

UNIVERSITÉ DU QUÉBEC À MONTRÉAL

DÉCOUVERTE DÉROUTANTE : L'ACTIVITÉ DES CHAUVES-SOURIS INSECTIVORES EST
NÉGATIVEMENT CORRÉLÉE AU BRUIT ROUTIER AINSI QU'À LA DISTANCE À LA ROUTE.

MÉMOIRE PAR ARTICLE

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RÉSUMÉ

Les chauves-souris jouent un rôle écologique important en tant qu'espèces bio-indicatrices de la santé des écosystèmes forestiers, donnant un indice de la résistance et de la résilience de ces derniers. Ce sont aussi des espèces longévives (ex. : 25-30 ans) qui nous rendent une panoplie de services écosystémiques, dont celui du contrôle des espèces d'arthropodes ravageurs des cultures agricoles. Malheureusement, leur survie est fragilisée par plusieurs menaces (ex. : syndrome du museau blanc, perte d'habitat, extermination, etc.) et le bruit provenant du réseau routier, en constant développement, pourrait constituer un stress supplémentaire. Toutefois, on connaît encore peu l'importance de son impact sur les chauves-souris insectivores, dont la survie dépend notamment du paysage sonore. L'objectif principal de cette recherche est d'identifier quelles caractéristiques de la route ont un impact sur l'activité des chauves-souris insectivores en suivant l'hypothèse que les routes perturbent le comportement des chauves-souris et que celles-ci adopteraient un comportement d'évitement en fonction de la largeur des routes, de leur proximité par rapport aux stations d'enregistrement des chauves-souris et du niveau de bruit de la circulation. La région au sud-est du Québec a été choisie pour ses habitats, ses points d'eau, ses nombreuses routes et surtout parce qu'on y trouve plusieurs espèces de chauves-souris présentes au Québec. Durant trois saisons estivales, de 2021 à 2023, 14 stations d'enregistrements en lisières boisées ont été installées en créant un gradient de distances à la route et de largeurs de route. Ces stations captaient des données acoustiques d'activité nocturne des chauves-souris ainsi que de bruit routier afin d'observer le lien entre ces deux variables et d'en déduire la résilience des espèces sensibles aux perturbations sonores anthropiques dans leur écosystème. Nous avons comparé deux groupes de chauves-souris dont les stratégies écologiques contrastaient : la grande chauve-souris brune (*Eptesicus fuscus*), très tolérante à l'environnement anthropisé, et les espèces du genre *Myotis*, plus sensibles à l'anthropisation, qui comprenaient la petite chauve-souris brune (*Myotis lucifugus*), la chauve-souris pygmée de l'Est (*Myotis leibii*) et la chauve-souris nordique (*Myotis septentrionalis*). Nos résultats suggèrent de fortes corrélations négatives entre l'activité nocturne des chauves-souris et le bruit routier pour les espèces du genre *Myotis* ($\beta = -2.25$ 95% CI : [-3.50, -0.99], $p = 0.0004$) ainsi que pour la grande chauve-souris brune ($\beta = -2.86$, 95% CI : [-4.36, -1.37], $p = 0.0001$). De plus, les espèces étudiées présentaient une activité légèrement plus élevée à proximité des routes (grande chauve-souris brune $\beta = -2.58$, 95% CI : [-5.41, 0.25], $p = 0.07$; genre *Myotis* $\beta = -2.00$, 95% CI : [-3.74, -0.26], $p = 0.02$), tandis que la largeur des routes n'avait aucun effet sur leur activité respective (grande chauve-souris brune $\beta = 0.30$, 95% CI : [-2.01, 2.61], $p = 0.80$; genre *Myotis* $\beta = 0.63$, 95% CI : [-0.81, 2.06], $p = 0.39$). Cette étude suggère que les chauves-souris utilisent les abords des routes comme des corridors facilitant leurs déplacements, mais les évitent aux moments où le niveau de bruit routier est élevé. Ces résultats soulignent l'importance de mettre en place des mesures de mitigation permettant de limiter spatialement la pollution sonore routière et favoriser les déplacements des chauves-souris.

Mots clés : Chauves-souris, Anthropisation, Bioacoustique, Bruit routier, Écologie routière, Fragmentation d'habitat.

