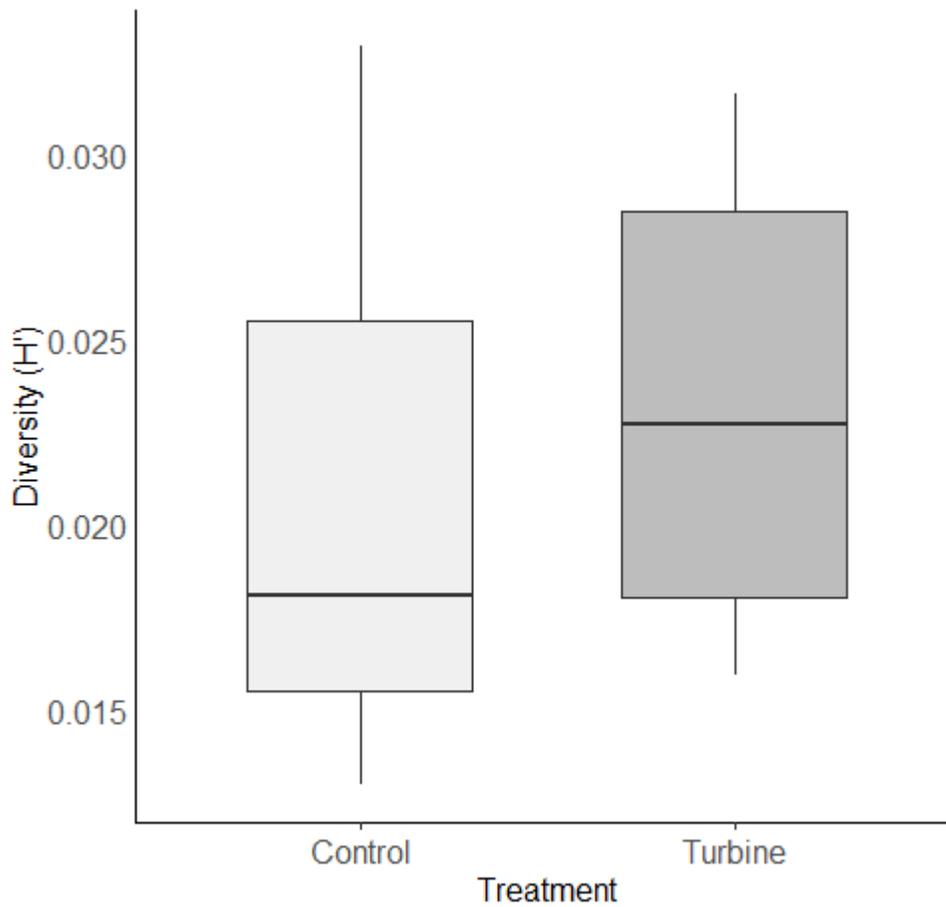


Figure 2.2. Cumulative Diversity (H') of amphibians found during transect surveys in four Control sites (>1.5 km from turbines, light grey) and four Turbine sites (<500 m from a turbine, dark grey) were not significantly different. Surveys of 100 m transects extending perpendicular from wetland edge were performed from May to September of 2018 and 2019. Surveys were conducted at the same site twice per visit, first at 08:30 then at 17:30. A Shannon-Weiner index was calculated for the cumulative counts of species found at each site over the 2018 and 2019 seasons and standardized by dividing H' by the number of surveys. A two-sample t-test revealed no significant difference between treatments.

