Impacts of wildfire and windfarm construction and operation on herpetofauna community ecology.

by

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Abstract

Climate change and its associated impacts threaten global biodiversity. Increases in wind energy harvesting to reduce carbon emissions, coupled with increases in wildfire frequency and severity, may pose risks to wildlife. I investigated potential impacts of windfarm operations and wildfire on herpetofauna biodiversity, anuran calling behaviour, and snake health, in an area where both impacts occurred simultaneously. I measured biodiversity metrics using squamate cover surveys and anuran acoustic monitoring across 4 replicated site treatments: Control, Wind, Burn, and WindBurn (double impact). Anuran diversity, evenness, richness, and relative abundances were lowest in Wind sites, whereas metrics in Burn sites did not differ from those in Control sites. Squamate total abundance was lowest in WindBurn sites, but other investigated metrics did not differ between impacted and non-impacted sites. More research using a Before-After-Control-Impact (BACI) study design is needed to understand the acute and chronic impacts of these disturbances on at-risk wildlife species.

Keywords

Community Ecology; Biodiversity; Windfarm; Wildfire; Bioacoustics; Noise Pollution; Reptiles; Amphibians

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General Introduction

Windfarms and Wildlife

Renewable energy production is widely recognized as a global strategy to transition away from dependence on fossil fuels (GWEC, 2019). Wind energy is one such form of renewable energy that provides several advantages over the burning of fossil fuels. Harvesting of wind energy on windfarms produces low lifetime carbon emissions and has a relatively high energy generation efficiency (Evans et al. 2009). Even more, investing in a non-finite energy source allows for greater energy security, a reduction of carbon extraction and consumption, as well as reductions in greenhouse gas emissions which can aid in the fight against climate change (Frondel et al. 2010). However, it is important to recognize that there are environmental consequences and potential impacts on wildlife associated with all forms of renewable energy (Lovich & Ennen, 2011), many of which are not well understood.

Windfarms require large tracts of land (McDonald et al. 2009) to implement extensive networks of wide service roads, electrical transmission lines, turbine pads and other associated infrastructure (e.g., substations, buildings), resulting in habitat loss, fragmentation, and degradation (Kuvlesky et al. 2007; Diffendorfer et al. 2019). The alteration of the landscape necessitated by windfarm infrastructure poses a significant threat to wildlife (Lovich & Ennen, 2013). These changes can elevate the risk of road mortality for slow-moving species (Lovich et al. 2011a; Lovich & Ennen, 2017; Keehn & Feldman, 2018a) and diminish the quality of remaining habitat (Kuvlesky et al. 2007; Diffendorfer et al. 2019). However, although the overall total project area of windfarms is large, as little as 1 – 10 % of the land may be directly altered for infrastructure (Denholm et al. 2009), which may leave behind suitable patches of wildlife habitat. Additional windfarm risks on wildlife include the effects of low-frequency noise, vibrations, and shadow flicker resulting from the wind turbines (Rabin et al. 2006; Lovich & Ennen, 2013; Dai et al. 2015; Schuster et al. 2015; Zwart et al. 2016; Szymański et al. 2017; Whalen et al. 2019; Teff-Seker et al. 2022; Trowbridge & Litzgus, 2022; Lehnardt et al. 2023). The collision of volitant wildlife (e.g., birds, bats) with wind turbines presents an additional direct mortality risk to wildlife and has been relatively well-studied (Kuvlesky et al. 2007; Hayes, 2013; Smallwood, 2013; Dai et al. 2015; Wang & Wang, 2015; Allison et al. 2019; Perold et al. 2020).

Windfarms, Wildfire, and Wildlife

Windfarms also pose an elevated risk of fire ignition, present through all stages of development, operation, maintenance, and decommissioning, as well as potential ignition from humans (e.g., cigarette smoking, welding), vehicles, and turbine malfunctions (Lovich & Ennen 2013, 2017; Lovich et al. 2018). Wildfires can directly and indirectly affect wildlife populations, via fire or smoked-induced mortality (Smith et al. 2012; Jordaan et al. 2020) and altered environmental conditions and resource availability post-fire (Batista et al. 2023). Furthermore, the ignition of a fire within a landscape already fragmented and degraded by windfarm implementation could multiply the risk to wildlife, compared to each impact occurring individually (Driscoll et al. 2021; Santos et al. 2022). There is currently an absence of regulations mandating the reporting of fires at windfarms (Uadiale et al. 2014), making it challenging to accurately evaluate the synergistic risks that windfarms and wildfires may pose to wildlife communities.

Herpetofauna and Windfarms

The majority of research involving wildlife and windfarm interactions have primarily focussed on volitant wildlife (e.g., birds, bats; Smallwood, 2013). Herpetofauna have largely been overlooked, with a few studies focusing on a small number of species' ecologies on windfarms (Lovich & Ennen, 2017). Trowbridge & Litzgus (2022) examined the potential effect of turbine noise pollution on anuran communities and chorusing behaviour in Ontario. Keehn & Feldman (2018b) and Keehn et al. (2019) explored the demography and predator avoidance of Side-Blotched Lizards (*Uta stansburiana*) on a California windfarm. Similarly, Thaker et al. (2018) examined body condition and community dynamics of Superb Large Fan-Throated Lizards (*Sarada superba*) on an Indian windfarm. Additionally, Law et al. (2020) studied avian attack rates on Common European Vipers (*Vipera burus*) within a Scotland windfarm. Even fewer studies have focused on the combined impacts of windfarm and wildfire, including an examination of habitat selection and spatial ecology of Spotted Turtles (*Clemmys guttata*) on an Ontario windfarm (Delay et al. 2023), and various ecological examinations of Desert Tortoises (*Gopherus agassizii*) on a California windfarm (Lovich et al. 2011a, 2011b, 2018; Agha et al. 2015).

The global decline of herpetofauna, primarily attributed to habitat loss, fragmentation, and degradation (Gibbons et al. 2000; Stuart et al. 2004; Böhm et al. 2013), underscores the need for more comprehensive research in the wind energy sector. Even more, as the demand for wind energy rises (IRENA, 2022) and the natural fire regime continues to alter due to climate change (Bowman et al. 2020; Pyne, 2020; Nimmo et al. 2021; Wu et al. 2021), it is possible that the combined effects of windfarms and wildfire on wildlife will become more frequent (Kelly et al. 2020). The need to understand if the development and operation of wind energy may be a further risk to the conservation and management of herpetofauna is of paramount importance (Lovich & Ennen, 2017).

Research and Rationale

To better understand the effects of windfarm construction and operation, wildfire, and their combined effects on herpetofauna, I investigated the community ecology of anurans (Chapter I) and squamates (Chapter II) within Central Ontario in an area where both impacts occurred simultaneously. I used four replicated site treatments – Control (no impacts), Wind (windfarm impact), Burn (wildfire impact), and WindBurn (combined windfarm and wildfire impact) – to investigate whether anuran call characteristics, snake health, and anuran and squamate community composition differed among treatments. More specifically, I investigated differences in anuran and snake abundance, diversity, richness, and evenness. I also investigated individual characteristics, including mean call frequency and call duration of Spring Peepers (*Pseudacris crucifer*) and Green Frogs (*Lithobates clamitans*), and body condition of Smooth Greensnakes (*Opheodrys vernalis*).

General Methods

Study Area

My study area is located in central Ontario, Canada, within a landscape characterized as a natural mosaic of wetlands, rock barrens and mixed forests (Crins et al. 2009) and is a recognized biodiversity hotspot for herpetofauna (UNESCO, 2004; Jalava et al. 2005). The study area is located within Anishinabek territory, including the Robinson-Huron Treaty of 1850 and Williams Treaty of 1923 lands, and the Métis Nation of Ontario Region 7. Much of this landscape had not been impacted by anthropogenic activities before the windfarm construction and wildfire occurred, meaning that any patterns observed in herpetofauna biodiversity and snake body condition were likely a direct result of the windfarm and wildfire (Delay et al. 2023).

Windfarm construction began in August 2017 and reached fully operational status in July 2019. The windfarm has an installed power capacity of 300 MW (87 Vestas turbines V136-3.45 MW®) and occupies a total area of 9,000 ha of which 7.7% (690 ha) has been directly altered for infrastructure (i.e., gravel roads, turbine pads, transmission lines, laydowns, and substations; Delay et al. 2023). In July 2018, during construction, a wildfire ignited on the windfarm. The fire was declared extinguished on 31 October 2018, burning a total of 11,300 ha, 1,900 ha of which were within the windfarm footprint and 9,400 ha of which were on public lands (Delay et al. 2023). Before the fire, this region had not had any large fires recorded since 1950 (Markle et al. 2020; Wilkinson et al. 2020).

Four site treatments, each replicated three times (n=12 sites in total), were used: Control (C) sites located out of the footprint of both impacts (>1.5 km); Burn (B) sites located within the footprint of the wildfire and outside of the windfarm footprint (>1.5 km); Wind (W) sites located within the footprint of the windfarm (<300 m from turbines and infrastructure); and WindBurn (WB) sites located within the footprints of both the wildfire and windfarm (Figure I). Sites were chosen based on the presence of target animal species and overwintering, foraging, and active season habitats. Each site consisted of a wetland complex to support active season and overwintering habitat for amphibians (Chapter I) and reptiles, as well as surrounding rock barrens to support active season habitat for squamates (Chapter II; Figure I).

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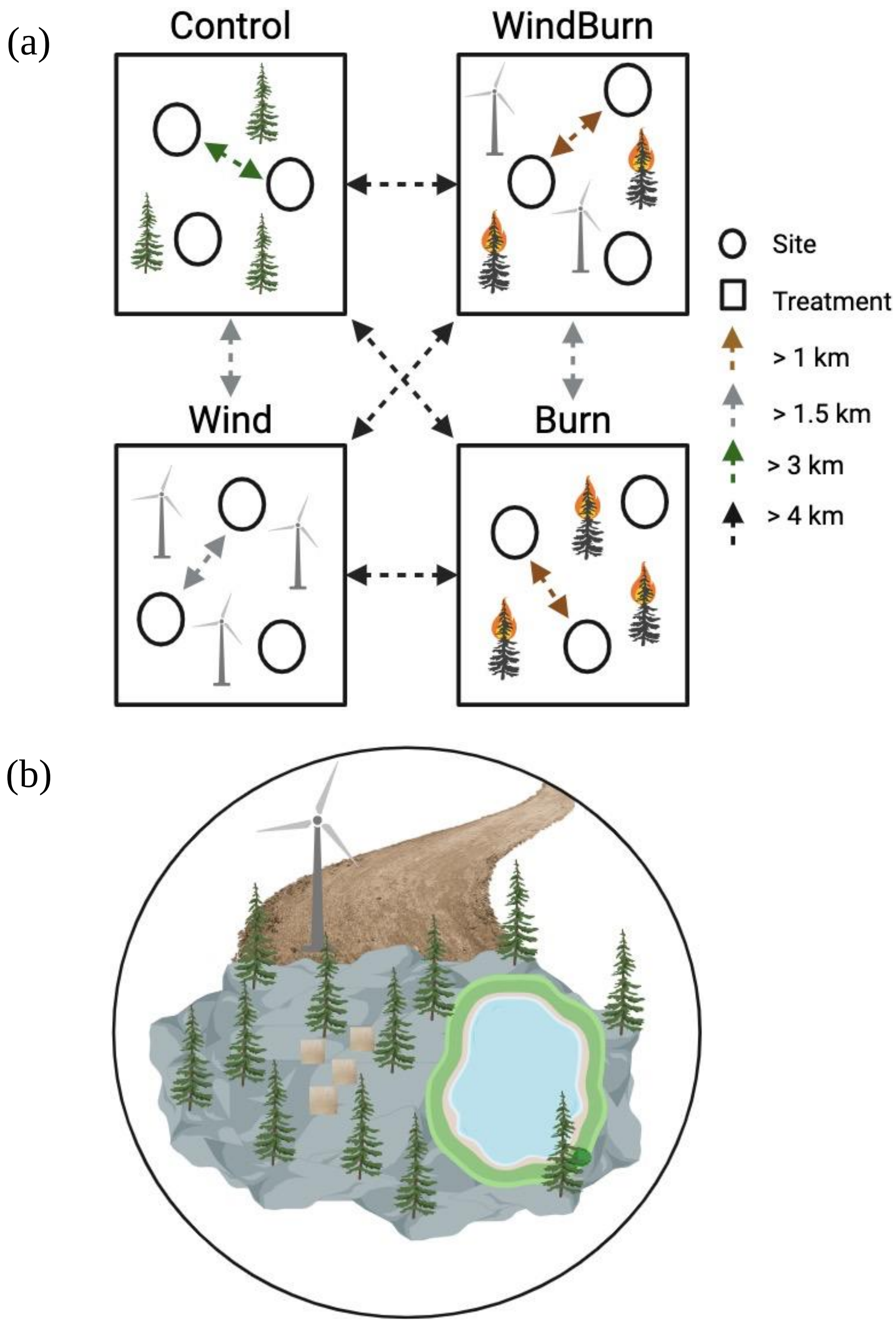

Figure I. a) Graphical representation of 4 treatments (Control, Burn, Wind, WindBurn), each replicated by 3 sites, in central Ontario. Each site consisted of a wetland complex surrounded by rock barrens. The provided distances represent the distance (km) between treatments and between sites within treatments. b) Layout of a standard Wind site, featuring a cluster of deployed cover boards and surrounding rock barrens where natural cover and visual encounter surveys for squamates occurred. Surveys were conducted in distinct areas within each site, during each study year. ARU's (autonomous recording units) were placed adjacent to the site's wetland complex to record chorusing anurans. Graphic created with Biorender.com.

Chapter I

Wind turbines impact anuran communities more than wildfire.

Abstract

Climate change is accompanied by a multitude of environmental effects, including a global increase in wildfires. A fast-growing way to mitigate the effects of climate change is through the incorporation of renewable energies, such as wind harvesting; however, windfarms can pose risks to wildlife. I hypothesized that habitat alterations in a landscape impacted by a wildfire, windfarm construction, and associated noise pollution generated from wind turbines, would negatively affect anuran communities, a taxonomic group dependent on acoustic communication for reproduction. I used autonomous recording units to examine anuran chorus community metrics (diversity, richness, evenness, relative abundance) and call characteristics (mean frequency, call duration) of Green Frogs (*Lithobates clamitans*) and Spring Peepers (*Pseudacris crucifer*), in 4 replicated site treatments: Control (no impacts), Wind, Burn, and WindBurn (double impact). To quantify differences in levels of noise pollution among windfarm sites, I created a Turbine Impact Score that incorporated the number and distance of turbines from wetlands with chorusing anurans. Anuran communities in Wind sites had lower richness, evenness, diversity, and species-specific relative abundances compared to Control and Burn sites. Green Frog mean call frequency was highest in Control sites while call duration was longest in Wind sites. Spring Peeper calls did not differ among treatments. Sites with greater turbine impact scores had lower richness, evenness, diversity, and relative abundances. My findings suggest that turbine noise negatively impacts anuran communities, particularly in low frequency calling species, whereas the wildfire seemed to have no impact on investigated

metrics. Future studies should more thoroughly examine the impacts of turbine noise on anurans, to establish the mechanism for the negative effects. I also recommend long-term monitoring of anuran communities affected by wildfire, to account for any time-lagged impacts.

Introduction

Climate change is one of the major drivers of severe and unpredictable weather and environmental effects, such as wildfires (Bowman et al. 2020), which can have negative consequences for humans and wildlife (Kelly et al. 2020). Recent work suggests that under future climate scenarios, extreme fire events will increase in frequency and become more variable in intensity (Pyne, 2020; Nimmo et al. 2021; Wu et al. 2021). Mitigating climate change means moving away from fossil fuels to renewable energy sources, such as harvesting wind energy, which more than tripled in global capacity from 2012 to 2021 (IRENA, 2022). However, windfarms are known to have negative consequences for wildlife, such as mortality from direct collisions of volitant wildlife (e.g., birds, bats) with wind turbines, as well as reductions in breeding success and altered habitat use (e.g., birds, bats, terrestrial and aquatic mammals; Kuvlesky et al. 2007; Hayes, 2013; Smallwood, 2013; Stevens et al. 2013; Dai et al. 2015; Wang & Wang, 2015; Russell et al. 2016; Łopucki et al. 2017; Fernández-Bellon et al. 2018; Allison et al. 2019; Perold et al. 2020).

Wildfires can affect wildlife populations and communities directly and indirectly (Engstrom, 2010). During an active fire, individuals that cannot locate refugia or flee may die due to the direct effects of heat, flames, or smoke inhalation (Meddens et al. 2018; Martinez et al. 2019; Steenvoorden et al. 2019; Tomas et al. 2021). If an individual animal survives the fire, they must subsequently survive the after-effects of the fire (i.e., the indirect effects). Indirect effects can include changes to the availability and quality of habitat and resources, changes to microclimatic conditions, and increased predation (Hradsky et al. 2017; Davis et al. 2019; Batista et al. 2023). Some species, however, can thrive in the modified landscape, taking advantage of the new environmental conditions (e.g., new habitat types, decreased competition; Cunningham et al. 2002; Guscio et al. 2007). The impacts of wildfire on anurans are not well understood (Hossack & Pilliod, 2011; Pastro et al. 2014; dos Anjos et al. 2021), and recent research has found mixed results. A large meta-analysis found that species and assemblages were both positively and negatively affected by fire (dos Anjos et al. 2021). Work on the 2019-2020 Australian bushfires found that occupancy and richness of both common and rare anuran species were negatively influenced by fire severity (Beranek et al. 2023). Other research found that fires resulted in reductions in anuran population sizes, species richness and genetic diversity (Hossack & Pilliod, 2011; Hossack et al. 2013; Potvin et al. 2017; Muñoz et al. 2019). Conversely, some research has found that certain anuran species benefit from fire and take advantage of the new environmental conditions (Guscio et al. 2008; Brown et al. 2014) or remain unaffected (Hossack & Corn, 2007).

In windfarms, turbine noise resulting from the spinning of turbines, may negatively affect wildlife (Rabin et al. 2006; Lovich & Ennen, 2013; Dai et al. 2015; Schuster et al. 2015; Zwart et al. 2016; Szymański et al. 2017; Whalen et al. 2019; Teff-Seker et al. 2022; Trowbridge & Litzgus, 2022; Lehnardt et al. 2023). Anthropogenically-induced noise pollution has increased substantially in recent decades and is a growing threat to wildlife across the globe, acting as a threat to breeding success and survival (Blickley & Patricelli, 2010; Francis & Barber, 2013; Kunc et al. 2016; Shannon et al. 2016). This is especially true for species whose reproductive ecology involves acoustic communication, including anurans which rely on male vocalizations to attract females to breeding sites (Wells, 1977). It has been well documented that noise pollution influences anuran calling behaviours (Cunnington & Fahrig, 2010; Simmons & Narins, 2018; Zaffaroni-Caorsi et al. 2023), which may lead to reduced fitness as reproductive success is linked to calling effort (Sun & Narins, 2005; Read et al. 2014). Anthropogenic noise (e.g., traffic noise on roads) has been shown to affect anuran calling activity (Lengagne, 2008), including call abundance (Grace & Noss, 2018), call rate and duration (Sun & Narins, 2005; Penna et al. 2005), call frequency (Parris et al. 2009; Cunnington & Fahrig, 2010; Hanna et al. 2014), and can interfere with the ability of females to differentiate between the calls of males (Wollerman & Wiley, 2002).

Most research on anthropogenic noise and anuran bioacoustics has mainly concentrated on road noise. However, the increase in wind harvesting (IRENA, 2022) may be introducing a new threat -- wind turbine noise pollution (Lovich & Ennen, 2013; Schuster et al. 2015; Dai et al. 2015; Teff-Seker et al. 2022) -- to these species whose populations are already declining globally (Stuart et al. 2004; IUCN, 2023). Wind turbines can generate loud persistent broadband sound (Dai et al. 2015), which increases with turbine size (Møller & Pederson, 2011). Unlike traffic noise, turbine noise is influenced by wind conditions (Rogers et al. 2006), thus can vary in intensity throughout the day and night. Turbine noise has been linked to changes in bird vocalizations (Zwart et al. 2016; Szymański et al. 2017; Whalen et al. 2019; Gómex-Castasús et al. 2022) and reduced bird abundance at migratory stopover sites when turbine noise is broadcasted in the absence of turbines (Lehnardt et al. 2023). Recent examinations of the effects of wind turbine noise on anurans have yielded mixed results. Trowbridge & Litzgus (2022) found significantly lower anuran chorus diversity in their turbine-affected sites in central Ontario whereas de Oliveira et al. (2023) found no relationships between anuran diversity patterns and windfarm noise in Brazil.

I examined the potential effects of wildfire and wind turbine proximity on anuran communities and acoustics in a location where both impacts occurred simultaneously. Using autonomous recording units (ARUs), I examined anuran chorus diversity and species-specific call characteristics in four treatment areas, each replicated three times (n = 12 sites): post-construction windfarm (Wind), post-wildfire (Burn), combined impacts of post-construction windfarm and post-wildfire (WindBurn), and no impacts (Control). My objectives were to compare (1) anuran chorus richness, diversity, evenness, and relative abundance and (2) the mean call frequency (Hz) and duration (s) of two ubiquitous anuran species, among treatments. If windfarm noise negatively affects anuran calling behaviour, then I expected to see lower diversity, richness, evenness, and relative abundance in windfarm sites compared to control sites. I also expected to see altered individual call characteristics within the windfarm sites as a result of competition with turbine noise. If wildfire presents significant direct and indirect threats to anuran communities, then I also expected to see lower diversity, richness, evenness, and relative abundance in wildfire sites compared to control sites. No previous studies to date have examined anuran community dynamics in areas affected by both a large wildfire and a large windfarm, thus my study is providing new data testing a novel combination of threats to anurans.