CHAPITRE 1

INTRODUCTION GÉNÉRALE

1.1 Les effets de la route sur la biodiversité

L'anthropisation de l'environnement par le changement d'utilisation des terres constitue l'une des causes premières de la perte de biodiversité (Jaureguiberry *et al.*, 2022 ; Richardson *et al.*, 2023). La création et l'utilisation du réseau routier, en particulier, constituent ensemble une forme d'anthropisation exerçant une influence négative sur plusieurs espèces fauniques (Garriga *et al.*, 2012 ; Bennett, 2017) à tel point que cela représente l'une des dix principales menaces pour la biodiversité (Maxwell *et al.*, 2016). Dès sa construction, la route cause une perte d'habitat et de biodiversité (Spellerberg et Morrison, 1998). Lorsqu'elle est en utilisation, les véhicules routiers causent des mortalités directes par collisions entre véhicule et faune (Underhill, 2003). Un autre effet négatif est la fragmentation de l'habitat impliquant la perte de connectivité structurelle due à la division ou la réduction de grandes parcelles d'habitat, ainsi que la perte de connectivité fonctionnelle au sein des métapopulations à cause de perturbations diverses (tel que le bruit de la circulation) qui dissuadent la faune sauvage de s'approcher des zones proches des routes (Fahrig et Rytwinski, 2009). En isolant les populations, la fragmentation de l'habitat causée par la route serait d'ailleurs la menace la plus importante pour la biodiversité, entraînant des conséquences au niveau de l'espèce, de la population et de la génétique (Spellerberg et Morrison, 1998 ; Dean *et al.*, 2019). La route peut aussi causer de la pollution lumineuse et sonore (Dean *et al.*, 2019). Cette dernière peut avoir divers impacts négatifs sur la faune sauvage, notamment en altérant la croissance (Zollinger *et al.*, 2019), la reproduction (Halfwerk *et al.*, 2011), le système immunitaire (Tennesen *et al.*, 2018), la recherche de nourriture (Senzaki *et al.*, 2016), ainsi que les comportements de communication (Gentry *et al.*, 2018) et d'alerte (Bee et Swanson, 2007 ; Templeton *et al.*, 2016). Même en l'absence d'infrastructure physique, plusieurs études sur les routes fantômes (enregistrement du trafic routier sans l'utilisation d'une route physique) indiquent que le bruit de la circulation à lui seul constitue un vecteur majeur des impacts des routes sur les populations animales (McClure *et al.*, 2013 ; Ware *et al.*, 2015 ; McClure *et al.*, 2017).

1.2 Les chauves-souris, des espèces uniques en danger

Il est important d'étudier les chauves-souris, car en plus d'être le seul mammifère capable de véritablement voler (U.S. National Park Service, 2024), elles procurent de nombreux bénéfices écosystémiques variés. En tant qu'espèces bio-indicatrices, elles permettent, par leur présence ou leur

état, de mieux comprendre les caractéristiques d'un écosystème ou les effets de changements naturels ou anthropiques (Jones *et al.*, 2009 ; Zukal *et al.*, 2015 ; Park, 2015). La majorité des chauves-souris utilisent l'écholocation pour naviguer et chasser (Diebold *et al.*, 2020), un comportement adaptatif sophistiqué qui permet par ailleurs d'inspirer la création et l'amélioration de nombreuses technologies dans les domaines de la robotique et de l'imagerie acoustique (Recchiuto *et al.*, 2014 ; Simmons *et al.*, 2017 ; Diebold *et al.*, 2020). De plus, les chauves-souris rendent une multitude de services écosystémiques (Kunz *et al.*, 2011). Les chauves-souris insectivores, par exemple, permettent le contrôle des insectes nuisibles aux cultures agricoles (Boyles *et al.*, 2011) et diminuent ainsi les pertes des récoltes (Rodríguez-San Pedro *et al.*, 2020) tout en offrant du guano, utilisé comme fertilisant naturel (Rahman *et al.*, 2023). La plupart des espèces de chauves-souris dans le monde se nourrissent principalement d'insectes et, en Amérique du Nord, la majorité des espèces sont insectivores (U.S. Geological Survey, 2016), incluant toutes les espèces de chauves-souris présentes au Québec (MELCCFP, s.d.).