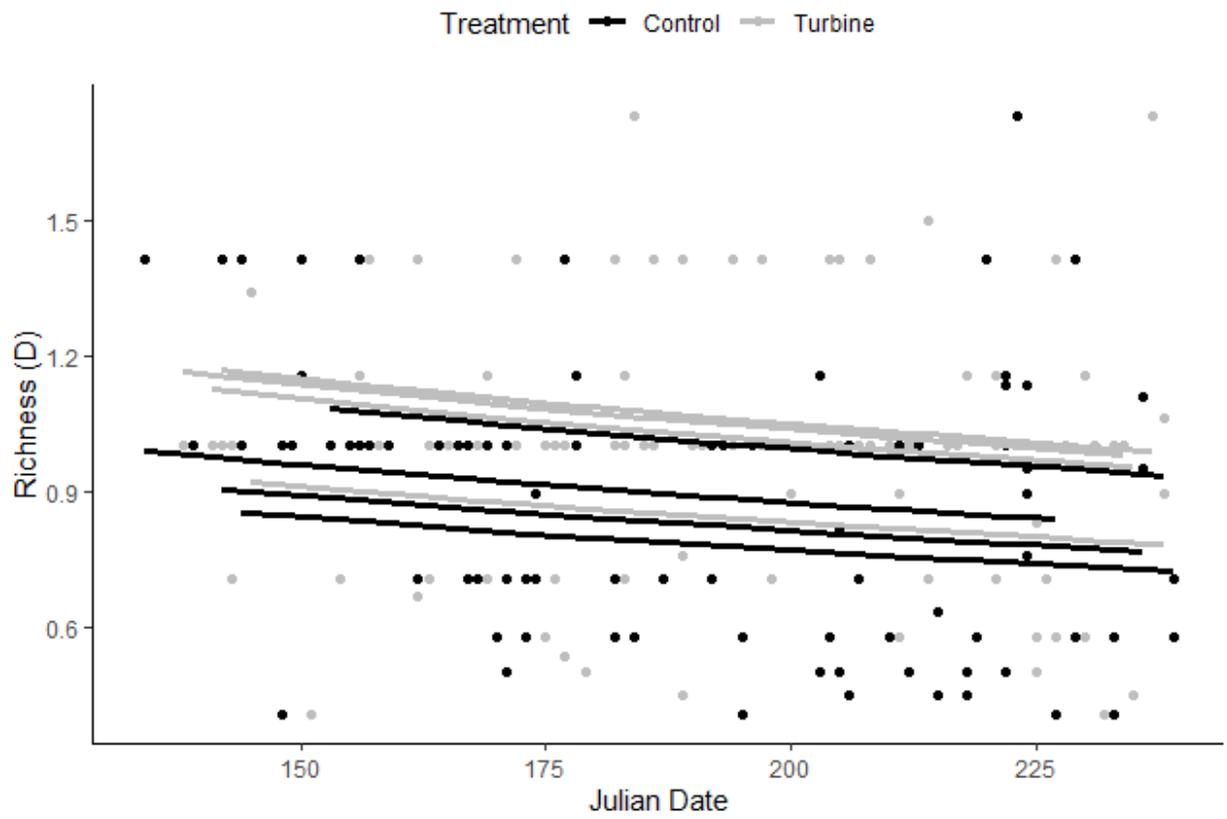


Figure 2.3. Generalized additive model for Menhinick’s Richness (D, log scale) of amphibian species found during transect surveys when at least one amphibian was found. Surveys were conducted on 100 m transects extending perpendicular from wetland edges in four Control sites (>1.5 km from turbines, light grey) and four Turbine sites (<500 m from a turbine, black). Sites were visited randomly between May and September of 2018 and 2019, with each visit consisting of a morning survey at 08:30 and evening surveys at 17:30. The model was fit with site and smoothing factor Julian date as significant predictors of D. Treatment (Control or Turbine) did not predict D.