Materials and Methods

Community Chorus Characteristics

Anuran call data were collected in 2023 from 8 May to 21 July using ARUs. A total of 6 SM4 Song Meters (Wildlife Acoustics, Maynard, MA) were rotated among the 12 sites within the 4 replicated site treatments on a weekly schedule. Each site received 6 sampling events, totally approximately 42 nights of recordings. SM4s were attached to trees within 5 m of wetlands containing calling anurans, scheduled to record for one hour at sunset and 5 mins every hour until sunrise, to capture the calling periods of all anurans located within the study area. In Ontario, anurans have peak calling periods ranging from late spring to mid-summer, depending on species' natural history (Ontario Nature, 2023). Chorus Richness, Shannon Weiner Chorus Diversity (H′) and Pielou's Chorus Evenness (E), as well as nightly relative species abundances (total number of species vocalizations/number of sampling nights), were compared amongst treatments using one-way ANOVAs, followed by Tukey's HSD post hoc tests. Vocalizations were used as proxies for relative abundance because the number of vocalizations positively correlates with the number of anuran individuals in a chorus (Nelson & Graves, 2004; Grace & Noss, 2018; Trowbridge & Litzgus, 2022). All data were analyzed using R statistical software (v4.2.3; R Core Team (2020), Vienna, Austria) in RStudio (RStudio Team (2016), Boston, MA).

Call Characteristics

The effect of treatment on call characteristics was examined using two ubiquitous species: Spring Peepers (*Pseudacris crucifer*) and Green Frogs (*Lithobates clamitans*). Individual calls with no background noise (i.e., interspecific and intraspecific calls, weather, anthropogenic, etc.), were extracted using individual species advanced classifiers, created with Kaleidoscope Pro v5.4.9. After the classifier extracted the individual detections, I examined two call characteristics: mean call frequency (Hz) and call duration (s), separately among treatments using a linear mixed effects model. Treatment, Julian date, time (converted to numeric values based on hh:mm format), wind speed (m/s; Markle & Waddington, unpublished data), and temperature (°C; collected using the SM4 internal thermometers) were predictor variables, while site (nested within treatment) was included as a random effect. A likelihood ratio test was employed to assess the impact of predictor variables in explaining call characteristics. If treatment was a significant predictor of call characteristics, then a Tukey HSD post hoc test was conducted with R package emmeans (Lenth, 2022) to further examine differences. The effect of site on call characteristics was analyzed using an ANOVA-like table using the function *ranova* within the lmerTest package in R (Kuznetsova et al. 2017; Trowbridge & Litzgus, 2022). Differences in call characteristics between sites within treatments were further compared using a one-way ANOVA followed by a Tukey HSD post hoc test.

Turbine Impact Score

I wanted to quantify potential differences in levels of noise pollution among windfarm sites, because turbines were more numerous and closer to wetlands in some sites compared to others. Turbine impact scores were created for each of the 6 sites (W x 3, WB x 3) located within the windfarm using the following formula:

$$\textbf{Impact} = (w_{\text{Turbines}} \text{ x Number of Turbines}) + (w_{\text{Distance}} \text{ x } (1 - \text{Transformed Combined Distance}))$$

where w_{Turbines} and w_{Distance} represented the weights (i.e., relative importance) I assigned to the number of turbines and distance from SM4 deployment location to the turbines. Number of Turbines represented the number of turbines within a 600-m radius (turbine noise remains even at 800 m; Katinas et al. 2016) of where I placed the SM4 and Transformed Combined Distance represented the mean distance (m) divided by 100 of all turbines in the same radius from where I placed the SM4 (Figure 1.1). I weighted the Number of Turbines (0.7) higher than the Transformed Combined Distance (0.3), as I expected turbine presence to have a greater impact on frog calling behaviour than distance, as more turbines leads to more noise pollution for anurans with which to compete. Distance to turbine, however, is still relevant, as turbine noise increases as distance from turbine decreases (Katinas et al. 2015). A site with a greater number of turbines and shorter distances to the turbines, for example, would have a higher impact score compared to a site with fewer turbines and farther distances. I used linear regressions to examine the relationships between turbine impact score (independent variable) and community metrics (diversity, richness, evenness) in addition to nightly relative abundances of 4 common anuran species (dependent variables).

Kaleidoscope Pro Software Classifiers

Kaleidoscope Pro's Cluster Analysis tool (Wildlife Acoustics, v5.4.9g) was used to extract anuran calls (also referred to as detections) from the nightly recordings. The tool groups detections together based on similarity between call properties and spectrogram patterns. To refine these groups, a user must manually identify the group sounds and rename them based on what species is calling. I created Advanced Classifiers for each anuran species occurring within the study area. In short, I first set target parameters for the species of interest (e.g., high frequency range for a Spring Peeper vs. low frequency range for a Green Frog). Next, I created basic classifiers, which grouped calls together into one large group (i.e., cluster) while ignoring non-targeted detections (birds, other anurans, mammals, etc.). This basic classifier was then manually fine-tuned by sifting through and listening to detections picked out by the classifier to confirm if the detection was the targeted species. This process increases accuracy, helping Kaleidoscope Pro to correctly identify calls produced by the target species. This process produced an Advanced Classifier, which is what I used to detect species-specific calls in the data. Separate classifiers were created for the windfarm impacted sites to ensure call properties potentially unique to one treatment were not missed in another (i.e., if a species calls at a higher frequency in one treatment vs. lower in another; Trowbridge & Litzgus, 2022). The number of detections for each species was used as an estimate of call abundance for the community analyses. Final classifiers were tested for accuracy following the same methodology as Trowbridge & Litzgus (2022).

Results

Chorus Characteristics

Calls of 8 anuran species were recorded in the 2023 field season (Table 1.1). Six of the 8 species were recorded in all treatment areas. Wood Frogs (*Lithobates sylvaticus*) were recorded in only 2 of the Control sites. Leopard Frogs (*Lithobates pipiens*) were not detected in the 3 Wind sites and 2 of the WindBurn sites. American Bullfrogs (*Lithobates catesbeianus)* were absent in 1 of each the Wind and WindBurn sites. I manually verified all detections for the infrequently calling and uncommon species (American Bullfrog, Leopard Frog, Wood Frog), resulting in 100% correct detection identification. The commonly vocalizing species (Green Frogs, Mink Frogs (*Lithobates septentrionalis)*, Spring Peepers, Grey Treefrogs (*Hyla versicolor*)) were correctly identified with over 90% accuracy using their dedicated advanced classifiers. American Toads (*Anaxyrus americanus*) were excluded from all analyses due to an insufficient number of detections to develop an accurate advanced classifier; however, manual scanning of the recordings confirmed Toad presence in each of the 12 study sites.

Chorus evenness, diversity and richness differed significantly among treatments (evenness: $F_{3,8} = 4.91$, $p < 0.05$; diversity: $F_{3,8} = 5.97$, $p < 0.05$; richness: $F_{3,8} = 9.22$, $p < 0.05$; Table 1.2; Figures 1.2 - 1.4). Post hoc analyses revealed that evenness in the Wind sites was significantly lower than the Burn sites ($p < 0.05$). Diversity was significantly lower in the Wind sites as compared to the Control and Burn sites ($p < 0.05$). Richness was significantly higher in the Control sites than the other three treatments ($p < 0.05$), which did not differ from one another. The Control treatment contained all 7 of the analyzed species, the Burn and WindBurn each contained the same 6 species, and the Wind treatment contained 5 species (Table 1.1). Species-level nightly relative abundances of Green Frog, Grey Treefrog, and Wood Frog differed significantly among treatments (Green Frog: $F_{3,8} = 3.94$, $p < 0.05$; Grey Treefrog: $F_{3,8} = 4.09$, $p < 0.05$; Wood Frog: $F_{3,8} = 4.00$, $p = 0.05$; Table 1.2). Post hoc analyses revealed that Green Frogs were significantly less abundant in the Wind sites compared to the Control sites ($p < 0.05$) and Grey Treefrogs were significantly less abundant in the Wind sites compared to the Burn sites ($p < 0.05$; Table 1.1).

Call Characteristics

A total of 24264 Green Frog calls were analyzed for individual call characteristics, 6458 from Burn sites, 14063 from Control sites, 816 from Wind sites and 2927 from WindBurn sites (Table 1.2). Mean frequency and call durations were significantly different among treatments (frequency: $x^2_3 = 11.43$ $p < 0.01$; duration: $x^2_3 = 9.27$, $p < 0.05$; Table 1.3). Post hoc analyses revealed that Green Frog mean call frequency was significantly higher in the Control sites compared to the Burn ($p < 0.05$) and WindBurn sites ($p < 0.01$), while call duration was significantly longer in the Wind sites compared to the Control sites ($p < 0.05$; Figures 1.5, 1.6). Date, time, temperature, and wind speed were all significant predictors of Green Frog call mean frequency and duration (Table 1.4). Temperature and wind speed were positively related to mean frequency and negatively related to call duration, indicating that higher temperatures and wind speeds resulted in higher call frequencies and shorter call durations (Table 1.4). The random effect of site significantly influenced the model for both mean call frequency and call duration (Table 1.4).

A total of 84700 Spring Peeper calls were analyzed for individual call characteristics, 18686 from Burn sites, 22377 from Control sites, 13239 from Wind sites and 30398 from WindBurn sites (Table 1.3). Mean frequency and call duration did not significantly differ among treatments (frequency: $x^2_3 = 1.06$, $p > 0.05$; duration: $x^2_3 = 1.71$, $p > 0.05$; Table 1.3; Figures 1.7, 1.8). Date, time, temperature, and wind speed were all significant predictors of Spring Peeper call mean frequency and duration (Table 1.4). Temperature was positively related to mean frequency and negatively related to call duration, indicating that higher temperatures resulted in higher call frequencies and shorter call durations (Table 1.4). Wind speed was negatively related to mean frequency and positively related to call duration, indicating that higher wind speeds resulted in lower call frequencies and higher call durations. The random effect of site significantly influenced the model for both mean call frequency and call duration (Table 1.4).

Because the random effect of site was significant, I used Tukey's HSD post hoc analyses to examine differences in call characteristics among sites, within treatments, for both Green Frogs and Spring Peepers. For Green Frogs in the Burn treatment, mean call frequency was significantly lower while call duration was significantly higher in B1 (i.e., Burn site #1) compared to the other two Burn sites ($p < 0.001$). Within the Control treatment, mean call frequency was significantly lower in C1 compared to the other Control sites ($p < 0.001$), while call duration was significantly lower in C3 as compared to the other Control sites ($p < 0.001$). Mean call frequency and call duration did not differ between any of the Wind sites ($p > 0.05$). Within the WindBurn treatment, mean call frequency was significantly higher in WB3 and WB2 compared to the other WindBurn site ($p < 0.001$), but call duration did not differ ($p > 0.05$)

(Figures 1.5, 1.6). For Spring Peepers in the Burn treatment, mean call frequency was significantly lower while call duration was significantly higher in B3 compared to the other Burn sites ($p < 0.001$). Within the Control treatment, mean call frequency was significantly higher in C2 and C3 compared to the other Control site ($p < 0.001$), while call duration was significantly lower in C3 compared to the other sites ($p < 0.001$). Within the Wind treatment, mean call frequency was significantly higher in W1 compared to the other Wind sites ($p < 0.01$), but call duration did not differ ($p > 0.05$). Lastly, within the WindBurn treatment, mean call frequency was significantly higher in WB1 compared to the other WindBurn sites ($p < 0.01$), but call duration did not differ ($p > 0.05$; Figures 1.7, 1.8).

Turbine Impact Score

Turbine impact scores ranged from 0 for the non-windfarm sites to 1.12 representing the site with the greatest potential noise pollution (Table 1.5; Figure 1.1). Impact scores were negatively related to Mink Frog ($y = 132.20 - 132.95x$, $R^2 = 0.39$, $p < 0.05$), Green Frog ($y = 97.78 - 110.40x$, $R^2 = 0.41$, $p < 0.05$) and Grey Treefrog ($y = 17.24 - 14.84x$, $R^2 = 0.53$, $p < 0.05$) nightly relative abundance (Figure 1.9). Nightly relative abundance of Spring Peepers was not related to turbine impact score ($y = 222.67 - 13.90x$, $R^2 = 0.0048$, $p > 0.05$; Figure 1.9). At the anuran community level, diversity ($y = 1.18 - 0.83x$, $R^2 = 0.80$, $p < 0.001$), evenness ($y = 0.67 - 0.43x$, $R^2 = 0.73$, $p < 0.001$), and richness ($y = 5.90 - 1.44x$, $R^2 = 0.41$, $p < 0.05$) were all negatively related to turbine impact score (Figure 1.10).

Discussion

The goal of my study was to determine if wildfire, and windfarm construction and operation, and the combination of these disturbances, impacted anuran communities and calling behaviour. In support of my hypothesis, my results suggest that windfarms have negative influences on anuran chorus richness, evenness, and diversity, while in contrast to my hypothesis, the wildfire did not impact these metrics. I also found relatively lower abundances of 2 of the 5 species living in the windfarm. I found differences in Green Frog mean call frequency and duration between treatments but no differences in the same call characteristics of Spring Peepers. Also in support of my hypothesis, sites within the windfarm that contained higher turbine impact scores had lower community metric values and lower relative abundances of 3 of the 4 species examined.

Community Metrics

The lower community richness, evenness, diversity, and relative abundances in the windfarm may have resulted from a variety of factors. Windfarm construction modifies habitats, leading to habitat loss, degradation, and fragmentation by service roads (Kuvlesky et al. 2007; Diffendorfer et al. 2019), which could then result in road mortality (Lovich et al. 2011). Roads are a ubiquitous disturbance to anurans, having been documented to be especially problematic during breeding migrations (Fahrig et al. 1995; Eigenbrod et al. 2008; Glista et al. 2008; Eigenbrod et al. 2009; Beebee, 2013). Anuran road mortality was likely at its highest during windfarm construction when traffic was high and service roads were being constructed. However, since construction ended, traffic volume has remained low, with the occasional researcher or turbine operator driving through. The combination of roads and habitat loss may explain the reduction in anuran community metrics (Lehtinen et al. 1999; Pillsbury & Miller, 2008). The impacts of the initial disturbance during windfarm construction may still be felt, as it is possible that not enough time has passed for populations and communities to have recovered, especially for species with longer generation times.

Noise pollution from turbines appears to be negatively impacting anuran populations and communities. Wind turbines produce loud broadband noise in the frequency range of 200 – 5000 Hz (Katinas et al. 2016) which overlaps the frequencies of the breeding calls of Ontario's anurans (Cunnington & Fahrig, 2010; Trowbridge & Litzgus, 2022). Low-frequency noise is higher for larger turbines (2.3 – 3.6 MW; Møller et al. 2010), which includes the range of turbines in my study site. Turbine noise is persistent, as turbines are typically operating throughout the day and night (Schuster et al. 2015), leaving few opportunities for anurans to escape the noise. Turbine noise also increases with wind speed and decreases with distance from the turbine (Katinas et al. 2016). I created a Turbine Impact Score to quantify turbine noise pollution so that I could examine its impacts on anuran communities. For 3 of the 4 species examined, nightly relative abundance decreased as Turbine Impact Score increased. Two of those 3 species, Mink Frog and Green Frog, call at lower frequencies (~200 – 1500 Hz), while the third species, Grey Treefrog, calls at mid frequencies (~1000 – 2000 Hz). Mink Frogs and Green Frogs would be directly competing with the low broadband noise of wind turbines, potentially explaining their decreased abundance, whereas Grey Treefrogs would not directly compete with turbine noise as often. The abundance of Spring Peepers was not related to Turbine Impact Score, and they call at high frequencies (~2500 – 3500 Hz) and would likely not have to compete with turbine noise, similar to results observed with other high call frequency species in the presence of road traffic noise (Vargas-Salinas et al. 2014).

Like abundance, community diversity, evenness, and richness were negatively related to Turbine Impact Score, suggesting that wetlands surrounded with a large number of close turbines are less suitable for anuran communities. It is possible that anurans are not calling as often in the presence of turbine noise, similar to the response observed in the presence of traffic noise

(Lengagne, 2008; Vargas-Salinas et al. 2014). Previous work has also found that anurans adjust their calling based on when traffic volume and noise decrease (Zelick & Narins, 1983; Vargas-Salinas et al. 2014); this adjustment would be difficult for anurans living in windfarms because turbines operate throughout the day and night (Schuster et al. 2015). The continuous low frequency noise, with few gaps in noise that can be taken advantage of (Zelick & Narins, 1982), may compromise an individual's ability to communicate and reproduce (i.e., male frogs are unable to advertise their position to females; Zelick & Narins, 1983), in turn leading to potential population declines.

Individuals may also avoid areas of wetlands where turbine noise is most prevalent. Noise pollution can lead to altered habitat selection, resulting in habitat avoidance by certain species (Shannon et al. 2016). For example, multiple songbird species display habitat avoidance in the presence of wind turbine noise (Lehnardt et al. 2023). However, breeding anurans do not necessarily avoid ponds with high traffic noise (Sun & Narins, 2005; Lengagne, 2008). Another possibility is that species with lower frequency calls are not able to adjust their calls to compete with the turbine noise, leading to decreased breeding success and gradual population declines in these wetlands (Zelick & Narins, 1983; Trowbridge & Litzgus, 2022). In contrast, Cunnington & Fahrig (2013) found that traffic noise did not negatively affect mate attraction in anurans.

The lack of community differences between the Burn and Control treatments may be explained by the presence of refugia. Fire can have both positive and negative effects on anuran communities, with effects primarily dependent on species' ecology and fire characteristics (dos Anjos et al. 2021). Boreal Toads, *Anaxyrus boreas*, for example, were found to use habitats burned to a high severity more than unburned or partially burned habitats (Guscio et al. 2008) due to warmer microclimatic temperatures in all suitable microhabitat types (Hossack et al.

2009). This result, however, may not be consistent across the entire anuran community exposed to the wildfire, as some species prefer cooler temperatures and stable environments (Hossack et al. 2009). Fire typically does not have an immediate, direct threat to anuran populations because individuals are able to seek refuge underground or in water (Russell et al. 1999; Mahoney et al. 2022). Species that disperse from their breeding ponds to terrestrial upland habitat (e.g., Spring Peepers, Grey Treefrogs), may have sought burn-resistant refugia by using auditory cues of the approaching fire, as suggested by Grafe et al. (2002). In the case of the wildfire at my study site, while it was high severity and widespread (Markle et al. 2022), personal anecdotal observations in the field provided confirmation that my wetland sites remained intact and suitable for anuran survival and persistence.

The majority of research investigating the impacts of wildfires on anurans has focussed on short fire-return intervals, often looking at the effects of a single wildfire (Hossack et al. 2013). The potential for time-lagged effects of wildfires on anuran communities needs consideration. Research on one wildfire found that occupancy of the Columbia Spotted Frog (*Rana luteiventris*) was not affected 6 years post fire, while occupancy of the Boreal Toad (*Anaxyrus boreas*) tripled 3 years post fire; however, occupancy of both species declined 7 – 21 years after the fire (Hossack et al. 2013). Numerous factors can influence anuran survival and persistence after wildfire. For example, droughts can lead to reproductive difficulties (Corn & Fogleman, 1984) and decreased juvenile size and immune function (Gervasi & Foufopoulos, 2007). An alteration of the surrounding vegetative areas can also lead to increased risk of desiccation and predation for species that disperse from breeding sites (Rittenhouse et al. 2009). The indirect alteration of breeding habitats and refugia availability could also lead to declines of anuran populations and communities (Hossack et al. 2013). These factors, combined with other potential lagged wildfire effects, could cause continuing gradual losses in populations. These declines could delay or impede the recovery of populations and communities, which may not be observable on short time intervals.

Call Characteristics

Anurans will alter their call frequency (Parris et al. 2009; Cunnington & Fahrig, 2010; Hanna et al. 2014) and call duration (Penna et al. 2005; Hanna et al. 2014) in the presence of abiotic noise. I followed the work of Trowbridge & Litzgus (2022) to further investigate if Green Frogs and Spring Peepers alter their calls in response to wind turbine noise or after wildfire. I found differences in mean call frequency and duration among treatments for Green Frogs but not for Spring Peepers (Table 1.3; Figures 1.5 – 1.8). This supports my hypothesis that low frequency calls, such as those of Green Frogs, would be more likely to change when exposed to low broadband turbine noise, compared to high frequency calls of Spring Peeper, which register at a higher frequency than the turbine noise, and would be more sensitive to high frequency noise rather than low frequency (Wilczynski et al. 1984).

While Green Frog call frequencies differed among treatments, they did not follow the predicted pattern. I predicted that mean call frequency would be highest in the windfarm sites because males would need to increase their call frequency to compensate for the noise of the turbines, much like the response to traffic noise (Cunnington & Fahrig, 2010), but this pattern was not observed, as frogs in the windfarm sites had lower call frequencies than in the Control (Table 1.3; Figure 1.5). Trowbridge & Litzgus (2022) found no differences in Green Frog call characteristics between their turbine and control sites, even though they reported a larger difference between means (~90 Hz versus ~35 Hz in my study). Further to that, Trowbridge & Litzgus (2022) found that the mean call frequency of Green Frogs at their sites ranged from ~366

- 583 Hz, which aligns closely with my detected mean call frequency range of ~415 - 525 Hz. These data help suggest that although there is a statistical difference in call frequency among treatments, it may not reflect a true biological difference in calls among treatments. Cunnington & Fahrig (2010) found that the difference in Green Frog call dominant frequency between low traffic and high traffic sites was ~375 Hz; if wind turbine noise was biologically affecting anuran call frequencies, I would expect to see a frequency difference of this magnitude.

Spring Peepers are known to decrease their call duration in response to noise (Hanna et al. 2014), while another study found that Chiloe Island Ground Frogs (*Eupsophus calcaratus*) increased their call duration (Penna et al. 2005). I observed the latter, with longer Green Frog call durations in windfarm sites (Table 1.3; Figure 1.6). The ability to produce longer calls is a strategy used by wildlife to increase detectability amid noisy interference (Brumm et al. 2004). Increased call duration within the windfarm may signify that Green Frogs are viewing the turbine noise as an intrusion within the acoustic environment and increasing their call length to compete with the background noise. Penna et al. (2005) found that Chiloe Island Ground Frogs decreased call duration between exposures to a given noise. With the persistent turbine noise in the windfarm, it is likely that anurans would not have a break from noise pollution, and they could theoretically have to continuously call at a lengthened duration to compete with the noise. Call duration could also increase on especially windy nights when turbine noise increases with the wind (Katinas et al. 2016). The increase in call duration within the wildfire sites could be attributed to increased wind-associated noise, due to a lack of canopy and vegetive buffers. Wind-generated noise is highest at the low frequency range of anuran calls (Bradbury & Vehrencamp, 1998). The increased background noise from increased wind speeds due to a loss of canopy cover (Ma et al. 2010) could result in longer call durations to compete with the wind noise.