Malgré tout, les chauves-souris sont parmi les espèces les plus mal comprises, en partie en raison de stéréotypes et de préjugés persistants à leur égard (Tuttle, 2015 ; U.S. Fish and Wildlife Service, 2020). Partout dans le monde, la survie des chauves-souris est compromise pour plusieurs raisons (Frick *et al.*, 2020) et en Amérique du Nord, les chauves-souris figurent parmi les vertébrés les plus menacés (Hammerson *et al.*, 2017). Une consultation internationale d'experts menée par Adams *et al.* (2024), portant sur l'évaluation de l'état de 153 espèces de chauves-souris au Canada, aux États-Unis et au Mexique, a révélé qu'au cours des 15 dernières années, 90 % de ces espèces ont connu un déclin de population et qu'au cours des 15 prochaines années, 53 % des espèces présentent un risque d'extinction modéré à très élevé. Parmi les principaux facteurs causant le déclin des espèces nord-américaines figurent notamment le syndrome du museau blanc (Cheng *et al.*, 2021), le dérangement d'hibernacles (Brown et Berry, 1997), la persécution (MacFarlane et Rocha, 2020), le développement urbain (Chen et Li, 2025), l'activité minière (Watkins, 2009), l'exposition aux contaminants (Cable *et al.*, 2022), l'agriculture (Hunninck *et al.*, 2022), la foresterie (Bats in forests: conservation and management, 2007), les feux de forêt intensifiés (Jung, 2020), la pollution lumineuse (Li *et al.*, 2024) et les changements climatiques (Sherwin *et al.*, 2013).

Il existe huit espèces de chauves-souris au Québec [la grande chauve-souris brune (*Eptesicus fuscus*), la chauve-souris argentée (*Lasionycteris noctivagans*), la chauve-souris cendrée (*Lasiurus cinereus*), la chauve-souris rousse de l'est (*Lasiurus borealis*), la pipistrelle de l'est (*Perimyotis subflavus*), la petite

chauve-souris brune (*Myotis lucifugus*), la chauve-souris pygmée (*Myotis leibii*), et la chauve-souris nordique (*Myotis septentrionalis*) (Animaux sauvages du Québec, 2024). Parmi celles-ci, sept espèces sont désignées comme ayant un statut légal de protection au niveau provincial (*Liste des espèces fauniques menacées ou vulnérables*, s. d.) et/ou au niveau fédéral (*Loi sur les espèces en péril*. L.C. 2002, ch. 29) et/ou faisant partie de la Liste rouge des espèces menacées de l'IUCN (Tableau 1.1). Durant la saison hivernale, la chauve-souris argentée, la chauve-souris cendrée et la chauve-souris rousse sont des espèces qui migrent vers le Sud, alors que les autres espèces sont résidentes et hibernent au Québec (Chauves-souris aux abris, s. d.). L'été, toutes les chauves-souris sont présentes au Québec pendant la période de mise à bas des petits et leur croissance (Chauves-souris aux abris, s. d.). Un inventaire des espèces présentes sur le lieu d'étude a été fait en 2018 (Lazure, 2018), confirmant la présence de trois espèces (soit la chauve-souris argentée, la grande chauve-souris brune et la chauve-souris cendrée) et la présence probable de cinq autres espèces (soit la petite chauve-souris brune, la chauve-souris pygmée, la chauve-souris nordique, la chauve-souris rousse de l'est et la pipistrelle de l'est, dont la présence est suspectée selon leur aire connue de répartition au Québec et au Canada) (Tableau 1.1). Parmi celles-ci, la grande et la petite chauve-souris brune peuvent être retrouvées dans une interface agricole-forestière (Tableau 1.1). La petite chauve-souris brune a un statut de protection au niveau fédéral, provincial et se retrouve sur la liste rouge de l'IUCN. La grande chauve-souris brune, quant à elle, semble s'accommoder aux perturbations anthropiques, avec une tendance démographique populationnelle à la hausse (Miller *et al.*, 2016). C'est par ailleurs la seule espèce à ne pas avoir de statut ni au niveau fédéral ni au niveau provincial.

Tableau 1.1 Caractéristiques des différentes espèces de chauves-souris présentes dans la MRC Brome-Missisquoi au sud-est du Québec. Les présences probables et suspectées, mais non confirmées durant l’inventaire de 2018 sont notées avec un « ? ».