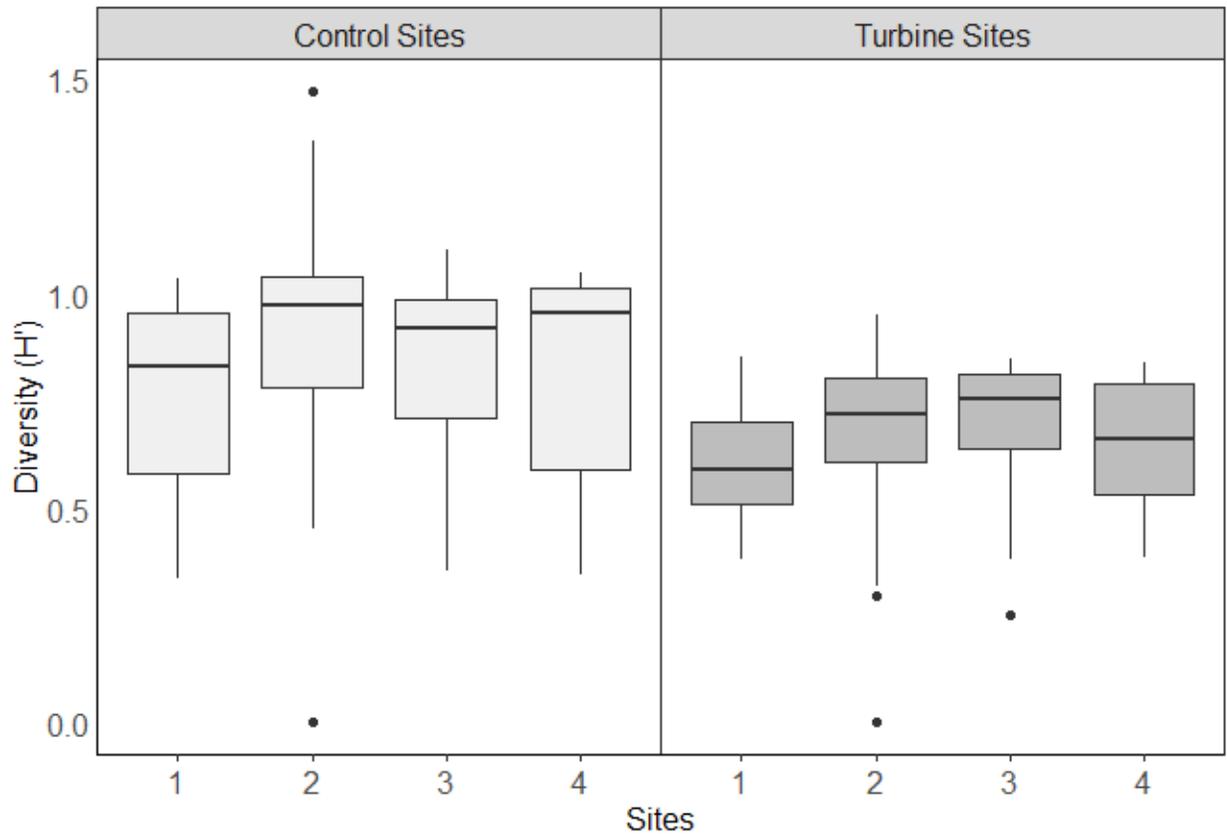


Figure 2.4. Chorus Diversity (H') of anurans in Control sites (>1.5 km from turbines, light grey) was higher than in Turbine sites (<500 m from a turbine, dark grey). Pseudo-random paired recordings in Control and Turbine sites were conducted nightly from April to July of 2018 and 2019. Calls of 6 different anuran species were identified using Kaleidoscope Pro (Wildlife Acoustics, v5.1.9g) and a Shannon-Weiner Diversity index was calculated for each recording night. An analysis of deviation on a linear mixed effects model accounting for repeated measures found diversity was significantly lower in Turbine sites and there were no differences among sites within treatments. Boxplot horizontal lines represent median and dots represent outliers.