The combination of wind speed and turbine noise likely also plays a role in anuran call characteristics. Increases in wind velocity lead to increases in wind turbine noise (Katinas et al. 2015; Figure 1.11). I found zero detections of Green Frogs within Wind and WindBurn sites when wind speeds exceeded 1.5 (m/s), although Green Frogs were detected in the Control the Burn sites at the same wind speeds (Figure 1.12). Spring Peepers on the other hand were present in all treatments at speeds including and exceeding 1.5 (m/s). It is possible that the combined effect of turbine noise and wind speed create a larger disruption to the acoustic environment, possibly preventing the low-frequency species from calling. However, it is difficult to determine the exact mechanism causing fewer Green Frogs to call. It is possible that they were unable to alter their calls and instead called less frequently in windfarm sites during periods of high wind, similar to periods of high traffic noise (Lengagne, 2008; Vargas-Salinas et al. 2014). Similarly, one study that found that American Bullfrog (*Lithobates catesbeianus*) call activity was negatively correlated with wind, possibly because of sound interference (Oseen & Wassersug, 2002). It is also possible that my results are just a by-product of Wind sites containing fewer Green Frogs compared to the non-impacted sites.

Conclusion

Most research to-date looking at relationships between anuran community dynamics and noise pollution has focused on traffic noise. These limited data can pose a challenge, as it is difficult to tease apart the effects of road mortality from noise pollution on populations. Grace & Noss (2018) examined the effect of traffic noise on anuran community call abundance in roadless areas and they found that broadcasting traffic noise (‘phantom road’) led to decreased call abundance, thus clearly showing that the reduction in calls was attributable to noise pollution alone and not road mortality. Similarly, Lehnardt et al. (2023) recently aimed to examine if wind turbine noise, and not the turbines themselves, could be a mechanism for habitat avoidance in songbirds. They found that when using a ‘phantom wind turbine’ (broadcasted turbine noise in a turbine-less area), bird abundance at a migratory stopover site was lower. These findings provide confirmation of my results showing that turbine noise negatively impacts anuran biodiversity. High levels of anuran road mortality do not exist within the windfarm where I conducted my study, suggesting that turbine noise is the most likely reason for reduced diversity and richness. Even more, anurans are not highly mobile organisms like mammals and birds and may not be able to avoid habitats where turbine noise is most prevalent. However, without clear patterns in call characteristics, it is difficult to determine the exact mechanism driving the observed reductions. I recommend future studies to continue examination of call characteristics and wind turbine noise, to try to tease apart potential mechanisms explaining community differences. Indirect effects, such as noise pollution, need to be addressed when planning renewable energy projects that involve noise-generating infrastructure, such as windfarms (Teff-Seker et al. 2022; Lehnardt et al. 2023). Wildlife noise sensitivity is not a priority during planning processes, even in biodiversity hotspots (Drewitt et al. 2006; Teff-Seker et al. 2022). I also recommend continued long-term monitoring of anuran communities affected by wildfires to account for any time-lagged effects. The continued growth of wind energy (IRENA, 2022) and wildfires (Bowman et al. 2009; Pyne, 2020; Nimmo et al. 2021; Wu et al. 2021) and decline of anurans (Stuart et al. 2004; ICUN, 2023), makes filling these gaps in knowledge more crucial than ever.

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Tables

Table 1. 1. Relative nightly abundance (total call detections/sampling nights) of seven species of anuran calls in four replicated treatments, Burn (sites B1, B2, B3), Control (sites C1, C2, C3), Wind (sites W1, W2, W3), and WindBurn (sites WB1, WB2, WB3), and raw total number of calls by that species indicated in parentheses. Bolded numbers represent the sum of all three sites within a treatment. Calls were collected nightly on a rotating weekly schedule from 8 May – 21 July 2023. Anuran calls were identified and extracted using Kaleidoscope Pro (Wildlife Acoustics, v5.4.9g).

		Species						
		American Bullfrog (*Lithobates casteianus*)	Green Frog (*Lithobates clamitans)*	Wood Frog (*Lithobates sylvaticus*)	Northern Leopard Frog (*Lithobates pipiens*)	Mink Frog (*Lithobates septentrionalis*)	Spring Peeper (*Pseudacris crucifer*)	Grey Treefrog (*Hyla versicolor*)
Treatment	Site							
	C1	4 (135)	124 (4202)	2 (55)	4 (124)	132 (4476)	207 (7033)	15 (510)
	C2	1 (29)	240 (8143)	2 (79)	0 (12)	262 (8923)	244 (8304)	16 (548)
	C3	0 (13)	60 (1988)	0 0	1 (30)	35 (1159)	231 (7639)	8 (276)
Control		**2** **(177)**	**142** **(14333)**	**1** **(134)**	**2** **(166)**	**144** **(14558)**	**227** **(22976)**	**13** **(1334)**
	B1	11 (396)	17 (627)	0 0	0 0	79 (2920)	65 (2422)	15 (550)
	B2	6 (175)	92 (2668)	0 0	13 (372)	221 (6400)	206 (5980)	23 (656)
	B3	1 (34)	99 (3252)	0 0	0 0	124 (4086)	320 (10574)	26 (862)
Burn		**6** **(605)**	**66** **(6547)**	**0** **0**	**4** **(372)**	**135** **(13406)**	**192** **(18976)**	**21** **(2068)**

	W1	0	0	0	0	1	112	10
		0	(3)	0	0	(37)	(4129)	(371)
	W2	0	14	0	0	11	225	1
		1	557	0	0	(417)	(8794)	(27)
	W3	1	7	0	0	132	176	2
		(45)	(256)	0	0	(5153)	(6880)	(64)
Wind		**0**	**7**	**0**	**0**	**49**	**172**	**4**
		(46)	**(816)**	**0**	**0**	**(5607)**	**(19803)**	**(462)**
	WB1	0	24	0	2	33	187	6
		0	(868)	0	(61)	(1187)	(6725)	(202)
	WB2	13	47	0	0	42	295	20
		(504)	(1882)	0	0	(1671)	(11791)	(781)
	WB3	1	4	0	0	14	348	5
		(43)	(157)	0	0	(486)	(12171)	(174)
WindBurn		**5**	**26**	**0**	**1**	**30**	**276**	**10**
		(547)	**(2907)**	**0**	**(61)**	**(3344)**	**(30687)**	**(1157)**

Table 1. 2. Results of the one-way ANOVAs of the various anuran community metrics compared among treatments, including Chorus Evenness, Chorus Diversity, Chorus Richness, and Species-Specific Relative Abundances. Bolded p-values indicate statistical significance ($p \leq 0.05$). See Appendix A for explanations of species acronyms.

	df	F	p
Chorus Evenness			
Treatment	3,8	4.91	**< 0.05**
Chorus Diversity			
Treatment	3,8	5.97	**< 0.05**
Chorus Richness			
Treatment	3,8	9.22	**< 0.05**
Species Nightly Relative Abundance x Treatment			
GRFR	3,8	3.94	**< 0.05**
GRTR	3,8	4.09	**< 0.05**
SPPE	3,8	0.93	> 0.05
BUFR	3,8	0.91	> 0.05
WOFR	3,8	4.00	**= 0.05**
MIFR	3,8	1.82	> 0.05
LEFR	3,8	0.70	> 0.05

Table 1. 3. Individual call characteristics (mean call frequency (Hz), call duration (s)) for *Lithobates clamitans* and *Pseudacris crucifer* by site, nested within treatment. Results of a likelihood ratio test, testing for differences between treatments for each call characteristic in a linear mixed effect model, are included. Bolded numbers represent cumulative treatment values.

Species	Site	**Mean call frequency (Hz)**					**Call duration (S)**			
		n	Mean (Hz)	±SE	x^2	p	Mean (s)	±SE	x^2	p
Lithobates clamitans	C1	4052	485.64	0.88	11.43	<0.01	0.28	0.88	9.27	<0.05
	C2	8023	515.28	0.58			0.27	0.58		
	C3	1988	525.44	6.74			0.17	6.74		
	Control	**14063**	**508.79**	**6.82**			**0.24**	**0.01**		
	B1	623	424.47	2.25			0.32	2.25		
	B2	2615	464.40	1.73			0.27	1.73		
	B3	3220	465.76	1.04			0.28	1.04		
	Burn	**6458**	**451.54**	**3.02**			**0.29**	**0.01**		
	W1	3	497.96	19.02			0.17	19.02		
	W2	557	457.96	1.79			0.38	1.79		
	W3	256	466.42	3.22			0.38	3.22		
	Wind	**816**	**474.11**	**19.38**			**0.31**	**0.07**		
	WB1	865	415.90	2.12			0.30	2.12		
	WB2	1839	432.61	1.23			0.30	1.23		
	WB3	223	478.64	4.99			0.31	4.99		
	WindBurn	**2927**	**442.38**	**5.56**			**0.31**	**0.01**		
Pseudacris crucifer	C1	6910	3034.66	2.48	1.06	>0.05	0.13	2.48	1.71	>0.05
	C2	7976	3067.43	2.63			0.14	2.63		
	C3	7491	2963.12	1.89			0.13	1.89		
	Control	**22377**	**3021.74**	**4.08**			**0.13**	**0.00**		
	B1	2353	3039.08	4.67			0.13	4.67		
	B2	5958	3028.31	2.55			0.13	2.55		
	B3	10375	2969.33	1.77			0.14	1.77		
	Burn	**18686**	**3012.24**	**5.61**			**0.13**	**0.00**		
	W1	2742	3043.57	4.28			0.13	4.28		
	W2	6018	3021.46	2.39			0.13	2.39		
	W3	4479	3024.77	3.36			0.13	3.36		
	Wind	**13239**	**3029.93**	**5.94**			**0.13**	**0.00**		
	WB1	6683	3007.23	2.61			0.13	2.61		
	WB2	11719	2989.02	1.85			0.14	1.85		
	WB3	11996	2996.02	1.75			0.13	1.75		
	WindBurn	**30398**	**2997.42**	**3.65**			**0.13**	**0.00**		

Table 1. 4. Linear mixed model summary of predictor variables not showing treatment results (see Table 1.3) for individual call characteristics of *Lithobates clamitans* and *Pseudacris crucifer* and likelihood ratio test results. Site was included as a random effect, using an ANOVA-like table for random effects (LRT = likelihood ratio test). Coefficient estimate of the variable indicates a positive or negative relationship to the tested call characteristic.

		Date			**Time**			**Temperature**			**Wind Speed**			**Site**	
Species	Call Characteristic	Coefficient Estimate	x^2	p	Coefficient Estimate	x^2	p	Coefficient Estimate	x^2	p	Coefficient Estimate	x^2	p	LRT	p
Lithobates clamitans	Mean Call Frequency (Hz)	-6.13E-01	22.77	<0.01	1.21E-02	179.71	<0.01	1.94E+00	206.46	<0.01	8.79E+00	103.4	<0.01	1016.7	<0.01
	Call Duration (s)	9.61E-04	107.15	<0.01	-8.94E-05	1241.9	<0.01	-1.22E-05	10.41	<0.01	-5.27E-03	4.83	<0.01	78.72	<0.01
Pseudacris crucifer	Mean Call Frequency (Hz)	-2.77E-01	20.78	<0.01	-4.26E-03	6.54	<0.01	7.18E+00	1679.4	<0.01	-8.81E+00	84.98	<0.01	1812	<0.01
	Call Duration (s)	6.27E-04	3362.2	<0.01	-2.20E-06	55.89	<0.01	-4.55E-04	218.74	<0.01	1.16E-03	46.93	<0.01	917.76	<0.01

Table 1. 5. Calculated Turbine Impact Scores with their respective site and treatment. Turbine Impact Scores were created using the following formula: *Impact* = (w_{Turbines} x Number of Turbines) + (w_{Distance} x (1 - Transformed Combined Distance)), see text for additional equation details. Sites with a higher value indicate a greater potential impact from the noise of the turbines. Sites with no turbines (Control, Burn) had calculated values of 0.

Treatment	**Site**	**Turbine impact score**
Control	C1	0
	C2	0
	C3	0
Burn	B1	0
	B2	0
	B3	0
Wind	W1	0.88
	W2	1.12
	W3	0.58
Windburn	WB1	0.56
	WB2	0.28
	WB3	0.62

Figures

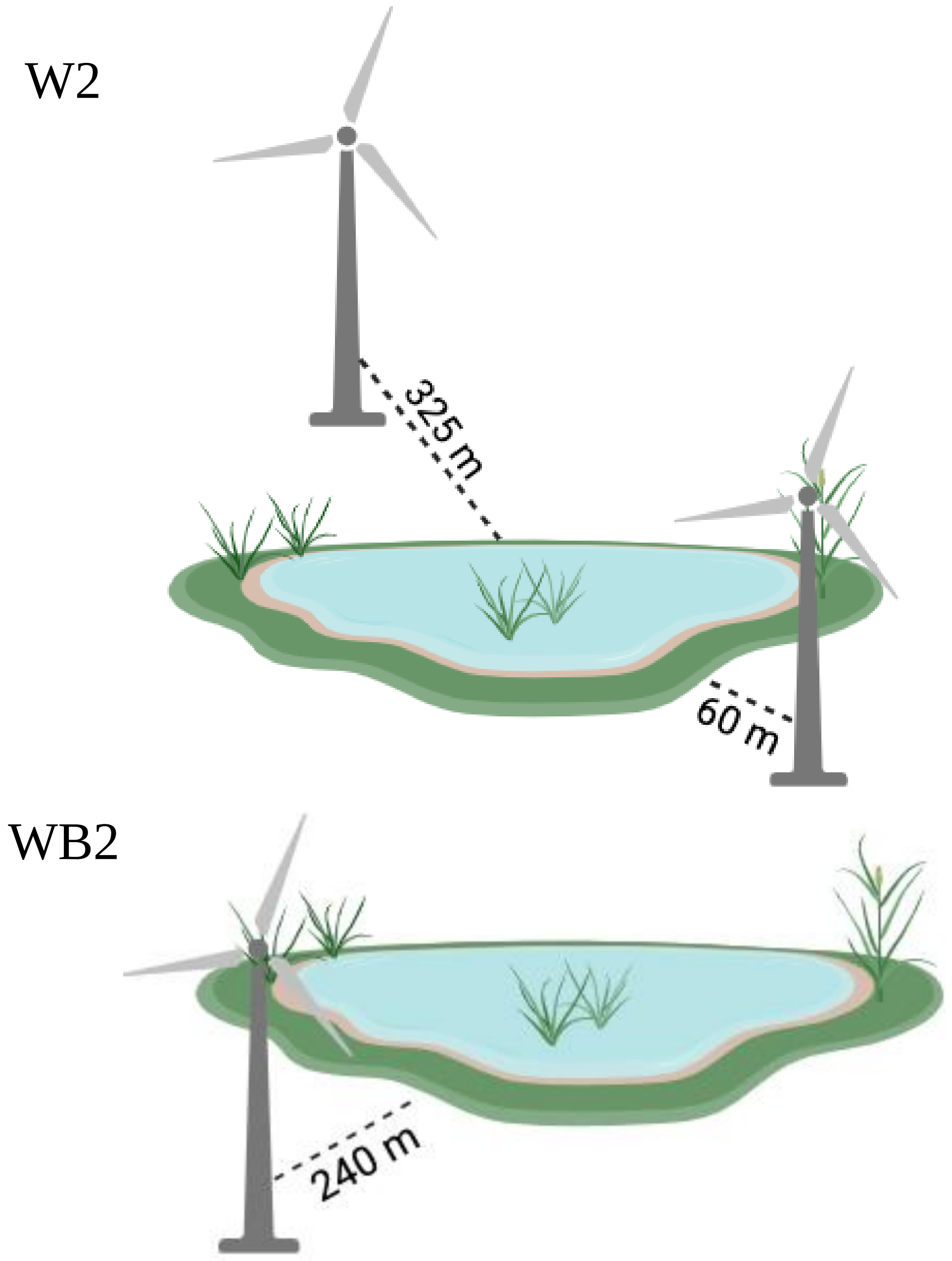

Figure 1. 1. Graphical representation of Turbine Impact Score; a score encompassing number and distance of turbines to a wetland site. Impact scores were calculated using the following formula: *Impact* = (w_{Turbines} x Number of Turbines) + (w_{Distance} x (1 - Transformed Combined Distance)). In this example, W2's *Impact* = (0.7 x 2) + (0.3 x (1 – ((385/2)/100)) = 1.12 vs. WB2's *Impact* = (0.7 x 1) + (0.3 x (1 – (240/100))) = 0.28. Higher scores indicate greater potential noise pollution from turbines, such that a wetland with 2 turbines in close proximity would experience greater noise pollution compared to a wetland with 1 turbine in further proximity. Graphic created with BioRender.com.

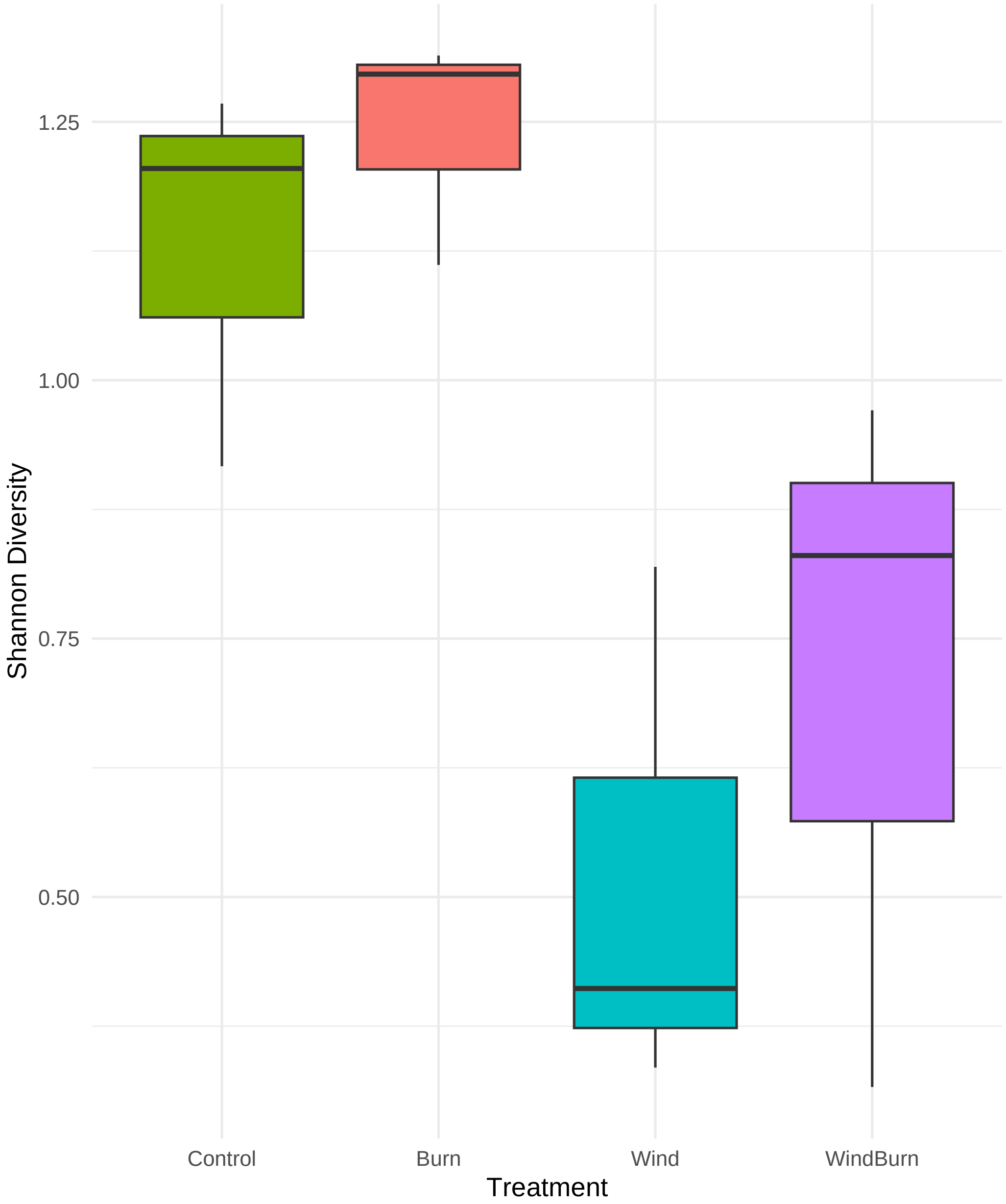

Figure 1. 2. Shannon Weiner Chorus Diversity (H′) of anurans in four treatments, Control, Burn, Wind, and WindBurn, each replicated three times (n=12 sites in total). Diversity was significantly higher in Control and Burn sites than in Wind sites ($p < 0.05$). Bi-weekly nightly recordings were conducted at each of the 12 sites from 8 May to 21 July 2023. Calls of 7 different anuran species were identified using Kaleidoscope Pro (Wildlife Acoustics, v5.4.9g). Boxplots contain the diversity values for the three sites per treatment. Horizontal lines represent treatment medians, while the vertical lines represent the spread of the data.