Nom commun (Nom scientifique)	Abréviation	Migratrice (M) Résidente (R)	Habitats estivaux et habitats d'alimentation préférés	Statut de protection présent ?			Présence détectée à Pike River en 2018 ?
				Canada	Québec	IUCN	
Chauve-souris argentée (<i>Lasionycteris noctivagans</i>)	Lano	M	Milieux forestiers		✓		Oui
Grande chauve- souris brune (<i>Eptesicus fuscus</i>)	Epfu	R	Milieux urbains/périurbains et pâturages				Oui
Chauve-souris cendrée (<i>Lasiurus cinereus</i>)	Laci	M	Milieux forestiers et clairières		✓		Oui
Petite chauve- souris brune (<i>Myotis lucifugus</i>)	Mylu	R	Milieux forestiers, lisière de clairières et de routes et milieux urbains/périurbains avec présence de boisés	✓	✓	✓	?
Chauve-souris nordique (<i>Myotis septentrionalis</i>)	Myse	R	Milieux forestiers	✓	✓	✓	?
Chauve-souris pygmée de l'Est (<i>Myotis leibii</i>)	Myle	R	Milieux forestiers		✓	✓	?
Chauve-souris rousse de l'Est (<i>Lasiurus borealis</i>)	Labo	M	Milieux forestiers, clairières et milieu périurbains		✓		?
Pipistrelle de l'Est (<i>Perimyotis subflavus</i>)	Pesu	R	Milieux forestiers nordiques et milieux urbains/périurbains au sud du Québec	✓	✓	✓	Non

(MELCCFP, s.d. ; Grindal *et al.*, 1999 ; *Loi sur les espèces en péril*. L.C. 2002, ch. 29 ; Ford *et al.*, 2005 ; Menzel *et al.*, 2005 ; Prescott et Richard, 2013 ; Miller *et al.*, 2016 ; Solari, 2018a ; Solari, 2018b ; Solari, 2021 ; Lazure, 2018 ; Charbonneau *et al.*, 2023)

1.3 Les effets des routes sur les chauves-souris

Le réseau routier est en constant développement (IEA, 2013) et pourrait constituer un stress supplémentaire particulièrement contraignant pour les déplacements des chauves-souris, qui sont déjà des espèces fragilisées par d'autres sources de perturbations anthropiques. En plus du risque de collision avec les véhicules (Ramalho *et al.*, 2021), l'impact des routes peut également s'étendre au-delà de leurs limites physiques par la « zone d'effet routier », qui désigne la distance à partir du bord de la route à l'intérieur de laquelle des impacts écologiques significatifs peuvent être observés (Forman et Alexander, 1998). En général, on remarque une activité réduite des chauves-souris à proximité des routes (Berthinussen et Altringham, 2012 ; Kitzes et Merenlender, 2014 ; Claireau *et al.*, 2019a). Toutefois, leur réaction par rapport à la distance à la route peut parfois varier selon les espèces et les préférences en matière d'habitat (Medinas *et al.*, 2019 ; Ramalho *et al.*, 2021). En effet, les espèces qui préfèrent voler dans des milieux ouverts (clairières, champs, etc.) sont adaptées pour faire de l'écholocation à longue distance et semblent donc mieux tolérer la proximité des routes (Medinas *et al.*, 2019). Elles présentent parfois même une activité plus élevée à proximité des routes qu'elles utiliseraient comme corridor de déplacement (Ramalho *et al.*, 2021). Aussi, la fragmentation de l'habitat induite par les routes est complexe, impliquant la perte de connectivité structurelle et la division ou la réduction de parcelles d'habitat, dissuadant généralement la faune sauvage de s'approcher des zones proches des routes (Fahrig et Rytwinski, 2009). Pour les chauves-souris, une fragmentation du territoire excessive aurait un impact négatif sur leur activité (Russo-Petrick et Root, 2023). Les routes provoqueraient des comportements d'évitement chez les chauves-souris et limiteraient ainsi l'accès aux ressources en raison des interruptions dans leurs itinéraires de déplacement (Bennett et Zurcher, 2013). Les routes de grandes tailles, telles que les autoroutes, dégradent particulièrement les habitats essentiels au maintien des populations de chauves-souris (Kitzes et Merenlender, 2014). Ces corridors de transports, en plus d'avoir des effets néfastes physiques : collisions, fragmentation du territoire, perte d'habitat, etc. (Damásio *et al.*, 2021), produisent aussi de la pollution sonore ayant des effets variés sur les chauves-souris (Schaub *et al.*, 2008 ; Siemers et Schaub, 2011 ; Allen *et al.*, 2021 ; Yantén *et al.*, 2022). En effet, le bruit provenant du trafic routier agirait à la fois comme une barrière sonore, soit un mur d'informations sensorielles que les chauves-souris auraient de la difficulté à franchir (Sołowczuk, 2019), et comme barrière physique à la dispersion, puisqu'une route « fantôme » (sans présence de trafic routier) affecterait négativement certaines espèces (Finch *et al.*, 2020). Le bruit de trafic routier pourrait également provoquer des changements comportementaux : les chauves-souris modifieraient leurs cris d'écholocation pour