Green Frog (*Lithobates clamitans*)

Spring Peeper (*Pseudacris crucifer*)

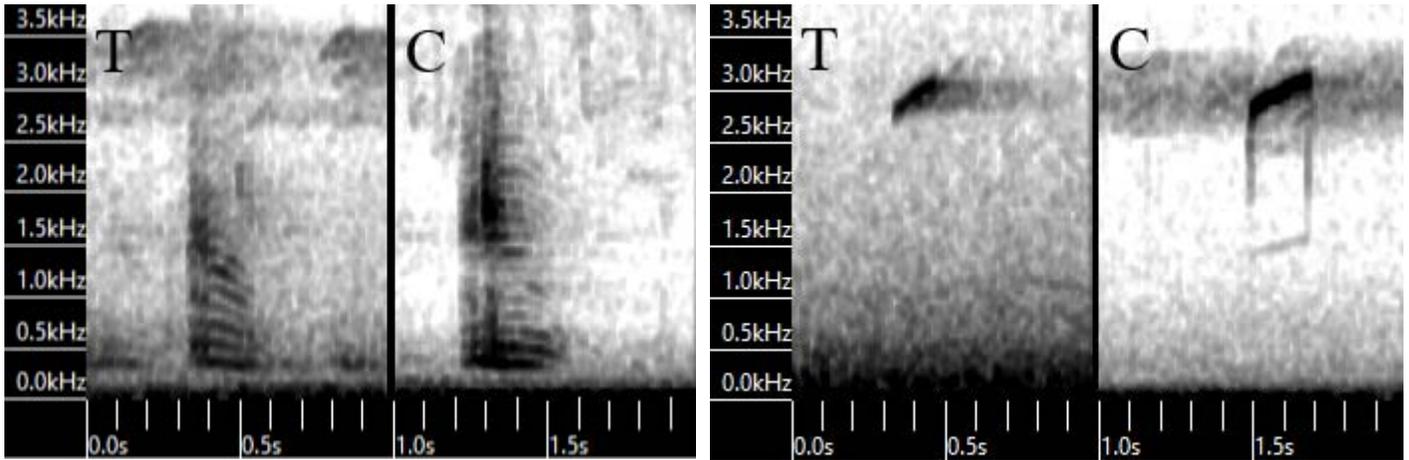


Figure 2.5. Spectrograms representations of average *Lithobates clamitans* (Left) and *Pseudacris crucifer* (Right) calls in Control sites (C, >1.5 km from turbines) and Turbine sites (T, <500 m from a turbine). Calls that best represented the average mean frequency, minimum frequency and duration (Table 2.6) for each treatment and species are displayed. Darker blacks represent amplitude or loudness of the sound, a characteristic that was not analyzed. Harmonics of Spring Peepers (as seen in the Control site call) were not included in the call characteristic measurements. Spectrograms were created with Kaleidoscope Pro software with the same brightness and contrast settings, then screen captures were cropped and overlaid to be represented on the same axis.