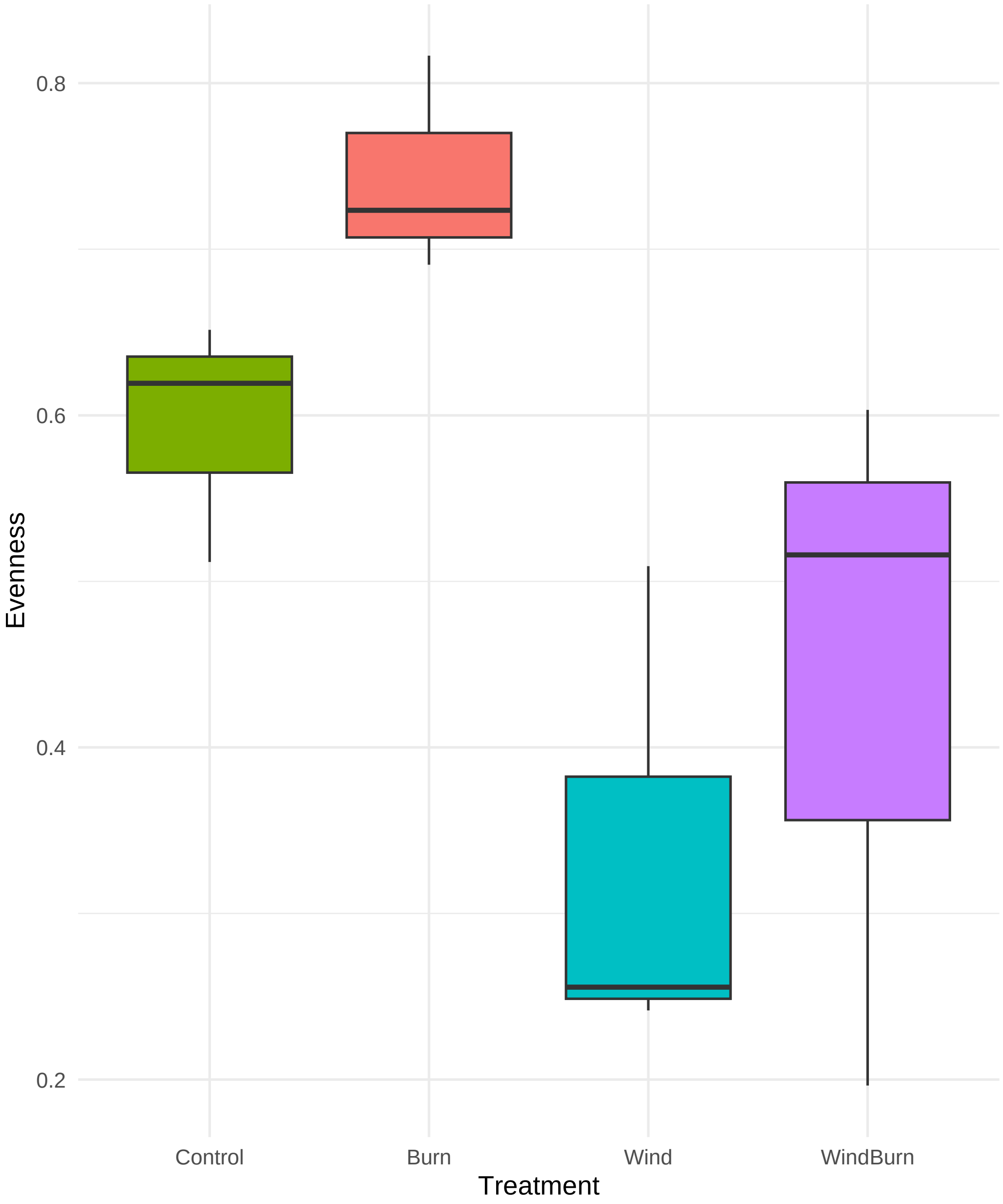

Figure 1. 3. Chorus Evenness (Pielou's Evenness, E) of anurans in four treatments, Control, Burn, Wind, and WindBurn, each replicated three times (n=12 sites in total). Evenness was significantly higher in Burn sites than in Wind sites ($p < 0.05$). None of the other pairwise comparisons were significant. Bi-weekly nightly recordings were conducted at each of the 12 sites from 8 May to 21 July 2023. Calls of 7 different anuran species were identified using Kaleidoscope Pro (Wildlife Acoustics, v5.4.9g). Boxplots contain the evenness values for the three sites per treatment. Horizontal lines represent treatment medians, while the vertical lines represent the spread of the data.

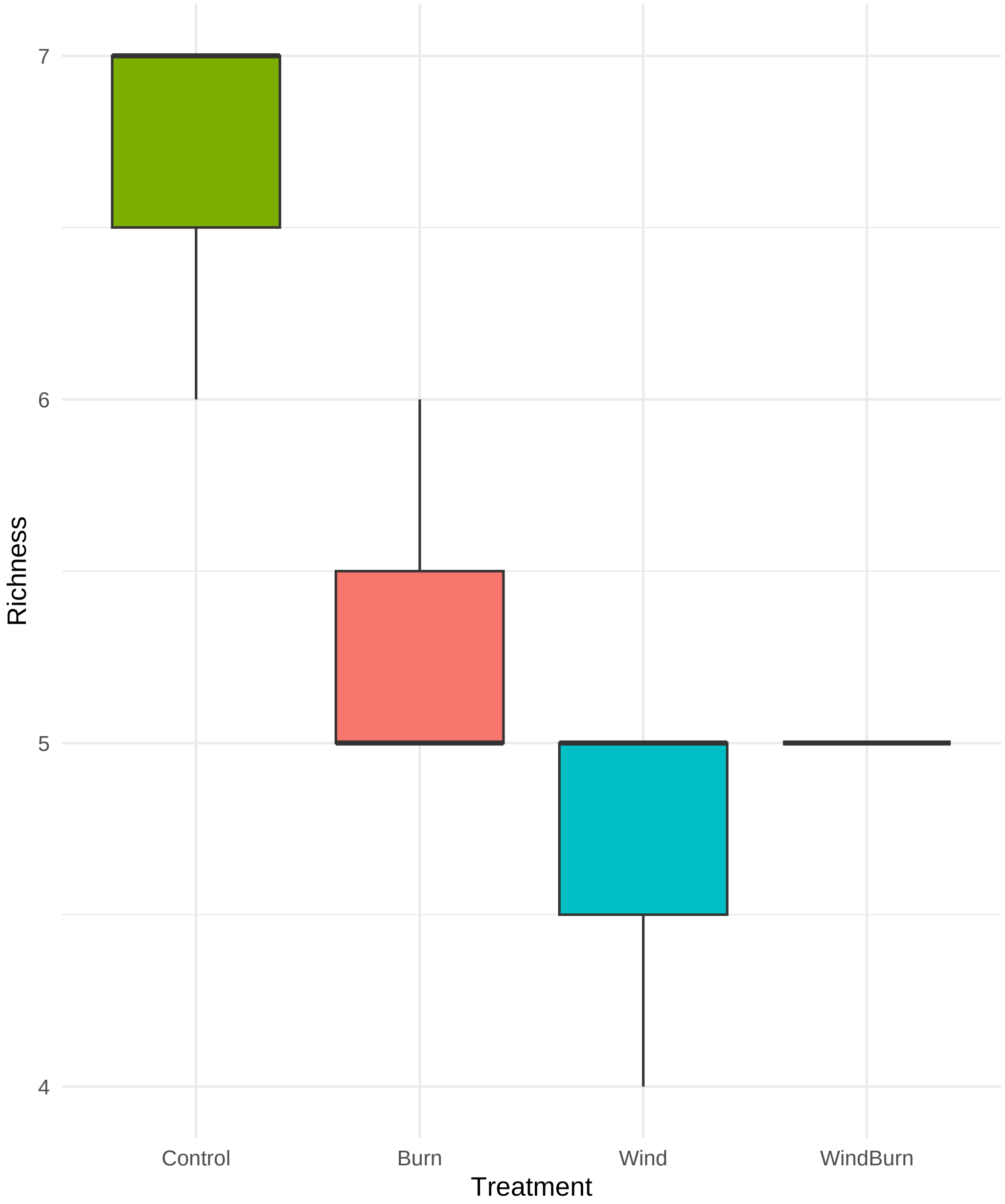

Figure 1. 4. Chorus Richness of anurans in Control, Burn, Wind, and WindBurn sites. Richness was significantly higher in Control than all other treatment sites (Burn: $p < 0.05$; Wind: $p < 0.01$; WindBurn: $p < 0.05$). Bi-weekly nightly recordings were conducted at each of the 12 sites from 8 May to 21 July 2023. Calls of 7 different anuran species were identified using Kaleidoscope Pro (Wildlife Acoustics, v5.4.9g). Boxplots contain the richness values for the three sites per treatment. Horizontal lines represent treatment medians, while the vertical lines represent the spread of the data.

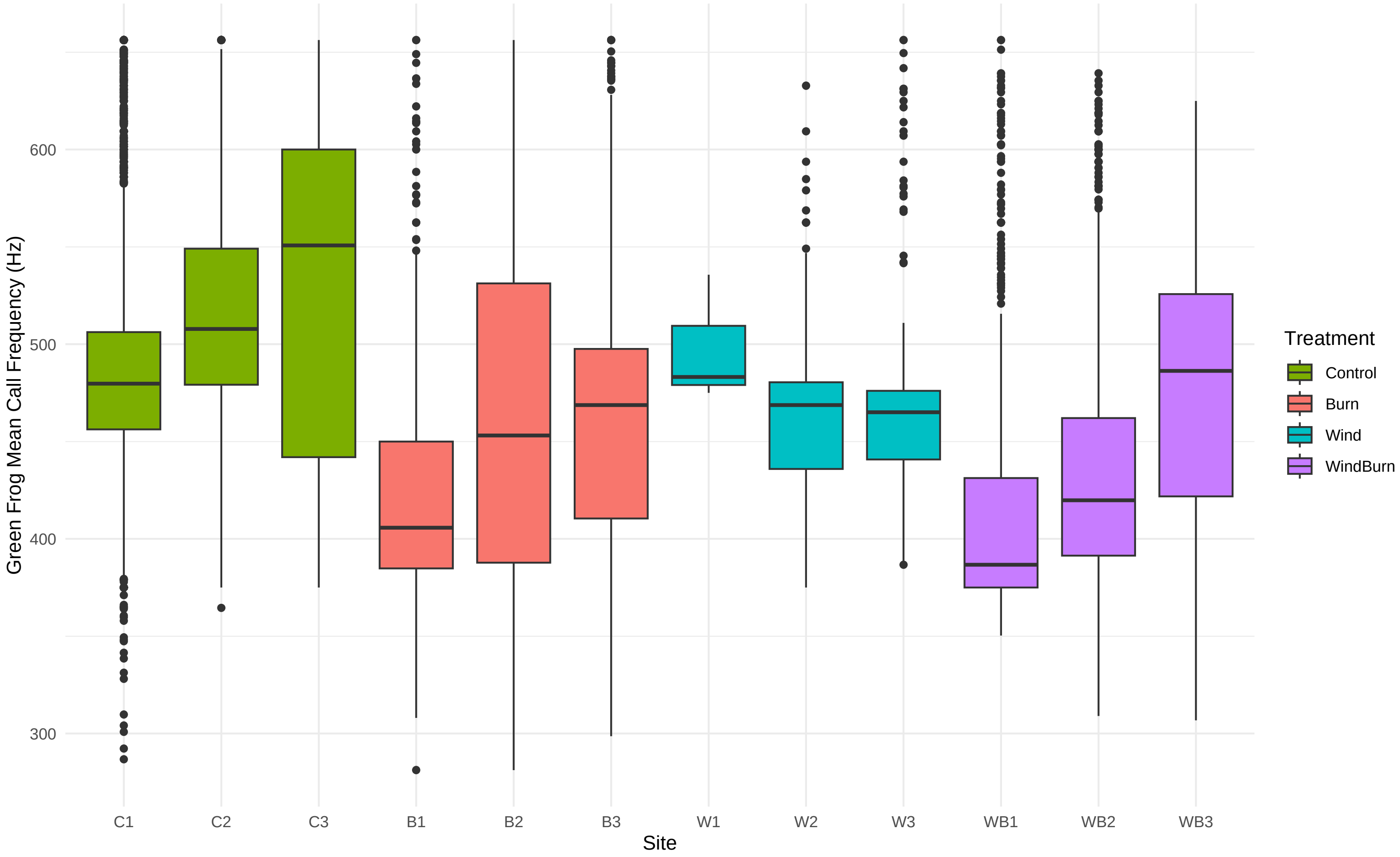

Figure 1. 5. Site-specific variation in mean call frequency (Hz) of *Lithobates clamitans* in Control, Burn, Wind, and WindBurn treatment sites. Boxplot horizontal lines represent site medians while dots represent outliers and vertical lines represent the spread of the data. Mean frequencies were significantly different among treatments ($x^2{}_3 = 11.43$, $p < 0.01$). Post hoc analyses revealed that mean call frequency was significantly higher in the Control sites as compared to the Burn ($p < 0.05$) and WindBurn sites ($p < 0.01$).

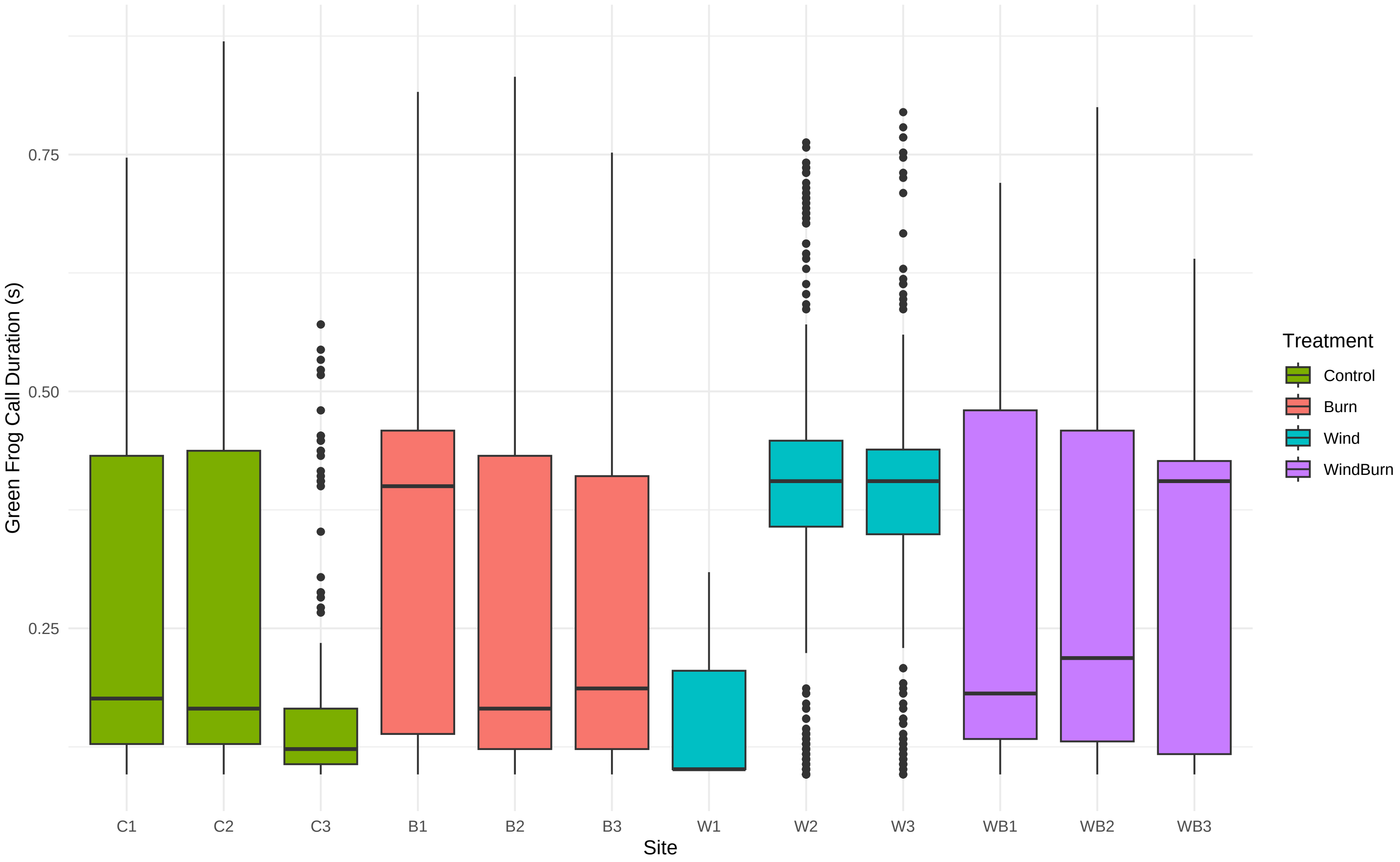

Figure 1. 6. Site-specific variation in call duration (s) of *Lithobates clamitans* in Control, Burn, Wind, and WindBurn sites. Boxplot horizontal lines represent site medians while dots represent outliers and vertical lines represent the spread of the data. Durations were significantly different among treatments ($x^2{}_3 = 9.27$, $p < 0.05$). Post hoc analyses revealed that that call duration was significantly longer in the Wind sites as compared to the Control sites ($p < 0.05$).

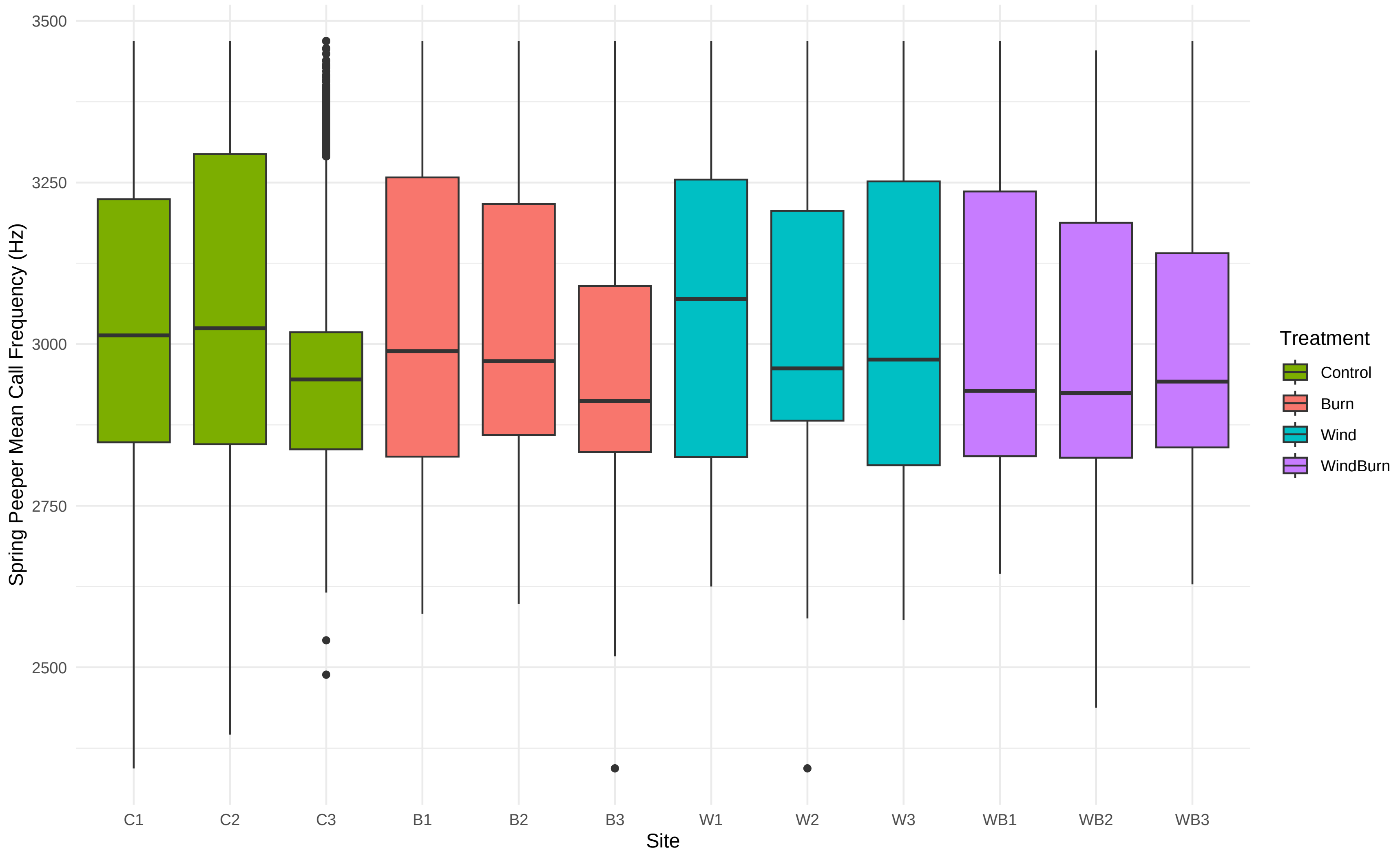

Figure 1. 7. Site-specific variation in mean call frequency (Hz) of *Pseudacris crucifer* in Control, Burn, Wind, and WindBurn sites. Boxplot horizontal lines represent site medians while dots represent outliers and vertical lines represent the spread of the data. Mean frequencies were not significantly different among treatments ($x^2_3 = 1.06$, $p > 0.05$).