compenser la perte d'efficacité dans des conditions bruyantes (Yantén *et al.*, 2022) ou adopteraient un comportement d'évitement de la zone impactée (Schaub *et al.*, 2008).

1.4 Lacunes scientifiques

À la suite d'une revue des connaissances actuelles sur les effets de la route sur les populations, Barrientos *et al.* (2021) ont souligné le fait que les études à l'échelle de la population ne représentent que 8 % des recherches dans le domaine de l'écologie routière. Ils recommandent de faire des études à long terme, d'utiliser une approche à l'échelle du paysage afin d'obtenir des données empiriques de qualité (Barrientos *et al.*, 2021). De plus, il existe des lacunes scientifiques au sein de la littérature au sujet de la pollution sonore routière et de ses effets sur les chauves-souris. Plusieurs études à ce sujet sont souvent effectuées en laboratoire dans un environnement expérimental contrôlé (Schaub *et al.*, 2008 ; Siemers et Schaub, 2011 ; Allen *et al.*, 2021 ; Yantén *et al.*, 2022), mais elles ne donnent pas une idée exacte des autres paramètres, tels que celui de la proximité des plans d'eau qui peut influencer l'activité nocturne des chauves-souris sur le terrain (Lehrer *et al.*, 2021). Même si certains articles dans la littérature traitent des effets de la pollution sonore routière (Finch *et al.*, 2020 ; Yantén *et al.*, 2022) ou de la fragmentation de l'habitat causé par une route physique sur les chauves-souris (Claireau *et al.*, 2019b ; Damásio *et al.*, 2021 ; Laforge *et al.*, 2022), peu d'études testent les effets relatifs de ces deux variables combinées sur le terrain (Sołowczuk, 2019 ; Ramalho *et al.*, 2021). De plus, la majorité des recherches sur l'impact des infrastructures routières sur les chauves-souris ont été menées en Europe (Ramalho et Aguiar, 2020). Il semble donc pertinent d'effectuer davantage d'études dans d'autres régions, notamment en Amérique du Nord, dont la circulation humaine est historiquement axée autour du réseau routier (Wells, 2014 ; Filion, 2018) et composée de véhicules routiers de plus en plus pesants (MacKenzie *et al.*, 2014), ce qui pourrait exercer une influence particulière sur la réponse des espèces présentes aux abords des routes.

1.5 Objectifs de l'étude

Pour mieux contribuer à l'avancement des connaissances sur les effets du réseau routier sur la faune, nous voulons effectuer une étude de l'effet des routes à l'échelle de la population en utilisant une approche à l'échelle du paysage sur plusieurs tronçons routiers par l'acquisition de données empiriques (Barrientos *et al.*, 2021). Nous voulons identifier quelles caractéristiques de la route ont un effet sur les populations de chauves-souris insectivores de la région du sud-est du Québec dans une seule étude de terrain. Notre hypothèse générale est que les routes perturbent le comportement des chauves-souris et que celles-ci adopteraient donc un comportement d'évitement des routes en fonction du bruit du trafic routier, de la

proximité à la route et de la largeur de celle-ci (Sołowczuk, 2019 ; Ramalho *et al.*, 2021). Selon cette hypothèse, nous voulons tester deux prédictions :

D'abord, nous prédisons que l'activité des chauves-souris diminuera quand le bruit routier augmentera en intensité (Schaub *et al.*, 2008 ; Ramalho *et al.*, 2021), quand la distance à la route diminuera (Berthinussen and Altringham, 2012) et quand la largeur de la route augmentera, car elle est généralement liée à une diminution du couvert forestier due à la fragmentation de l'habitat (Kitzes et Merenlender, 2014).