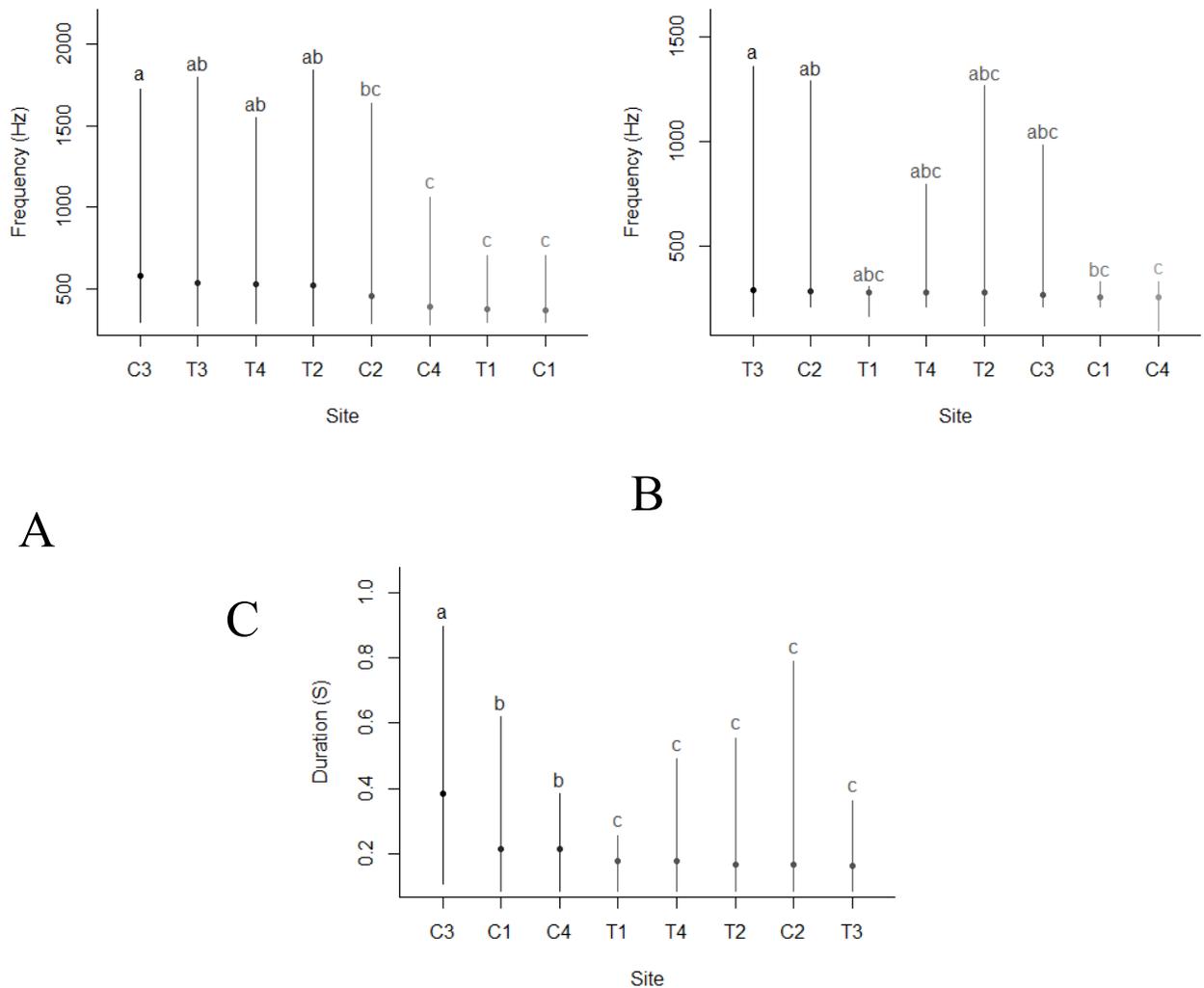


Figure 2.6. Site-specific variation in call characteristics of *Lithobates clamitans* in Control sites (located >1.5 km from turbines; C1, C2, C3, and C4) and Turbine sites (located <500 m from a turbine; T1, T2, T3, and T4). Mean call frequency (A), minimum call frequency (B), and call duration (C). Lines represent the full range of data and dots represent the site mean. Shared letters indicate similarity between sites (i.e., groupings) within each call characteristic based on a Tukey HSD test ($p > 0.05$). Sites are arranged in descending order of means to best represent groupings.