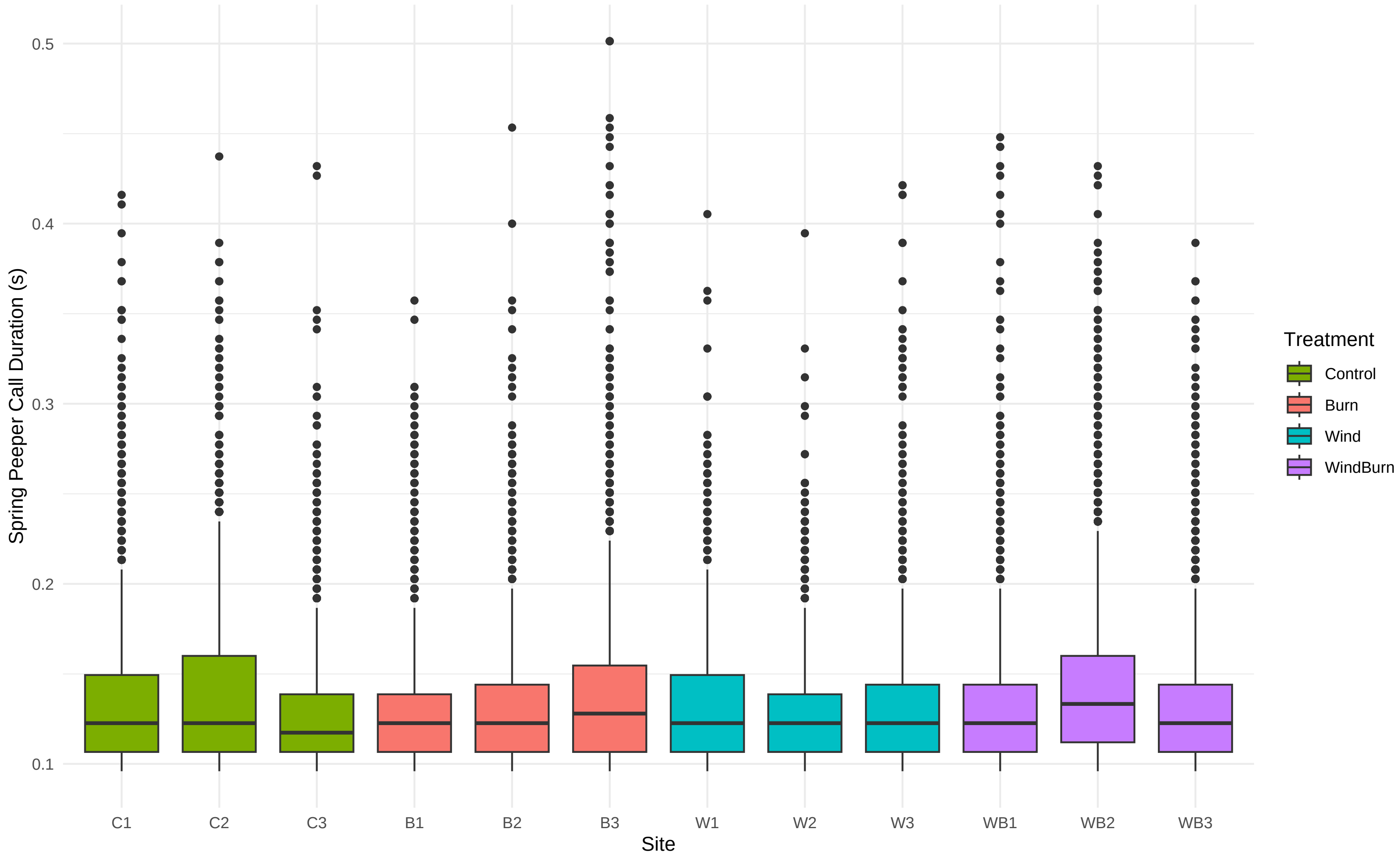

Figure 1. 8. Site-specific variation in call duration (s) of *Pseudacris crucifer* in Control, Burn, Wind, and WindBurn sites. Boxplot horizontal lines represent site medians while dots represent outliers and vertical lines represent the spread of the data. Durations were not significantly different among treatments ($x^2_3 = 1.71$, $p > 0.05$).

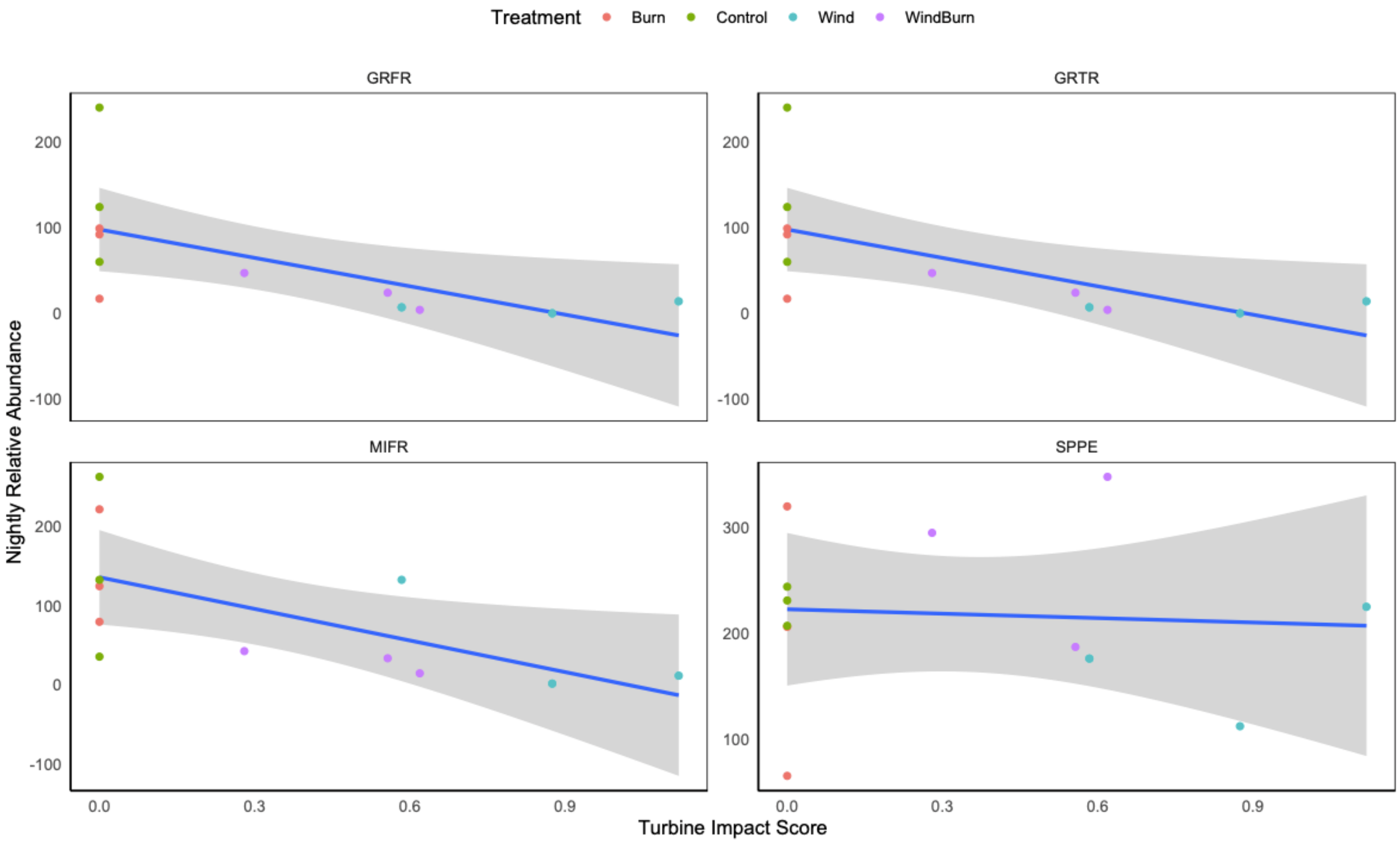

Figure 1. 9. Linear regressions displaying the relationships between Nightly Relative Abundance (number of species detections/number of sampling nights) of 4 common species and Turbine Impact Score, by site. Significant negative relationships were observed for *Lithobates clamitans* (GRFR), *Hyla versicolor* (GRTR), and *Lithobates septentrionalis* (MIFR) (GRFR: $y = 97.78 - 110.40x$, $R^2 = 0.41$, $p < 0.05$; GRTR: $y = 17.24 - 14.84x$, $R^2 = 0.53$, $p < 0.05$; MIFR: $y = 132.20 - 132.95x$, $R^2 = 0.39$, $p < 0.05$). No significant relationship was observed for *Pseudacris crucifer* (SPPE) ($y = 222.67 - 13.90x$, $R^2 = 0.0048$, $p > 0.05$). Sites with no noise pollution from turbines (Control, Burn) had calculated turbine impact score values of 0, while sites with turbine impacts (Wind, WindBurn) had calculated values based on number and distance of turbines within proximity (Table 1.5; Figure 1.1). See text for equation details.

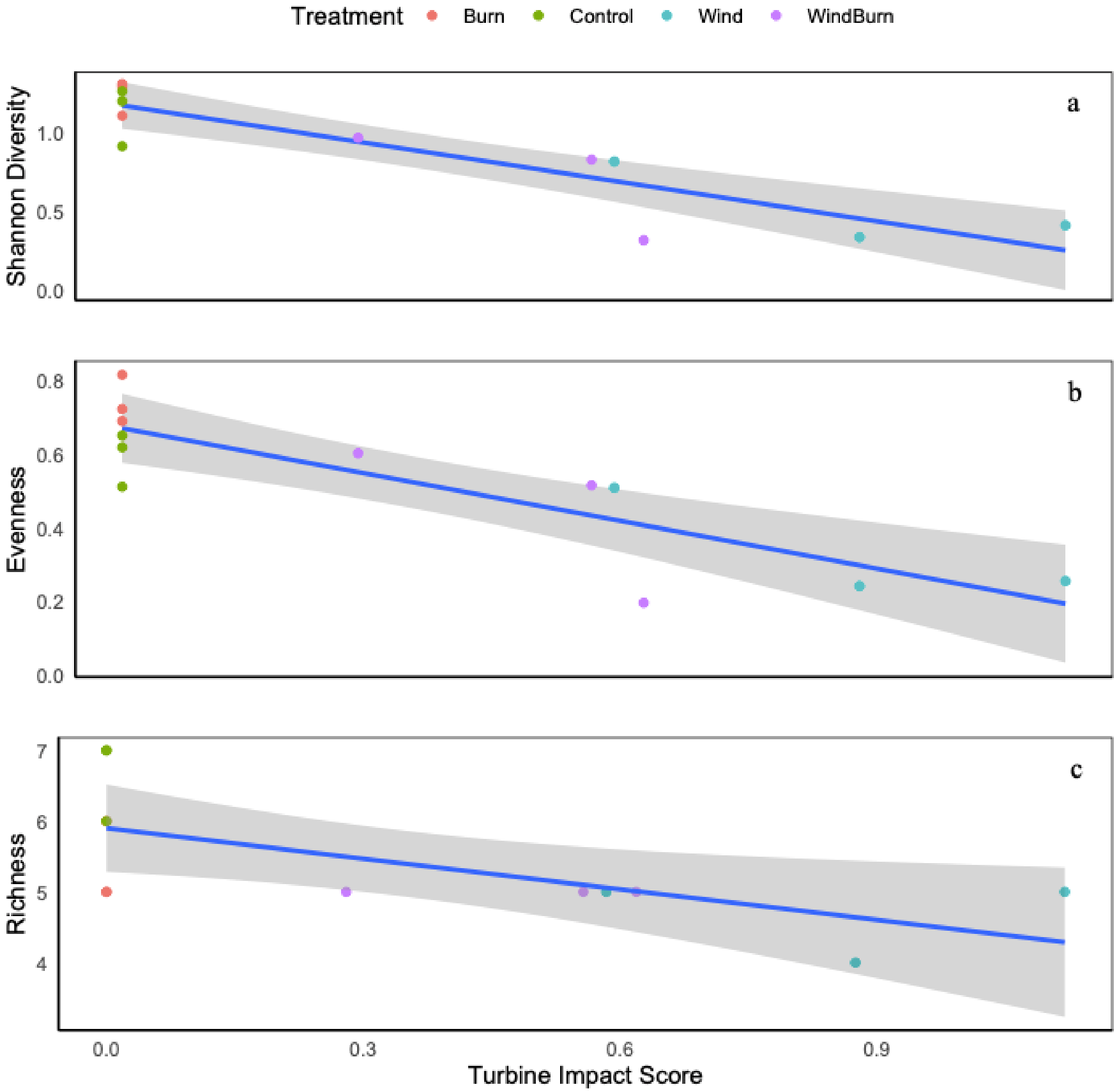

Figure 1. 10. Linear regression displaying the significant negative relationships between Shannon Weiner Chorus Diversity, Chorus Evenness, Chorus Richness and Turbine Impact Score ($y = 1.18 - 0.83x$, $R^2 = 0.80$, $p < 0.001$; $y = 0.67 - 0.43x$, $R^2 = 0.73$, $p < 0.001$; $y = 5.90 - 1.44x$, $R^2 = 0.41$, $p < 0.05$, respectively). Sites with no noise pollution from turbines (Control, Burn) had calculated turbine impact score values of 0, while sites with turbines (Wind, WindBurn) were assigned values based on number and distance of turbines from the wetland (Table 1.5; Figure 1.1). See text for equation details.

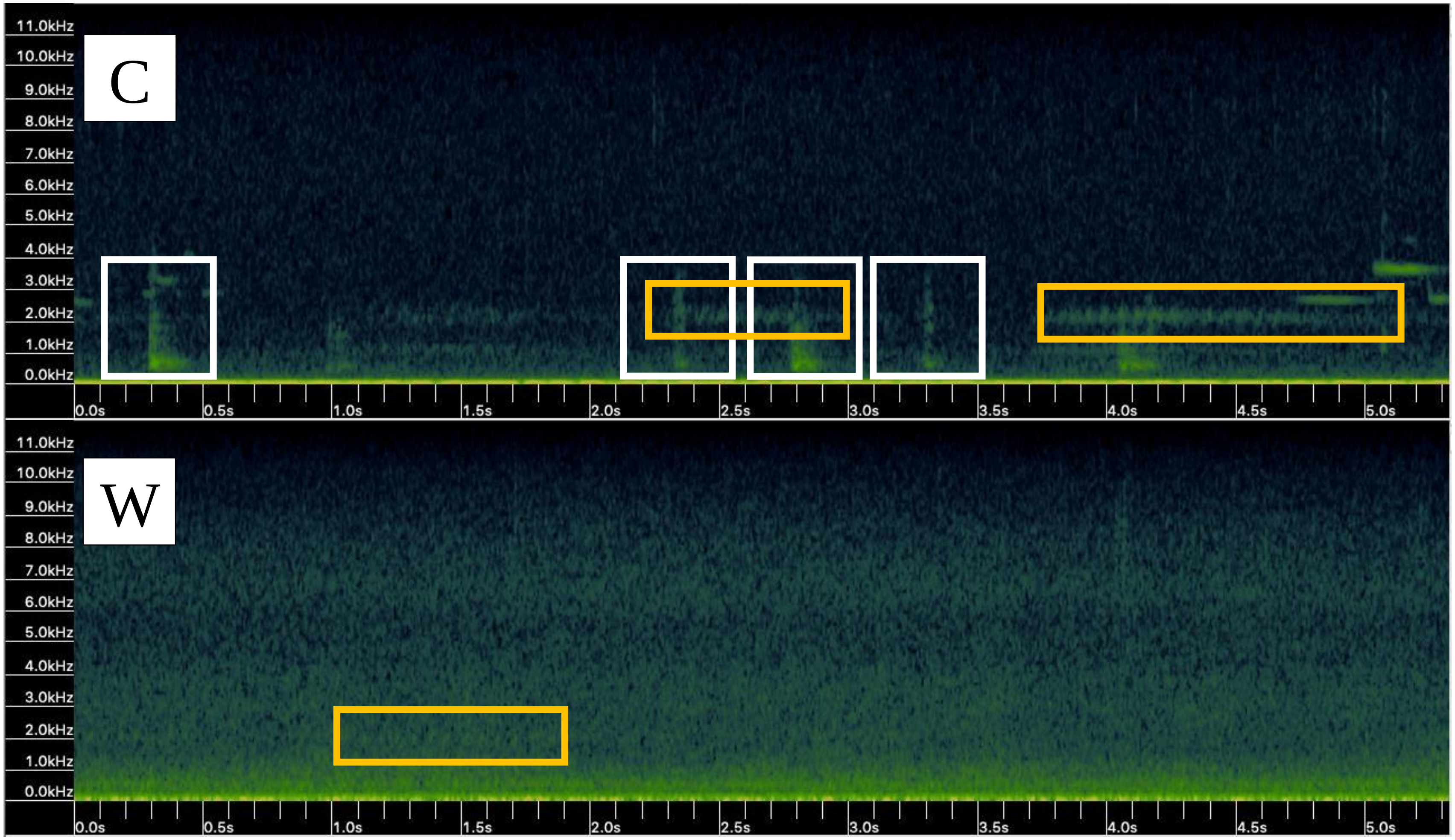

Figure 1. 11. Spectrogram representations of an average windy night (>1.5 m/s) in a Control (C) and Wind (W) site. Both spectrograms were captured from the same night and time to visualize the noise interference from wind turbines during periods of high wind. *Lithobates clamitans* (white square box) and *Hyla versicolor* (yellow rectangle box) calls are easily observable in the Control site, while *Hyla versicolor* calls are slightly visible in the Wind site, as most of the spectrogram is displaying the wind turbine noise during periods of high wind. Spectrograms were created with Kaleidoscope Pro software (Wildlife Acoustics, v5.4.9g) with the same brightness and contrast settings, then screen captures were cropped and overlaid to be represented on the same axis.

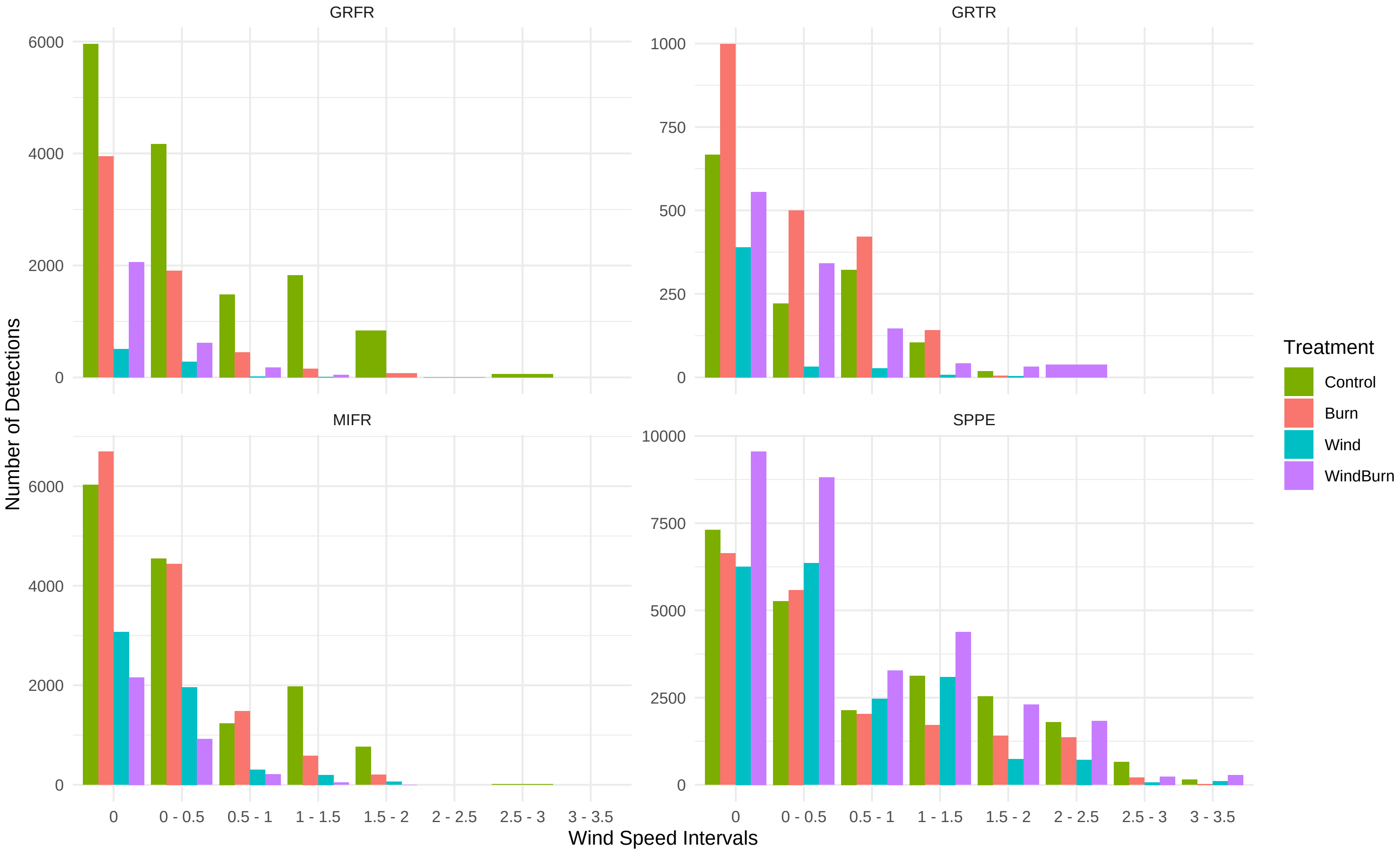

Figure 1. 12. Number of total detections of calls of 4 anuran species (GRFR; *Lithobates clamitans*, GRTR; *Hyla versicolor*, MIFR; *Lithobates septentrionalis*, SPPE; *Pseudacris crucifer*) extracted using Kaleidoscope Pro (Wildlife Acoustics, v5.4.9g), from 8 May – 21 July 2023, at varied wind speed intervals (m/s). *Pseudacris crucifer* were detected in each treatment at every wind speed interval, while *Lithobates clamitans* were absent from Wind and WindBurn sites when wind speeds exceeded 1.5 m/s.

Chapter II

The synergistic impacts of wildfire and a windfarm may exceed individual impacts on squamate communities.

Abstract

Land-use changes and their effects on wildlife are a growing concern for the worldwide conservation of biodiversity. Squamates are especially sensitive to landscape modifications, including habitat loss, fragmentation, and degradation, due to their reduced dispersal abilities, smaller home ranges and thermoregulatory needs. The goal of my study was to understand the individual and potentially synergistic effects of windfarm construction and operation and wildfire on squamate communities. I used a combination of cover board, natural cover, and visual encounter surveys to examine squamate community composition (abundance, diversity, richness), and body condition of a ubiquitous insectivorous snake species (*Opheodrys vernalis*) in 4 replicated site treatments: Control (no impacts), Wind (windfarm), Burn (wildfire), and WindBurn (double impact). If both the windfarm and wildfire resulted in direct and indirect mortality to squamates and reduced resource availability, then I expected to see lower community metrics and a reduced body condition relative to unimpacted areas. If the windfarm and wildfire effects are synergistically negative, then I expected to see the lowest community metrics and body condition in the combined impact treatment. I did not detect any significant differences in community composition or body condition between single impacted sites and Control sites. However, WindBurn sites had significantly lower snake and lizard abundance and overall lower community metrics. At the individual species level, I detected lower abundances of *Diadophis punctatus* within the wildfire and double impact treatments. My findings suggest that habitat alterations resulting from the combination of windfarm development and wildfire

negatively affected squamate communities. As the demand for wind energy rises and natural fire regimes are altered due to climate change, it is probable that such negative interactions will become more frequent. As such, it is crucial to understand the implications for biodiversity, to inform future land-use planning and mitigation strategies.