Ensuite, nous prédisons que l'activité de la grande chauve-souris brune, plus tolérante à l'environnement urbain, aura moins de corrélations négatives entre son activité nocturne et les effets liés à la route comparativement aux espèces du genre *Myotis* qui devraient être plus sensibles aux perturbations anthropiques du fait de leur stratégie de déplacement et d'alimentation (Schaub *et al.*, 2008).

CHAPITRE 2

TRAFFIC NOISE, NOT PROXIMITY TO ROADS, REDUCES BAT ACTIVITY

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2.1 Abstract

Bats face numerous threats, contributing to their decline. Expanding road networks could exacerbate this situation. However, we still know little about the impacts of roads and associated noise on insectivorous bats, whose survival depends on the soundscape they use for orientation and hunting. Our objective was to analyze which road characteristics influence bat behavior, with the expectation of decreased bat activity with increased traffic noise, increased road proximity, and wider roads. Over three summers, we installed 14 recording stations near roads in southeastern Quebec, creating a gradient of varying distances to the road and road widths. We measured bat activity (bat passes per night) using ultrasonic acoustic detectors. We recorded ambient noise using SM4 acoustic recorders and then filtered it to retain only the frequencies associated with road-related noise. We characterized the environment (i.e., forests, water bodies, roads) around the sampled recording stations using GIS tools. We compared nocturnal bat activity responses to road characteristics using a generalized linear mixed model. We compared two groups of bats with contrasting ecological strategies: the urban-tolerant big brown bat (*Eptesicus fuscus*) and more sensitive species from the *Myotis* genus, which mainly included the little brown bat (*Myotis lucifugus*), along with the eastern small-footed bat (*Myotis leibii*), and the northern long-eared bat (*Myotis septentrionalis*) to a lesser extent. Our results suggest strong negative correlations between nocturnal bat activity and traffic noise for the non-threatened big brown bat (*Eptesicus fuscus*) ($\beta = -2.86$, 95% CI: [-4.36, -1.37], $p = 0.0001$) as well as for threatened species in the *Myotis* genus ($\beta = -2.25$, 95% CI: [-3.50, -0.99], $p = 0.0004$). However, both studied species showed slightly higher activity near roads (big brown bat $\beta = -2.58$, 95% CI: [-5.41, 0.25], $p = 0.07$; *Myotis* genus $\beta = -2.00$, 95% CI: [-3.74, -0.26], $p = 0.02$), while road width had no effect on their activity (big brown bat $\beta = 0.30$, 95% CI: [-2.01, 2.61], $p = 0.80$; *Myotis* genus $\beta = 0.63$, 95% CI: [-0.81, 2.06], $p = 0.39$). This study suggests that bats use roads as corridors to facilitate movement but avoid them when nocturnal traffic noise levels are high. These results highlight the importance of minimizing traffic noise and provide insight into how different features of a road network influence bat activity.

Keywords: Chiroptera, roads, insectivorous bat activity, road ecology, bioacoustics, traffic noise.

2.2 Introduction

Insectivorous bats specifically play an important role in insect pest control, using bio-sonar to effectively hunt insect prey (Kunz *et al.*, 2011). Hence, we receive financial and ecological benefits from bats, saving money on agricultural pest control worldwide (Cleveland *et al.*, 2006; Boyles *et al.*, 2011; Wanger *et al.*, 2014). Unfortunately, bats are among the least understood animals (Frick *et al.*, 2020), and their survival is compromised for several reasons (Hammerson *et al.*, 2017). In Quebec (Canada), eight insectivorous bat species [the big brown bat (*Eptesicus fuscus*), silver-haired bat (*Lasionycteris noctivagans*), the hoary bat (*Lasiurus cinereus*), the eastern red bat (*Lasiurus borealis*), the tricolored bat (*Perimyotis subflavus*), the little brown bat (*Myotis lucifugus*), the eastern small-footed bat (*Myotis leibii*), and the northern long-eared bat (*Myotis septentrionalis*)] face threats from multiple sources: white-nose syndrome (Frick *et al.*, 2010; Alves *et al.*, 2014), urban development (Chen and Li, 2025), contaminants exposure (Cable *et al.*, 2022), climate change (Sherwin *et al.*, 2013), etc.