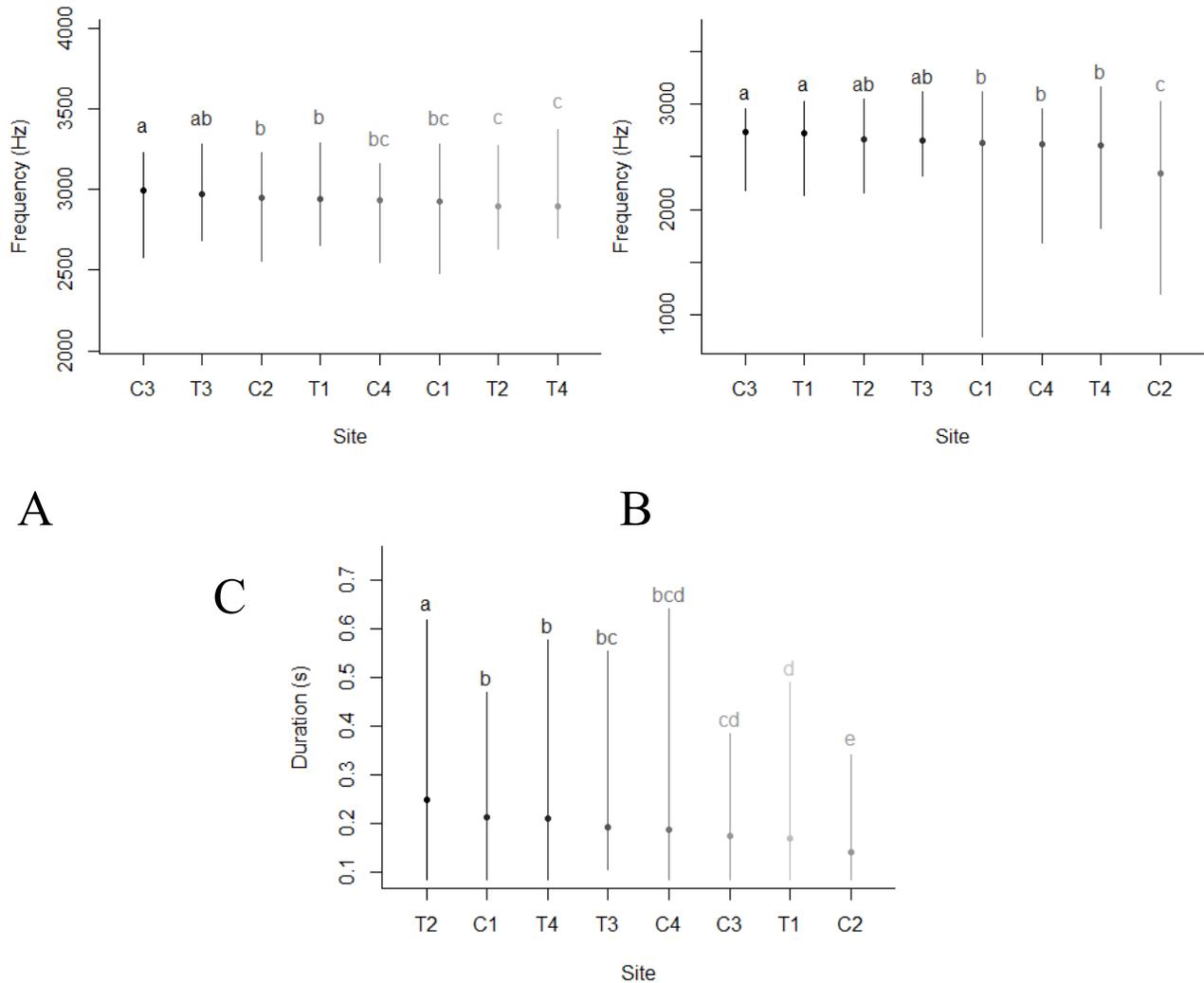


Figure 2.7. Site-specific variation in call characteristics of *Pseudacris crucifer* in Control sites (located >1.5 km from turbines; C1, C2, C3, and C4) and Turbine sites (located <500 m from a turbine; T1, T2, T3, and T4). Mean call frequency (A), minimum call frequency (B), and call duration (C). Lines represent the full range of data and dots represent the site mean. Shared letters indicate similarity between sites (i.e., groupings) within each call characteristic based on a Tukey HSD test ($p > 0.05$). Sites are arranged in descending order of means to best represent groupings.