Introduction

Landscape level changes, caused primarily by anthropogenic activities, are known to negatively impact reptiles via habitat loss, fragmentation, and degradation (Gibbons et al. 2000; Row et al. 2007; Böhm et al. 2013; Rytwinski & Fahring, 2015; Keinath et al. 2017; Doherty et al. 2020). One such landscape change is the construction of windfarms, which are becoming more prevalent as governments look for ways to reduce carbon footprints (IRENA, 2022). The landscape alterations posed by windfarm implementation have the potential to impact populations and communities of reptiles (Lovich & Ennen, 2013, 2017; Keehn & Feldman, 2018a; Diffendorfer et al. 2019). Windfarms modify habitats through the creation of service roads, turbines pads, and other associated infrastructure (e.g., substations, laydowns, transmission lines; Kuvlesky et al. 2007; Dai et al. 2015; Diffendorfer et al. 2019) and have the second greatest land-use intensity per unit of energy compared to all other forms of energy generation (e.g., hydropower, coal, natural gas, nuclear; McDonald et al. 2009). Windfarms can also lead to landscape level changes through the increased risk of fire ignition, present through all stages of development, operation, maintenance, and decommissioning, as well as potential ignition from humans (e.g., cigarette smoking, welding), vehicles, and turbine malfunctions (Lovich & Ennen 2013, 2017; Lovich et al. 2018a). Wildfires can result in landscape level changes, altering habitat conditions and the availability and quality of resources for reptiles

(Russell et al. 1999; Driscoll & Henderson, 2008; Greenberg & Waldrop, 2008; Lindenmayer et al. 2008; Costa et al. 2020).

Windfarms require a large network of wide roads, suitable for transportation of large turbines and because electrical transmission lines are often placed beneath the roads (Kuvlesky et al. 2007; Diffendorfer, 2019). The impacts of windfarms on squamate (lizard and snake) communities are largely dependent on the degree to which individuals, populations, and communities were directly impacted during construction (e.g., direct mortality from machinery, habitat loss, increased traffic) and indirectly post construction (e.g., altered habitat, resource availability, continued road mortality). The creation of service roads, turbine pads, and electrical transmission lines results in habitat loss and fragmentation, leading to an increased risk of road mortality for individuals moving between habitats (Lovich et al. 2011a; Lovich & Ennen, 2017; Keehn & Feldman, 2018a), and presenting an ongoing threat as long as the windfarm continues to operate. However, although wind energy requires larger tracts of land compared to other renewable energy projects (McDonald et al. 2009), the direct physical footprint of windfarm infrastructure may be less than 1% of the total project area (Denholm et al. 2009), as the areas surrounding turbine pads are often not cleared of vegetation, leaving behind usable wildlife habitat (Lovich & Ennen, 2011). The suitability of the remaining habitat, however, is dependent upon the diverse responses of individual species to disturbances (Lovich & Ennen, 2013). Numerous studies have highlighted the severe effects of habitat loss and fragmentation, particularly due to roads, on squamate communities (Gibbons et al. 2000; Row et al. 2007; Andrews et al. 2008; Böhm et al. 2013; Rytwinski & Fahring, 2015; Doherty et al. 2020). The impacts of windfarm roads on squamate communities would likely mirror those observed for roads outside of windfarms, with similar traffic volumes (Lovich & Ennen, 2017).

The construction and operation of windfarms can also lead to increased risks of fire ignition (Lovich & Ennen 2013; Lovich & Ennen, 2017; Lovich et al. 2018a), which can result in further landscape-level alterations to habitats (Greenberg et al. 1994; Rochester et al. 2010; Elzer at al. 2013; Bosso et al. 2018; Costa et al. 2020). The documented impacts of wildfire on reptile communities are varied. After two megafires in Spain, Santos et al. (2022a) found that reptile abundance decreased in burnt plots relative to unburnt plots. Additionally, alterations in reptile community composition post-fire, including changes in richness and diversity, have been documented (Abom & Schwarzkopf, 2016; Ferreira et al. 2016; Santos et al. 2016). Other studies found that community composition and abundance remained unchanged or even increased following fire (Moseley et al. 2003; Greenberg & Waldrop, 2008; Brown et al. 2014a; Hromada et al. 2018). Although the direct effects of fire (e.g., flames, heat, smoke inhalation) can result in direct mortality (Smith et al. 2012; Jordann et al. 2020), certain squamate species can survive by seeking refuge under cover objects, in rock crevices, climbing trees, or utilizing shelters like mammal burrows (Russell et al. 1999; Smith et al. 2012). However, there could be indirect effects post-fire via altered resources, habitat, and thermoregulatory opportunities (Elzer et al. 2013; Santos & Cheylan, 2013; Brown et al. 2014b; Pastro et al. 2014; Hu et al. 2016; Hromada et al. 2018).

Multiple threats can work synergistically to negatively impact wildlife populations when compared to the individual effects of each threat (Brook et al. 2008). It is possible that the combined impacts of wildfire and a windfarm pose a threat to squamates, a group of species experiencing global declines (Gibbons et al. 2000). Squamates are especially vulnerable to habitat degradation, including that which may result from the combined impacts of a windfarm and wildfire, due to their reduced dispersal abilities, smaller home ranges and thermoregulatory needs (Böhm et al. 2013; Doherty et al. 2020). While little is known about these combined impacts on squamates, they have been studied in Desert Tortoises (*Gopherus agassizii*) on a California windfarm (Lovich et al. 2011a, 2011b, 2018a; Agha et al. 2015) and in Spotted Turtles (*Clemmys gutatta*) on an Ontario windfarm (Delay et al. 2023), revealing predominantly neutral effects. The unique slow life histories of testudines (turtles and tortoises) make it challenging to transfer any of those population- and community-level findings to squamates who tend to have faster life histories (Shine, 2005), creating a significant gap in knowledge. Moreover, like turtles (Lovich et al. 2018b), snakes can act as bioindicators for ecosystems (Beaupre & Douglas, 2009), offering broader insights into how changes associated with windfarm implementation and wildfire may impact various processes related to ecosystem health. The growing worldwide use of wind energy (IRENA, 2022) parallels the escalating occurrence of wildfires globally (Bowman et al. 2020; Pyne, 2020; Nimmo et al. 2021; Wu et al. 2021), along with increased interactions between fire and various other land-use processes (Kelly et al. 2020). This underscores the importance of comprehending their potential combined impacts on vulnerable species.

I examined the potential effects of wildfire and windfarm construction and operation on squamate communities in a location where both impacts occurred independently and simultaneously. Using a combination of cover board, natural cover, and visual encounter surveys, I examined squamate community diversity and snake body condition in four treatment areas, each replicated three times (n = 12 sites): post-construction windfarm (Wind), post-wildfire (Burn), combined impacts of post-construction windfarm and post-wildfire (WindBurn), and no impacts (Control). My objectives were to compare (1) snake richness, diversity, and abundance, (2) lizard abundance and (3) the body condition of a ubiquitous snake species, among treatments. If the windfarm and wildfire resulted in direct and indirect mortality of squamates, then I expected to see lower community richness, diversity, and abundance in impacted sites. If the windfarm and wildfire caused a decline in habitat quality and prey availability, then I expected to see lower body condition of individual snakes in impacted sites. If the combined effects of the windfarm and wildfire act synergistically in a negative manner, then I expected to see the lowest community metrics in the double impact sites. No previous studies to date have examined squamate community dynamics in areas affected by both a large wildfire and a large windfarm, thus my study is providing new data testing a novel combination of threats to squamates.

Materials and Methods

Squamate Surveys

I collected animal data over 2 field seasons: from 14 May to 25 August in 2022 and from 8 May to 24 August in 2023, using cover board, natural cover, and visual encounter surveys. Clusters of 4 cover boards (2 plywood, 2 tin; ~ 60 x 60 cm; Halliday & Blouin-Demers, 2015; Figure I) were placed within a 60 m radius of each wetland complex, at each site (n = 48 boards in total). Boards were deployed in August 2021 (allowing for 8 months to season), with deployment locations chosen based on 1) suitable snake habitat in the immediate area, 2) slope facing the rising sun, and 3) microhabitat connectivity (i.e., avoidance of placement on open rock barren devoid of ground vegetation). I conducted a total of 20 surveys per site (9 in 2022, 11 in 2023; n = 240 surveys in total). Surveys occurred when air temperatures were predicted to be cooler than temperatures under cover objects, causing snakes to seek thermal refuge under cover, thereby increasing their capture probability during surveys (Grant et al. 1992). In May and June, surveys occurred between 8 and 11 am, while in July and August, surveys occurred between 7 and 10 am. Surveys consisted of looking under the cover boards followed by a 30 minute visual encounter survey (2 persons totalling 1 survey hr per visit; 20 total survey hrs per site), consisting of walking slowly and searching for animals seeking refuge underneath natural cover objects (e.g., logs, rocks, and bark) or on the ground surface (e.g., active, basking; Sasaki et al. 2015). Looking under the same natural cover objects within the same survey season was avoided to minimize probabilities of finding the same individuals, in addition to limiting disturbance to microclimatic conditions under cover objects (Sasaki et al. 2015). Survey timing at a given site was alternated to reduce potential effects of time of day on animal detectability (Grant et al. 1992; Sasaki et al. 2015). To further limit recapturing the same individuals, surveys were conducted in a different area within the respective site, in each given year (i.e., 9 different areas in 2022, 11 different areas in 2023).

Each snake found was captured by hand (if possible) and measured. Snout-vent length (SVL) and tail length (TL) were measured to the nearest millimeter in the field with a flexible measuring tape. Three measurements of each length were recorded and then averaged, to limit sampling errors. The average SVL and TL were subsequently combined to obtain snake total body length (TBL). Body mass (BM) was measured using appropriately sized spring scales (Pesola AG, Barr, Switzerland). Sex was determined based on sexually dimorphic characteristics, if applicable (i.e., longer TL relative to TBL in male vs. female Smooth Greensnakes (*Opheodrys vernalis*); Rowell, 2012). Age class was determined based on body size and any distinctive adult characteristics (Rowell, 2012). To avoid resampling the same individuals, snakes were marked on their venter with a permanent marker. When Common Five-lined Skinks (*Plestiodon fasciatus*) were encountered during surveys, only presence and basic demographic data (e.g., age class and sex) were recorded. No morphometrics were collected, as skinks were not captured or handled.

Community Composition Analyses

Snake community diversity was calculated using two metrics: species richness (i.e., total number of species) and the effective number of species (ENS; Jost, 2006). I derived ENS from the Shannon Entropy Index (ENS = exp(Shannon Entropy Index)):

$$\mathrm{ENS} = \exp\left(-\sum_{i=1}^{S} p_i \ln p_i\right)$$

where S represents the number of species and p_i is the proportion of species i within a community (Jost, 2006). ENS counts each individual equally and weighs them in proportion to their abundance, placing emphasis on common and abundant species (Jost, 2006). Differences in species richness and ENS amongst treatments were tested using one-way ANOVAs. All data were analyzed using R statistical software (v4.2.3; R Core Team (2020), Vienna, Austria) in RStudio (RStudio Team (2016), Boston, MA).

Two metrics were used to compare snake abundance among treatments: 1) species specific abundance and 2) total snake abundance (summed number of snakes of all species observed at a site). Both metrics were compared among treatments using one-way ANOVAs, followed by Tukey's HSD post hoc tests if the ANOVA revealed significant differences. Community differences between treatments were further examined by creating individual-based rarefaction curves which plot species richness against number of pooled individuals. These curves were created using the rarecurve function in the iNEXT package (Hsieh et al. 2016).

I compared the number of Five-lined Skink observations among treatments. Skinks were completely absent from the Burn treatment and from one of the Control sites, likely as a result of a species range constraint (Ontario Nature, 2023) due to a geographic barrier, rather than an impact of the treatments. Because of this, I excluded the Burn treatment and the single Control site from all statistical analyses of skink observations. Skink observations from the remaining sites were summed (per site) and compared amongst treatments using a one-way ANOVA followed by a Tukey's HSD post hoc test.

Body Condition Analyses

I used Scaled Mass Index (SMI; Peig & Green, 2009) to estimate body condition, where a greater SMI indicates an accumulation of energy capital, suggesting that the individual is in greater health (Peig & Green, 2009). I calculated SMI_i, using:

$$\mathrm{SMI}_i = \mathrm{M}_i\,(\mathrm{L}_0/\mathrm{L}_i)^{bsma}$$

where M_i is the mass (BM) of the individual i, L_0 is mean total body length (TBL) of all individuals included (n = 46), L_i is the total body length of individual i, and b_{sma} is the scaling component derived by dividing the slope of the log length versus log mass ordinary least squares (OLS) regression (b_{ols}) by Pearson's coefficient r (Peig & Green, 2009). SMI has previously been successfully used for estimating body condition in snakes (Sasaki et al. 2016; Frank & Dudás, 2018) and lizards (Thaker et al. 2018). SMI was compared amongst treatments and sexes using a type III two-way ANOVA, to account for an unequal number of individuals amongst treatments. Gravid females, juveniles, and neonates were excluded from SMI analysis.

I estimated body condition of individual Smooth Greensnakes (*Opheodrys vernalis*). Due to their unique insectivorous diet (Ernst & Ernst, 2003), reliance on habitat with dense vegetation (Cook, 1984; Ontario Nature, 2023), and widespread occurrence in the study area, I felt Smooth Greensnakes would make a good indicator species to understand if the treatments influenced habitat quality and prey availability, thus leading to an effect on body condition (Stevenson & Woods, 2006; Beaupre & Douglas, 2009; Baron et al. 2013). Furthermore, Smooth Greensnakes spend a considerable portion of their active season under cover (Redder et al. 2006), providing a greater chance of finding a large enough sample size via natural cover and cover board surveys for my analysis. Because only one Smooth Greensnake individual was observed within the WindBurn, body condition comparisons were limited to the other 3 treatments. Total body length (TBL) and body mass (BM) were natural log transformed prior to SMI analysis.

Results

Squamate Community Composition

A total of 198 targeted snake individuals representing 7 different species were found during the 2022 and 2023 field seasons (Table 2.1; Figure 2.1). Additionally, 254 observations of Five-Lined Skinks were recorded (Table 2.1; Figure 2.2). Twenty-five Eastern Massasauga Rattlesnakes (*Sisturus catenatus*) and 3 Eastern Foxsnakes (*Pantherophis vulpinus*) were opportunistically observed during surveys; however, they were excluded from analyses due to distinct survey techniques required to accurately confirm their presence and abundance (i.e., Eastern Massasauga Rattlesnakes are not surveyed for by checking cover objects; Casper et al. 2001). Additionally, because of their rarity and specific habitat usage, Eastern Foxsnakes may not have been available for capture at each of the sites, regardless of treatment.

The Burn and Wind treatments contained all 7 snake species, while the Control contained 6 and the WindBurn contained 5 (Table 2.1; Figure 2.1). Eastern Gartersnake (*Thamnophis s. sirtalis*) and Smooth Greensnake were the most abundant species, appearing in 11 and 9 of the 12 sites, respectively. Eastern Milksnake (*Lampropeltis triangulum*) was the least abundant species, appearing in 4 sites. Only 4 snake individuals were located under my deployed cover boards (1 Smooth Greensnake, 1 Northern Red-Bellied Snake (*Storeria o. occipitomaculata*), 1

Eastern Gartersnake, 1 Dekay's Brownsnake (*Storeria dekayi*)), while the rest were located during natural cover and visual encounter surveys. A female Five-lined Skink laid her eggs under a wooden cover board at a Wind site (W2) in both field seasons; to limit disturbance, that board was not checked after discovering the nest, until enough time had passed for the eggs to hatch.

The Effective Number of Species (ENS) and snake richness did not differ significantly among treatments (ENS: $F_{3,8} = 0.90$, $p > 0.05$; richness: $F_{3,8} = 0.99$, $p > 0.05$; Tables 2.2, 2.3, Figures 2.1, 2.3). The total abundance of snakes differed significantly among treatments ($F_{3,8} = 4.84$, $p < 0.05$; Tables 2.1, 2.3; Figure 2.1). Post hoc analyses revealed that total abundance was significantly lower in the WindBurn than the Control sites ($p < 0.05$; Table 2.1; Figure 2.1). Abundance of only one species, Northern Ring-Necked Snake (*Diadophis punctatus*), differed among treatments ($F_{3,8} = 6.34$, $p < 0.05$; Table 2.3; Figure 2.1). Post hoc analyses revealed that Northern Ring-Necked Snake abundance was significantly higher in the Control than in the Burn ($p < 0.05$) and WindBurn ($p < 0.05$). Species abundance and richness distributions among and within treatments are displayed in individual-based rarefaction curves (Figure 2.4). The Control treatment curve was closest to reaching a plateau followed by the Wind, Burn, and WindBurn treatments.

The number of skinks observed differed significantly among treatments ($F_{2,5} = 7.14$, $p < 0.05$; Tables 2.1, 2.3; Figure 2.2). Post hoc analyses revealed that skink observations were significantly lower in the WindBurn than in the Control ($p < 0.05$) and Wind sites ($p = 0.05$).

Body Condition

Smooth Greensnake body condition (SMI) did not differ significantly among treatments (mean ± SD (n): Control = 11.7 ± 2.69 (18), Burn = 12.3 ± 1.84 (5), Wind = 13.1 ± 2.84 (23), $F_{2,43} = 1.42$, $p > 0.05$; Table 2.3; Figure 2.5a). Male and female SMI did not differ significantly (mean ± SD (n): ♀= 13.3 ± 3.24 (16), ♂ = 12.0 ± 2.34 (30), $F_{1,44} = 2.65$, $p > 0.05$; Table 2.3; Figure 2.5b). The interaction between treatment and sex was not significant (♀ $F_{2,13} = 0.51$, $p > 0.05$; ♂ $F_{2,27} = 1.38$, $p > 0.05$; Table 2.3; Figure 2.5c).

Discussion

The goal of my study was to determine if wildfire, windfarm construction and operation, and the combination of these disturbances, impacted squamate communities and snake body condition. Contrary to my hypothesis, I found no differences in snake richness and diversity between unimpacted and impacted sites, and no variation in body condition of one representative snake species among treatments. However, in support of my hypothesis, I found that the cumulative effects of a windfarm and wildfire resulted in lower total snake abundance and skink abundance. I also found lower abundances of one snake species within the wildfire and double impacted sites. Finally, the greatest variation in community metrics among sites was observed within the treatments affected by wildfire.

Snake Community Composition

The lack of difference in snake diversity, richness, and abundance between Wind and Control sites may be explained by the characteristics of available snake habitat within the windfarm footprint (Keehn & Feldman, 2018a). Law et al. (2020) suggested that a windfarm in Scotland could act as a refuge for Common European Vipers (*Vipera burus*) because of a reduction of avian predators. Although the windfarm where I conducted my study is large and contains a substantial amount of infrastructure, relatively undisturbed habitat remains. Of the 9000-ha windfarm footprint, 690 ha (7.7%) has been directly altered for infrastructure (Figure

2.6b; Delay et al. 2023). Even if the remaining habitat can support snake populations, it is difficult to truly understand the effect of windfarm construction, and the associated habitat loss, without pre-construction data. However, it is also plausible that because the study area region has high snake diversity (UNESCO, 2004; Jalava et al. 2005), even if direct mortality was high during construction, snake populations could persist post-impact. Although the lack of community differences among treatments is a positive finding, habitat loss can lead to eventual population declines (Thomas et al. 2001), suggesting that continued long-term monitoring is crucial to understand if snake populations can persist on this windfarm.

Even if the remaining habitat allow snake populations to persist, road mortality within the operating windfarm may pose an ongoing threat. Snake road mortality represents a consequence associated with windfarm operation, which also warrants consideration, especially considering the positive correlation between wider roads and wildlife vehicle collisions (Valero et al. 2015). I opportunistically recorded numerous instances of alive on road (AOR) and dead on road (DOR) snakes while driving the windfarm service roads (i.e., 47 AOR, 13 DOR individuals observed May – August 2023; Figure 2.7), which is of concern, as continued snake road mortality can lead to future population declines (Row et al. 2007; Winton et al. 2020). Interestingly, Law et al. (2020) detected no evidence of snake roadkill on their windfarm service roads; their site similarly experiences minimal traffic, with enforced speed limits, suggesting that responses to windfarm roads may vary depending on various factors. Winton et al. 2020 studied a population of Western Rattlesnakes (*Crotalus oreganus*) in British Columbia within a protected area with a low road density and traffic volume, much like my study area. They projected that the current road mortality rate at their site would lead to substantial population declines over the next 100 years, while any increases in road mortality could lead to extirpation (Winton et al. 2020). Their results suggest that road mortality can still be a significant contributor to snake population declines, even in areas with low traffic volume and road density (Winton et al. 2020). The number and diversity of snakes that I observed on windfarm roads without using a standardized road survey protocol suggests that all snake species present in the area (all 9 species observed on roads in 2022 and 2023) are using the roads. Similar to Winton et al. (2020), it is possible that continual mortalities on windfarm roads, even in a low traffic area, could present a risk to the continued persistence of the local snake populations.

As may be the case for lack of community differences between Wind and Control sites, the lack of community compositional differences between Burn and Control sites may also be due to the presence of refugia, which is a key factor in determining the resistance of wildlife to fire (Robinson et al. 2013; Hale et al. 2022). The study landscape is characterized by large expanses of rock barrens with countless rocks and boulders that could act as greater flame-resistant structures for snakes to use as refuge when compared to vegetation (Santos et al. 2016; Ferreira et al. 2019). Because these rocky cover objects are highly abundant in the study landscape, snakes may have exploited these structures during the fire, increasing their chances of surviving the flames and heat. Patches of unburnt areas (i.e., intact habitat islands) may have also served as refugia and helped to sustain populations post-fire (Steenvorden et al. 2019). All snake species within the study area (including Eastern Massasauaga Rattlesnake and Eastern Foxsnake), were found within the Burn sites, suggesting there were no local extinctions of any snake species due to fire.

The altered habitat post-fire (Figure 2.6c; Costa et al. 2020) could have favoured species with a preference for open habitat, while species relying on forested habitat may have been hindered (Hu et al. 2016). This result was observed for Northern Ring-Necked Snakes, whose abundance was significantly lower in the wildfire-affected sites than the Control sites (Table 2.1; Figure 2.1). Ring-Necked Snakes are habitat specialists that rely on moist, heavily-shaded habitats (Rowell, 2012), suggesting that the species would be less resilient to the direct and indirect effects of fire. The resiliency of organisms to fire is largely dependent on their functional and life-history traits, which makes some species, such as Ring-Necked Snakes, more vulnerable to fire and less adapted to burnt landscapes (Rochester et al. 2010; Westgate et al. 2012; Chergui et al. 2020). However, over the long-term as wildfire succession progresses, species relying on forested habitat should eventually replace species with preferences for open habitat (Santos & Cheylan, 2013).

Although snake community metrics in the Burn treatment did not differ from those in Control sites, there was variation in metrics among Burn treatment replicates (Tables 2.1, 2.2; Figures 2.3, 2.4). One site specifically (B2) was an outlier, accounting for 71% of the total number of snakes encountered within the Burn treatment (Figure 2.4b), yet it is difficult to explain the reason for this site outlier. Site B2 may have had a greater availability of refugia compared to the other Burn sites, supporting survival from the direct effects of the fire (Robinson et al. 2013; Steenvorden et al. 2019; Hale et al. 2022). It is also possible that site B2 experienced a lower fire severity, which has influenced small mammal abundance post-fire in California (Roberts et al. 2008). However, recently, Lindsay et al. (2023) found no influence of fire severity on the herpetofaunal community in a Florida scrub habitat. Another possibility for the higher snake encounters in B2 is increased colonisation of burnt areas from adjacent unburnt areas, as site B2 was the closest to the edge of the wildfire footprint. However, Santos et al. (2022a) argued that reptile resilience to wildfire is due directly to their persistence and survival of the fire, not by their colonization abilities from unburnt areas. They found a lack of post-fire spatial response of lizards from the edge to the center of their burnt area (Santos et al. 2022a). Similarly, the mechanism driving the recovery of mammal populations following Australian wildfires was also identified as post-fire survival rather than recolonization (Banks et al. 2011; Hale et al. 2022), despite mammals having larger home ranges and greater dispersal capacities than many reptiles. Without before-fire or associated environmental data, it is difficult to conclude the exact reasons for the large within-Burn-treatment differences; therefore, the absence of snake community differences between the Burn and Control treatments should be considered with caution.

The cumulative impacts of windfarm development and operation and wildfire may result in a greater effect on snakes than each disturbance on its own. In support of my hypothesis, snake abundance and richness were lowest in the WindBurn treatment. Threats can be deemed synergistic if their cumulative effects on a population are larger than the sum of the individual impacts (Brook et al. 2008; Paterson et al. 2021) and can drive populations to extinction (Doherty et al. 2015; Driscoll et al. 2021). Fire can interact with fragmented and degraded landscapes, exacerbating the effects of fire, resulting in negative effects on species richness and abundance (Hossack et al. 2013; Driscoll et al. 2021; Lazzari et al. 2022; Santos et al. 2022b). Even species accustomed to co-existing with fire may display altered responses in fragmented landscapes, as they could encounter challenges when dispersing the landscape to colonise habitat patches at their preferred successional stage (Nimmo et al. 2019; Driscoll et al. 2021).

Hossack et al. (2013) discovered that road and development fragmentation amplified the negative effects of a high severity wildfire on the population size of Long-Toed Salamanders (*Ambystoma macrodactylum*). Population sizes in fragmented areas were similar to those in wetlands in protected landscapes. However, in high severity burned areas, high road densities exacerbated the negative effects of wildfire on salamanders, resulting in a greater reduction in population size (Hossack et al. 2013). In a recent study in Australia, Mulhall et al. (2024) found that the response of reptiles to fire varied depending on the structure of the surrounding landscape. For snakes at my study site, it is possible that increased direct and indirect mortality from windfarm fragmentation and habitat loss were not enough to cause noticeable population declines on their own but may have made the populations more vulnerable to additional mortality. When combined with the adverse effects of wildfire, populations already impacted by the presence of the windfarm may face additional reduction, possibly explaining the observed lower values of community metrics (Figure 2.6d).

Body Condition

Fires can alter the composition and abundance of prey, which can alter the range and abundance of reptiles feeding on those prey (Pastro et al. 2013; Santons & Cheylan, 2013; Hu et al. 2016). In contrast to my hypothesis, the body condition of Smooth Greensnakes did not differ among treatments (Figure 2.5), suggesting that windfarms and wildfire do not have a negative effect on food resource availability for these primarily insectivorous snakes. The burning of vegetative material leads to a pulse of nutrients into soil and plants (Bond & Van Wilgen, 2012), increasing food availability which can then be exploited by consumers. Teasdale et al. (2013) found invertebrate abundance increased after a fire, leading to increased body condition in an insectivorous gecko (*Nephrurus stellatus*) in burnt areas compared to unburnt areas (Smith, 2018). Similarly, Santos & Cheylan (2013) found an increased frequency of insectivorous reptiles post-fire, suggesting that invertebrate prey availability was not negatively affected by fire. The creation of more open vegetative areas post-fire can lead to increased foraging efficiency (Vernes & Haydon, 2001), which may also explain the lack of effect on Smooth

Greensnake body condition. Anecdotally, I observed a higher abundance of Pennsylvania Wood Cockroaches (*Parcoblatta pensylvanica*) under cover objects in the wildfire affected areas, which may have been prey for Smooth Greensnakes. It is possible that net resource availability for Smooth Greensnakes did not change but rather the composition of resources changed (i.e., increased abundance of Wood Cockroaches). Alterations in resource composition may have a neutral effect on generalist snake species which are adept are exploiting various resource types, while specialist species may be disproportionally affected if they are unable to readily adapt to different resources (Böhm et al. 2016). Future research should investigate the response of prey-specialist snake species to fire events, particularly considering the potential limitation of resource availability.

The body condition of snakes in the windfarm did not differ from those in the unimpacted sites. Similarly, Spotted Turtles on an Ontario windfarm (Delay et al. 2023) and Desert Tortoises on a California windfarm (Lovich et al. 2011b) did not display relatively lower body condition. This lack of difference may be explained by the presence of suitable habitat for Smooth Greensnakes, as well as their terrestrial invertebrate prey as well. Pustkowiak et al. (2018) found no differences in pollinator communities on a windfarm compared to in grassland patches. Similarly, Millidine et al. (2015) found no effect of the construction of a windfarm on aquatic invertebrate community structure. While the diet of Smooth Greensnakes was not directly assessed in my study, there is no indication that prey items such as aquatic or terrestrial invertebrates were reduced in the windfarm areas. Nonetheless, future research is needed to specifically understand any potential effects of windfarms on invertebrates in the short- and long-term.

Five-Lined Skink Observations

The number of Five-Lined Skink observations did not differ between the windfarm and unimpacted sites (Figure 2.2), suggesting that the lizards can successfully persist on windfarms. A few recent studies have conducted examinations on the impacts of windfarms on lizard community ecology, yielding similar findings. Keehn & Feldman (2018b) and Keehn et al. (2019) studied populations of Side-Blotched Lizards (*Uta stansburiana*) revealing that 1) predator attack rates were reduced at windfarm sites and 2) the windfarm had minimal impacts on lizard demography and behaviour, indicating that healthy populations can persist on windfarms. Likewise, Thaker at al. (2018) observed that Superb Large Fan-Throated Lizards (*Sarada superba*) living on an established windfarm in India experienced decreased mortality from avian predators whose abundance and predation attempts were reduced on the windfarm, leading to a rise in the lizard population, which in turn resulted in a density-dependent reduction of body condition for individual lizards.

Consistent with my snake abundance results, there were also fewer skink observations in the WindBurn sites compared to the Control and Wind sites (Table 2.1; Figure 2.2). Because no skinks naturally occur in the Burn footprint due to a range constraint, and because there is no literature on the effects of fire on this lizard species, it is difficult to determine if my findings are a result of the wildfire or the cumulative impacts of both the fire and windfarm, as previously discussed. Like snakes, several studies have documented beneficial and detrimental effects of fire on lizard communities (Griffiths & Christian, 1996; Cunningham et al. 2002; Pastro et al. 2011; Pianka & Goodyear, 2012; Pastro et al. 2013), with responses largely dependent on species’ functional and life-history traits (Rochester et al. 2010; Santos & Cheylan, 2013). I speculate that my findings are a result of direct mortality during the wildfire. During cover surveys, skinks were typically beneath small cover objects (e.g., small rocks). When the fire swept through, it is plausible that skinks sought refuge under their usual smaller cover objects, which may not have shielded them from the intense heat and flames of the fire. I recommend future studies conduct more robust examinations of Five-Lined Skink population dynamics post-wildfire, as this represents a large knowledge gap in the literature. Being the sole lizard species in Ontario, where they exist at their northern limit, Five-Lined Skinks might respond differently to wildfires compared to other lizard species in landscapes more traditionally susceptible to fire.

Conclusion

My study is the first to assess the impacts of a windfarm, wildfire, and the cumulative impacts of both, on squamate communities. I did not observe any community compositional differences between areas impacted by the windfarm compared to unimpacted areas. However, I did detect a species-specific difference in abundance for the fire affected sites, suggesting that snake responses to wildfire are species-dependent. Furthermore, I observed lower squamate abundance and community metrics in the double impact, indicating that the effects of wildfire could be amplified in a landscape previously fragmented and degraded by the establishment of a windfarm. My study serves as a starting point for future research aimed at understanding the immediate and long-term impacts, driven by climate change, of an increasing green energy source (IRENA, 2022) and escalating frequency of wildfires (Pyne, 2020; Nimmo et al. 2021; Wu et al. 2021) on squamate communities, which are facing global declines (Gibbons et al. 2000). Even more, my research identifies the need to understand the complex interactions between fire and land-use changes, and how they may interact to influence species persistence. To better inform future data-driven mitigation strategies, future windfarm projects should employ multi-year BACI (Before-After-Control-Impact) designs (Kuvlesky at al. 2007; Delay et al. 2023), with an emphasis on the effects of habitat loss and road mortality on squamate population abundance and survivorship in the short- and long-term.

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Tables

Table 2. 1. Species-specific and total snake abundance of 7 snake and 1 lizard species in four replicated treatments Burn (sites B1, B2, B3), Control (sites C1, C2, C3), Wind (sites W1, W2, W3), and WindBurn (sites WB1, WB2, WB3). Bolded values represent the sum of all three sites within a treatment. Snakes were encountered through cover board, natural cover, and visual encounter surveys, from May – August 2022 and 2023, in central Ontario. Each site was sampled 20 times for a total of 60 sampling events per treatment.
* denotes the lack of abundance due to a species range constraint rather than an effect of the treatment.

		Species							
Site	Total Snake Abundance	Dekay's Brownsnake (*Storeria dekayi*)	Eastern Gartersnake (*Thamnophis s. sirtalis*)	Smooth Greensnake (*Opheodrys vernalis*)	Eastern Milksnake (*Lampropeltis triangulum*)	Northern Red-Bellied Snake (*Storeria o. occipitomaculata*)	Northern Ring-Necked Snake (*Diadophis punctatus*)	Northern Watersnake (*Nerodia s. sipedon*)	Common Five-Lined Skink (*Plestiodon fasciatus*)
C1	25	0	8	8	0	4	4	1	*
C2	25	0	8	5	1	1	10	0	36
C3	29	0	5	7	0	6	10	1	68
Control	**79**	**0**	**21**	**20**	**1**	**11**	**24**	**2**	**104**
B1	8	0	2	2	0	2	2	0	*
B2	30	1	15	5	1	5	1	2	*
B3	4	0	3	0	0	1	0	0	*
Burn	**42**	**1**	**20**	**7**	**1**	**8**	**3**	**2**	*****
W1	17	0	0	11	0	0	4	2	42
W2	23	3	3	1	1	5	10	0	65
W3	23	1	4	13	0	2	3	0	28
Wind	**63**	**4**	**7**	**25**	**1**	**7**	**17**	**2**	**135**
WB1	3	1	2	0	0	0	0	0	4
WB2	4	0	4	0	0	0	0	0	8
WB3	7	1	3	1	1	0	0	1	3
WindBurn	**14**	**2**	**9**	**1**	**1**	**0**	**0**	**1**	**15**

Table 2. 2. Effective number of species (ENS) and snake species richness in four replicated treatments: Control (sites C1, C2, C3), Burn (sites B1, B2, B3), Wind (sites W1, W2, W3), and WindBurn (sites WB1, WB2, WB3), in central Ontario. Metrics did not differ among treatments, as indicated by the results of ANOVAs. Bolded values represent treatment means with standard deviation shown in parentheses. Snakes were encountered through cover board, natural cover, and visual encounter surveys, from May – August 2022 and 2023. Each site was sampled 20 times for a total of 60 sampling events per treatment.

Site	Species Richness	p	ENS	p
C1	5	>0.05	4.24	>0.05
C2	5		3.71	
C3	5		4.29	
Control	**5** (0)		**4.08** (0.16)	
B1	4		4.00	
B2	7		4.33	
B3	2		1.75	
Burn	**4.33** (2.52)		**3.36** (1.15)	
W1	3		2.40	
W2	6		4.47	
W3	5		3.46	
Wind	**4.67** (1.15)		**3.44** (0.85)	
WB1	2		1.89	
WB2	1		1.00	
WB3	5		4.37	
WindBurn	**2.67** (2.08)		**2.42** (1.43)	

Table 2. 3. Results of the ANOVAs of the various snake and lizard community metrics compared among treatments, including diversity measures (Effective Number of Species, Snake Richness), abundance measures (Total Snake Abundance, Species-Specific Abundances, Five-Lined Skink Observations), and body condition measures (Smooth Greensnake (*Opheodrys vernalis*); Scaled Mass Index). Bolded p-values indicate statistical significance ($p \leq 0.05$). See Appendix A for explanations of species acronyms.

	df	F	p
Effective Number of Species (ENS)			
Treatment	3,8	0.90	> 0.05
Snake Richness			
Treatment	3,8	0.99	> 0.05
Total Snake Abundance			
Treatment	3,8	4.84	**< 0.05**
Species-Specific Abundance x Treatment			
BRSN	3,8	1.30	> 0.05
EAGA	3,8	1.16	> 0.05
SMGR	3,8	3.29	> 0.05
MILK	3,8	0.00	> 0.05
NOWA	3,8	0.10	> 0.05
REBE	3,8	1.70	> 0.05
RING	3,8	6.34	**< 0.05**
Five-Lined Skink Observations			
Treatment	2,5	7.14	**< 0.05**
SMGR Body Condition (SMI)			
Treatment	2,43	1.42	> 0.05
Sex	1,44	2.65	> 0.05
Treatment x Sex			
Male	2,13	0.51	> 0.05
Female	2,27	1.38	> 0.05

Figures

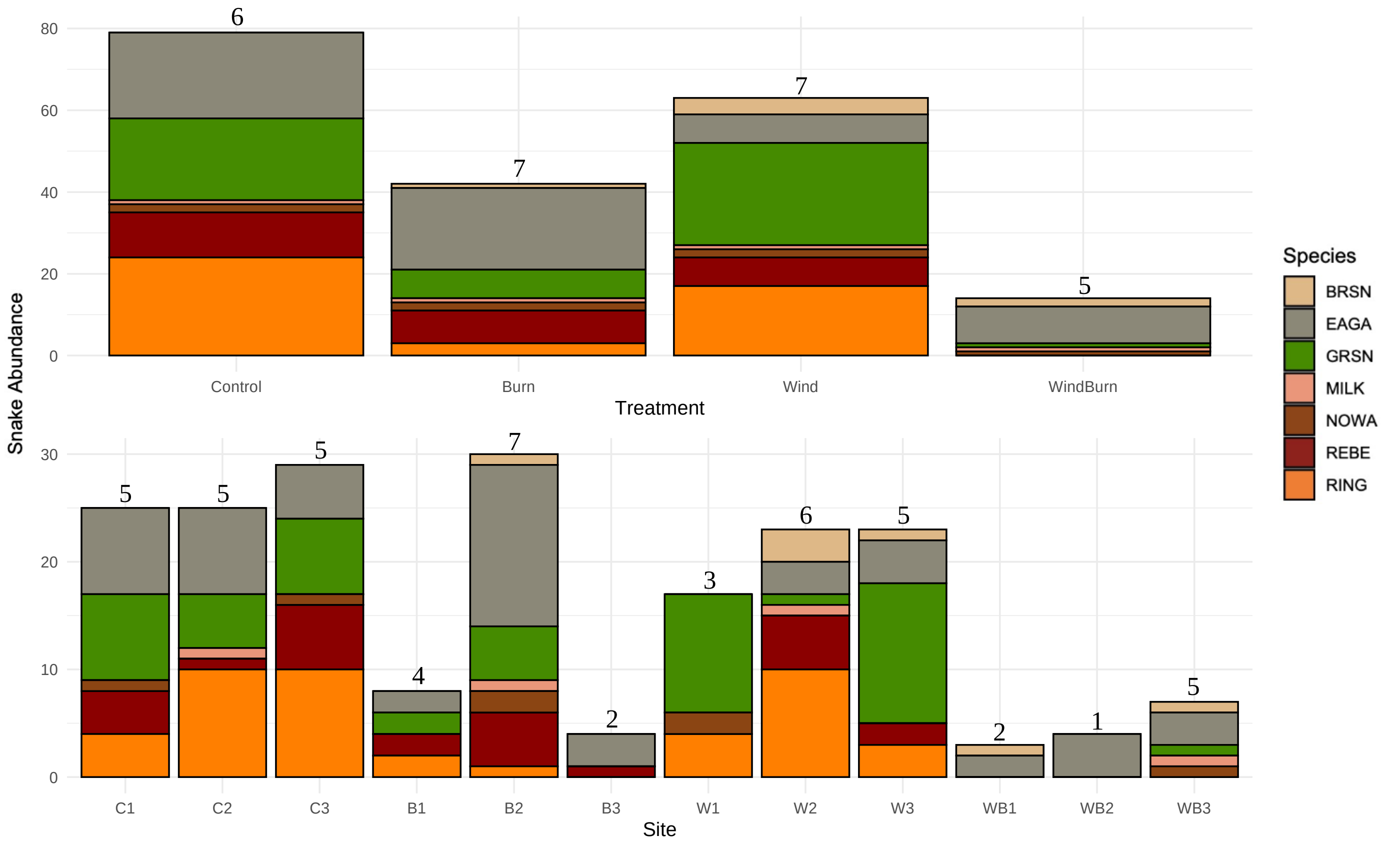

Figure 2. 1. Snake richness and abundance, by treatment (Control, Burn, Wind, WindBurn; top panel) and site (3 replicated per treatment; bottom panel), encountered using cover board, natural cover, and visual encounter surveys, from May – August 2022 and 2023, in central Ontario. Each site was sampled 20 times for a total of 60 sampling events per treatment. Total snake abundance was significantly lower in WindBurn than in Control ($p < 0.05$), while Northern Ring-Neck (orange fill) abundance was significantly lower in Burn and WindBurn than in Control ($p < 0.05$). Values above bars represent snake richness. See Appendix A for explanations of species acronyms.

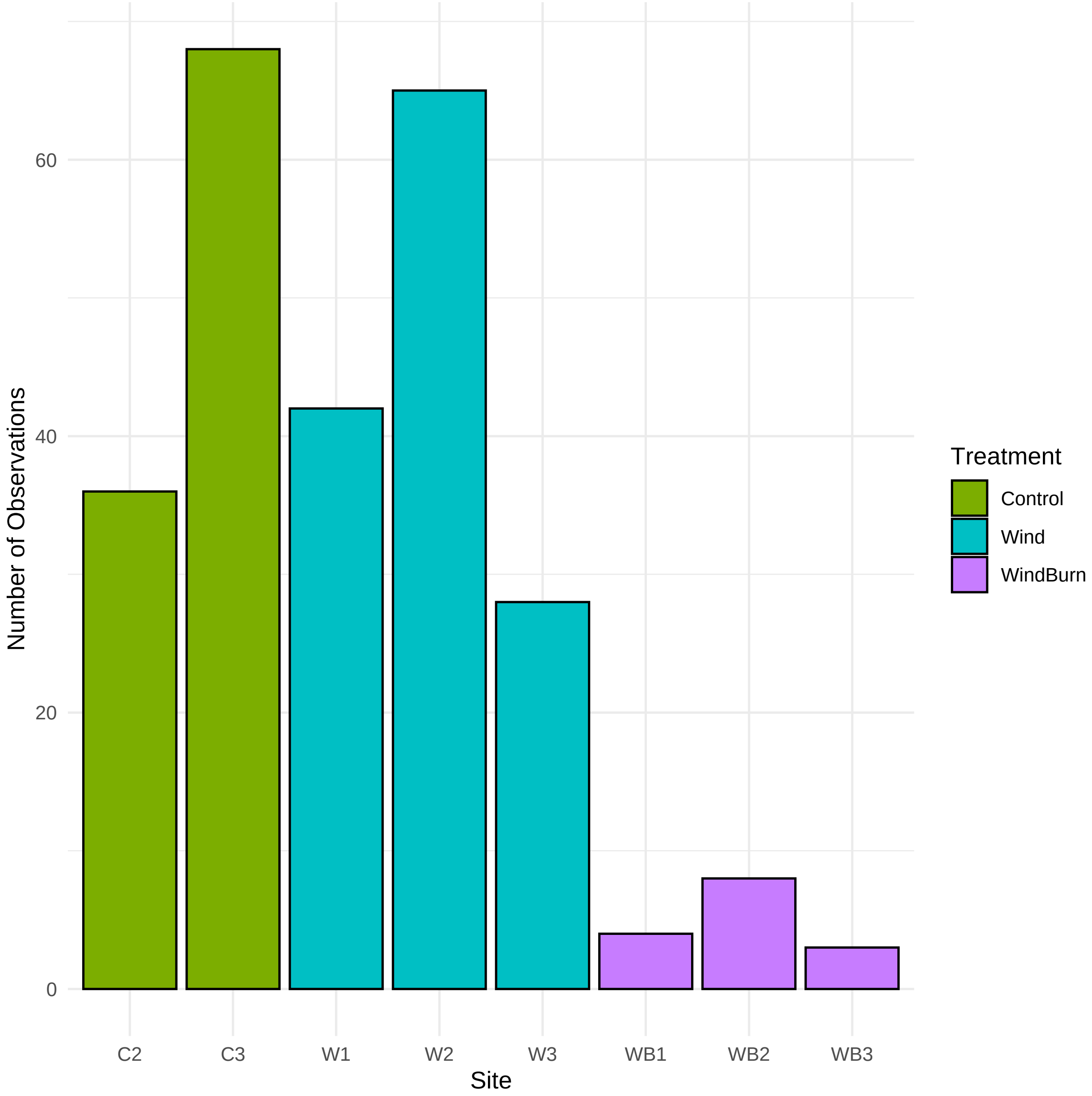

Figure 2. 2. Number of Common Five-Lined Skink (*Plestiodon fasciatus*) observations in 3 treatments (Control, Wind, WindBurn), each replicated in 3 sites, in central Ontario. Skinks were located using cover board, natural cover, and visual encounter surveys from May – August 2022 and 2023, over a total of 20 sampling events per site. Skink observations were significantly lower in the WindBurn than the Control and Wind sites ($p < 0.05$; $p = 0.05$), respectively. Skinks were absent from Control site C1 and all 3 sites in the Burn treatment, due to a range constraint rather than an effect of the treatments, thus these sites were excluded from the figure and analysis.

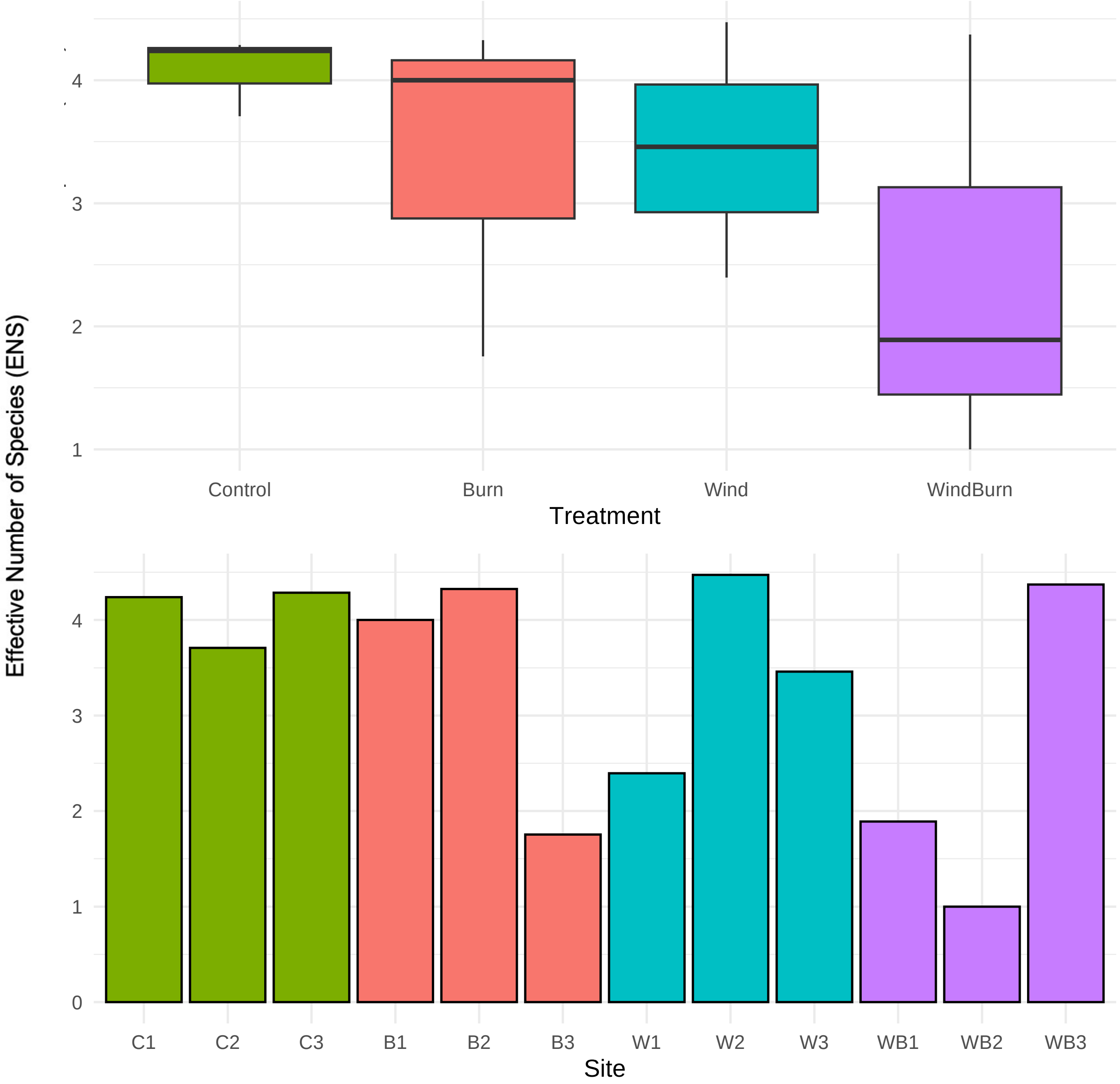

Figure 2. 3. Effective number of species (ENS) by treatment (Control, Burn, Wind, WindBurn) and site (3 replicates per treatment), in central Ontario. ENS counts each individual equally and weighs them in proportion to their abundance, placing emphasis on common and abundant species. ENS was not significantly different among treatments. Boxplots (top panel) contain the ENS values for treatment replicates, with horizontal lines representing treatment medians, and the vertical lines representing the spread of the data. The frequency histograms (bottom panel) visualize the magnitude of differences of sites, within treatments.

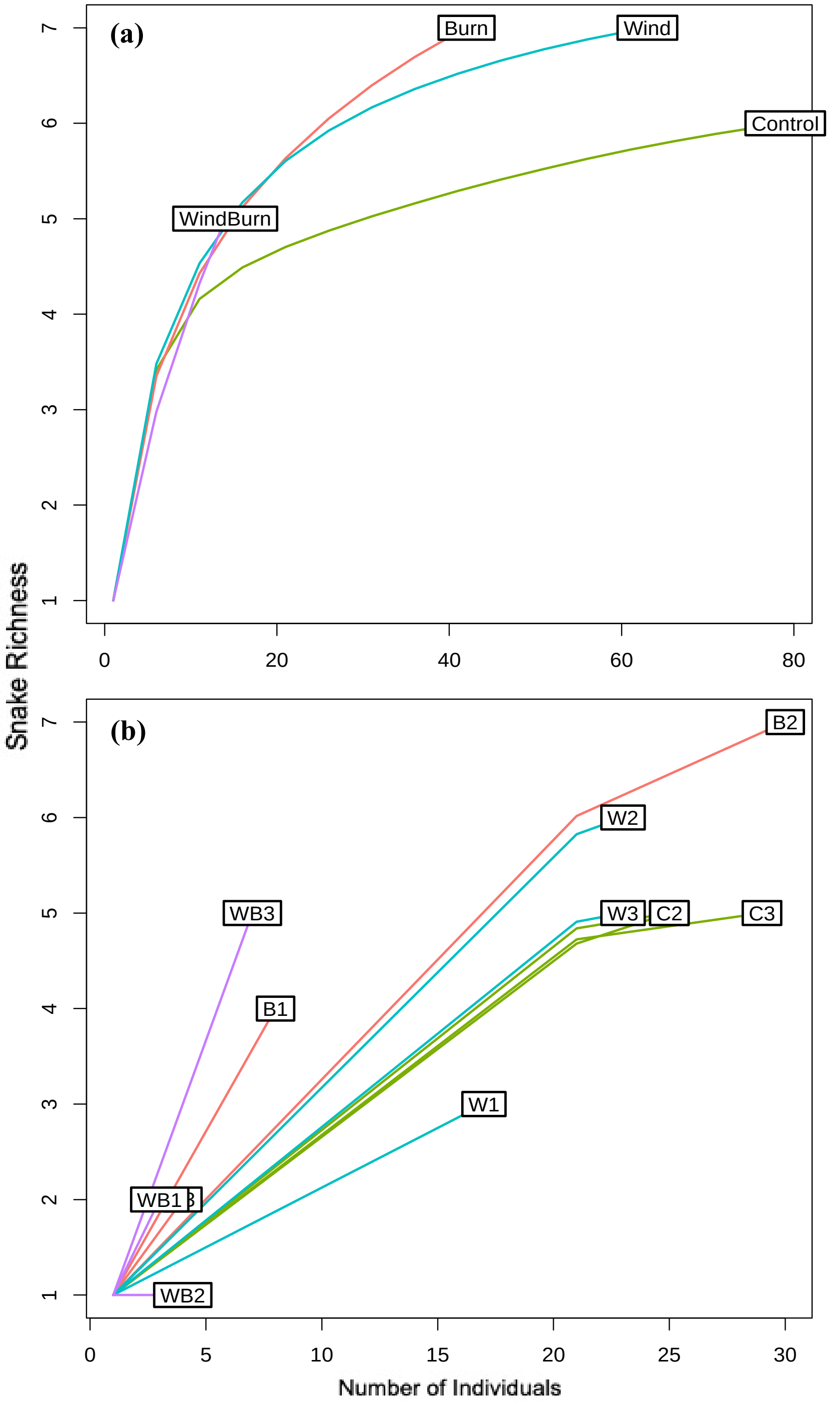

Figure 2. 4. Individual-based rarefaction curves depicting the relationships between snake richness (y-axis) and sample-based abundance (x-axis), by (a) treatment (Control, Burn, Wind, WindBurn) and (b) site (e.g., Control sites; C1, C2, C3), in central Ontario. Each point represents the species richness and number of individuals encountered, at a given level of sampling effort (60 surveys per treatment, 20 surveys per site). Treatments and sites with a high diversity (richness and abundance) appear in the upper right quadrant while low diversity treatments appear in the lower left quadrant.

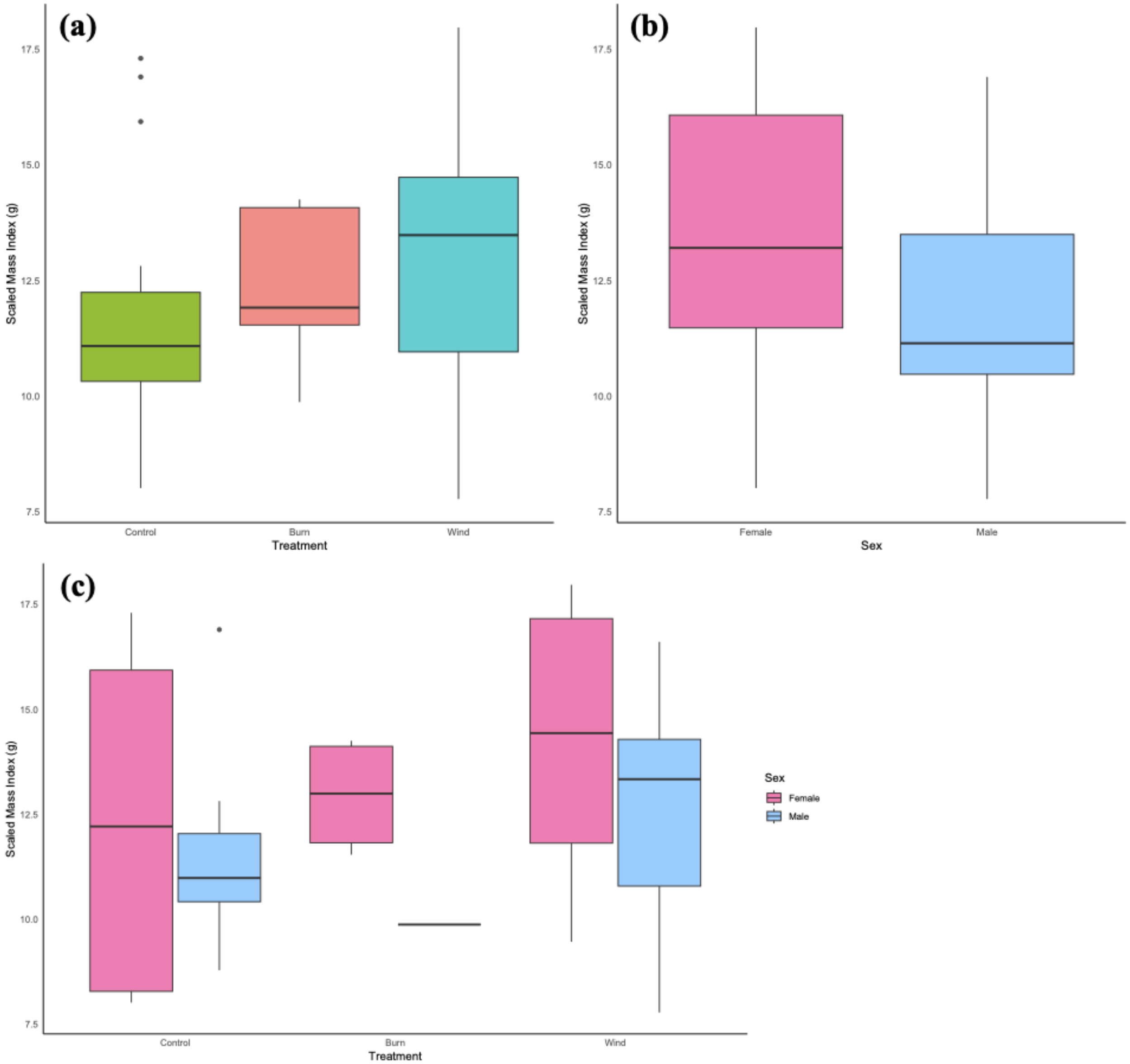

Figure 2. 5. Scaled mass index (SMI) for Smooth Greensnakes (*Opheodrys vernalis*; n = 46) by (a) treatment (Control, Burn, Wind; replicated in 3 sites), (b) sex (Male, Female), and (c) sex and treatment. Captured snakes were measured to the closest mm from snout to vent (SVL) and vent to tail (TL) with a measuring tape, which were then summed for total body length (TBL). Body mass (BM) was taken with appropriately sized spring scales. None of the comparisons were statistically significant. Horizontal bars represent group medians while vertical bars represent the spread of the data.

Figure 2. 6. Typical natural cover and visual encounter survey locations in the (a) Control, (b) Wind, (c) Burn, and (d) WindBurn treatments, in central Ontario. Surveys consisted of walking slowly and searching for animals seeking refuge underneath natural cover objects (e.g., logs, rocks, and bark), deployed cover boards, or on the ground surface. Wind sites closely resembled Control sites visually, except for the presence of infrastructure and fragmentation via service roads. Burn sites significantly lacked ground vegetation and canopy cover, whereas WindBurn sites not only lacked vegetation but also contained windfarm infrastructure and road fragmentation.

Figure 2. 7. Opportunistic encounters of alive on road (AOR) squamates, observed on Windfarm roads from May – August 2022 and 2023, in central Ontario. All snake and lizard species known to occur within the study area were observed on roads in the 2022 and 2023 seasons. Pictured species and average total lengths: (a) Eastern Gartersnake (*Thamnophis sirtalis sirtalis*; ~ 55 cm), (b) Northern Red-Bellied Snake (*Storeria o. occipitomaculata*; ~ 22 cm), (c) Eastern Foxsnake (*Pantherophis vulpinus*; ~ 120 cm), (d) Common Five-Lined Skink (*Plestiodon fasciatus*; ~ 15 cm; Rowell, 2012; Ontario Nature, 2023). Species exhibit a wide range of sizes, making it particularly challenging to spot smaller individuals such as the (b) Northern Red-Bellied Snake on the wide unpaved roads.

General Conclusion

The goal of my study was to examine the impacts of landscape modifications resulting from wind energy harvesting and wildfire on herpetofauna communities. Instead of concentrating solely on a single population of a particular species, I used a broader community approach. This approach provides a greater understanding of the impacts of disturbances on multiple species of conservation significance. I explored differences in anuran and squamate community metrics, snake body condition, and anuran vocalizations among sites characterized as relatively undisturbed, windfarm impacted, wildfire impacted, and sites with both windfarm and wildfire impacts. My findings suggest that anurans exhibit greater responses to windfarm impacts, with lower community metrics observed in windfarm sites compared to undisturbed sites. Squamates on the other hand, displayed greater responses to the combined effects of both disturbances, with the lowest community metrics observed in the double impact sites. My study offers new insights into the impacts of a rapidly emerging green energy source and wildfires on herpetofauna, paving the way for future research and potential mitigation strategies to protect animal taxa already at risk of extinction from several other threats (Gibbons et al. 2000; Stuart et al. 2004; Böhm et al. 2013).

Anurans

Noise pollution from wind turbines is a large concern for wildlife (Rabin et al. 2006; Lovich & Ennen, 2013; Schuster et al. 2015; Dai et al. 2015; Zwart et al. 2016; Teff-Seker et al. 2022; Trowbridge & Litzgus, 2022), that is largely ignored compared to the more obvious impacts associated with windfarm construction and operation. One of the objectives of my study was to investigate whether turbine noise influenced the acoustic environment of anurans, potentially affecting their calling behaviour and community dynamics. Acoustic analyses revealed lower chorus richness, diversity, evenness, and species-specific abundance within the windfarm, particularly notable in wetlands with a higher density of nearby wind turbines. If the noise pollution generated by the turbines interferes with the ability of anurans to vocalize effectively, they may face difficulties in locating mates and reproducing, potentially resulting in decreased reproductive success and diminished fitness (Sun & Narins, 2005; Blickley & Patricelli, 2010; Read et al. 2014), which could further result in gradual species population declines and local extirpation (Trowbridge & Litzgus, 2022).

The impact of turbine noise pollution has generally not been considered during the windfarm planning processes (Teff-Seker et al. 2022), creating a challenge to inform future mitigation recommendations. To better understand potential impacts, I recommend that future studies investigate the effects of wind turbine noise on anuran communities by using audio play-back experiments (Cunnington & Fahrig, 2010; Hanna et al. 2014). Although my study detected community differences possibly due to wind turbine noise, I did not confirm a clear-cut mechanism for the differences. Broadcasting turbine noise in a controlled setting to calling anurans, similar to various studies with other anthropogenic noise (i.e., traffic noise; Cunnington & Fahrig, 2010; Hanna et al. 2014), would allow for call characteristics (e.g., call frequency, call duration, call abundance) to be accurately measured and compared against a control. These tests would help verify whether the low-frequency noise from the turbines directly hinders the ability of anurans to vocalize, potentially affecting mate attraction and reproductive success. Given that turbine noise is positively correlated to wind speed (Katinas et al. 2015), leading to increased energy production (IRENA, 2022), devising mitigation strategies to minimize noise impacts on wildlife poses a significant challenge. This challenge is likely to be most effectively addressed during the planning phase when deciding on the placement of turbines (i.e., away from productive wetlands). Further to that, anuran breeding call activity is highest at night (Wells, 1977), meaning that a timing shift in turbine operation could be implemented as a potential mitigation strategy to limit the overlap of turbine noise with peak anuran calling periods.

Squamates

Although turbine noise represents an indirect consequence of windfarm implementation, the direct impacts such as habitat loss, degradation, and fragmentation can pose significant threats to squamates (Gibbons et al. 2000; Row et al. 2007; Böhm et al. 2013; Rytwinski & Fahring, 2015; Doherty et al. 2020). Another objective of my study was to assess whether the construction and operation of a windfarm, along with a wildfire, impacted the composition of squamate communities and snake health (inferred from body condition of a representative species). I detected lower total snake and skink abundance in the combined wildfire and windfarm impacted areas, compared to undisturbed areas. I also detected lower abundances of a snake habitat specialist (*Diadophis punctatus*) in the wildfire impacted areas, compared to undisturbed areas. However, no differences were observed in areas affected solely by the windfarm, suggesting that squamate communities can persist on the windfarm. Wildfires are expected to increase in frequency and severity, principally due to climate change (Bowman et al. 2020; Pyne, 2020; Nimmo et al. 2021; Wu et al. 2021), suggesting that more research is needed to understand how squamate populations will respond and persist. To my knowledge, no other research studies in Ontario have specifically examined the community ecology of squamates post-wildfire. This is concerning given that in 2023, the amount of forest burned in Ontario was almost 3 times higher than the 10-year average, with 51 more fires than the same average (OMNRF, 2023).

Future windfarm and associated research projects should consider the use of Before-After-Control-Impact (BACI) designs (Kuvlesky et al. 2007; Lesbarrères & Fahrig, 2012; Delay et al. 2023) when implementing windfarms in areas of high biodiversity. BACI designs provide before data, allowing direct comparisons to population or community metrics post-construction. Although my post-construction study provides useful data, the results may not reflect full picture of impacts of windfarm development on squamate populations (Kuvlesky et al. 2007). Without pre-construction data, my findings should be used cautiously to inform management decisions regarding future windfarm development in areas of high squamate diversity.

My study is one of the first to examine the impacts of windfarms and wildfire on herpetofauna communities. My findings can be used to inform research on other amphibian and reptile species and to inform management decisions for future windfarm projects in biodiverse areas. Reptiles and amphibians are in decline globally (Gibbons et al. 2000; Stuart et al. 2004; Böhm et al. 2013), meaning that continued research is needed to examine the potentially negative consequences associated with wind energy on the persistence of these species. Achieving a mutually beneficial outcome for both wind energy and wildlife (Kiesecker et al. 2011) is only possible through ongoing research and monitoring, which informs the development and implementation of mitigation strategies aimed at preventing negative effects on wildlife, while maximizing the benefits of renewable energy.

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Appendix

Appendix A: Anuran and snake species acronyms and associated common and scientific names.

Group	Acronym	Common Name	Scientific Name
Snake	BRSN	Dekay's Brownsnake	*Storeria dekayi*
	EAGA	Eastern Gartersnake	*Thamnophis s. sirtalis*
	GRSN	Smooth Greensnake	*Opheodrys vernalis*
	MILK	Eastern Milksnake	*Lampropeltis triangulum*
	NOWA	Northern Watersnake	*Storeria o. occipitomaculata*
	REBE	Northern Red-Bellied Snake	*Diadophis punctatus*
	RING	Northern Ring-Necked Snake	*Nerodia s. sipedon*
Anuran	BUFR	American Bullfrog	*Lithobates casteianus*
	GRFR	Green Frog	*Lithobates clamitans*
	GRTR	Grey Treefrog	*Hyla versicolor*
	LEFR	Northern Leopard Frog	*Lithobates pipiens*
	MIFR	Mink Frog	*Lithobates septentrionalis*
	SPPE	Spring Peeper	*Pseudacris crucifer*
	WOFR	Wood Frog	*Lithobates sylvaticus*

Appendix B: ARU (autonomous recording unit) attached to a charred tree adjacent to a wetland containing calling anurans, at a Burn treatment site.

Appendix C: Wetland containing calling anurans surrounded by wind turbines, at a Wind treatment site.