A major threat to biodiversity arises from the anthropization of the environment (Jaureguiberry *et al.*, 2022; Richardson *et al.*, 2023). One large anthropogenic transformation negatively affecting numerous terrestrial wildlife species is the development and subsequent use of road networks (Garriga *et al.*, 2012; Bennett, 2017). Transportation (including roads and railways) is recognized as one of the ten major threats to biodiversity (Maxwell *et al.*, 2016), and if current trends continue as predicted, passenger and freight should double from 2010 to 2050 levels, and to accommodate this growth, nearly 25 million paved road lane-kilometres are expected to be built worldwide (without counting new parking spaces) (IEA, 2013). Bats are important bioindicator species and umbrella species in the ecosystems they inhabit (Jones *et al.*, 2009; Boyles *et al.*, 2011; Park, 2015). They could be useful bioindicators of road impacts (Berthinussen and Altringham, 2012), as they respond to anthropogenic environmental alterations (Russo and Ancillotto, 2015). To tailor mitigation and conservation measures (Ramalho and Aguiar, 2020), it is crucial to quantify the extent of the effects of roads on bats.

Road noise also interferes with species' capacity to perform essential biological functions (Kight and Swaddle; 2011; Shannon *et al.*, 2016), causing various negative effects on wildlife including delayed offspring growth (Zollinger *et al.*, 2019), reduced reproductive success (Halfwerk *et al.*, 2011), reduced foraging efficiency (Senzaki *et al.*, 2016), modified immune response (Tenessen *et al.*, 2018), as well as modified behaviour in communication (Gentry *et al.*, 2018) and antipredatory behaviour (Bee and Swanson, 2007; Templeton *et al.*, 2016). Studies on phantom roads (applying traffic noise playbacks to a

roadless landscape) even suggest that traffic noise alone, without the existence of a physical road, constitutes a major pathway by which roads exert effects on animal populations (McClure *et al.*, 2013; Ware *et al.*, 2015; McClure *et al.*, 2017), including bats (Finch *et al.*, 2020). Insectivorous bats use sound for communication and alimentation (Berthinussen and Altringham, 2012; Lehrer *et al.*, 2021), and thus anthrophony can modify bat behavior via two mechanisms: (1) by interference with echolocation when the human-caused sounds occur as ultrasounds or (2) by inundating sound in other wavelengths, which increases the overall decibels of noise and disturbs bats (Allen *et al.*, 2021). Schaub *et al.* (2008) found that bats are responsive to a range of environmental sounds, including highway noise at audible frequencies (20 Hz to 20 kHz), which they detect through passive listening. Thus traffic noise is too low to jam echolocation calls (The California Department of Transportation, 2016), but can rather reduce bat hunting efficiency due to a decreased capture success during their foraging behavior by masking the noise of natural prey or creating a sensory overload (Schaub *et al.*, 2008; Siemers and Schaub, 2011; Bunkley and Barber, 2015; Yantén *et al.*, 2022). Borkin *et al.* (2020) studied bats in New Zealand using fixed points and found a negative correlation between bat activity and traffic volume at highway-adjacent detector units, while no significant relationship was observed further from the road.

The distance to the road is another variable often studied to examine the impact of roads on bats. Generally, bat species tend to exhibit reduced activity levels at short distances from roads (Berthinussen and Altringham, 2012; Kitzes and Merenlender, 2014; Claireau *et al.*, 2019a). In a study in California, four common bat species (Brazilian free-tailed bat (*Tadarida brasiliensis*), big brown bat (*Eptesicus fuscus*), hoary bat (*Lasiurus cinereus*), and silver-haired bat (*Lasionycteris noctivagans*)) exhibited consistently reduced activity in proximity to three major highways, where predicted bat activity was lowest at 0 m, intermediate at 100 m, and highest at 300 m from the road, consistently across all transects and survey nights (Kitzes and Merenlender, 2014). In the United Kingdom, Berthinussen and Altringham (2012) also found positive correlations between bat activity and distance from the road, with a total bat activity increasing over three times between 0 and 1600 m from the road. Meyer *et al.* (2024) noted in research near our study location that bat activity declined within a 200 m radius of high-traffic roads. However, response to road proximity may sometimes vary among species and habitat preferences (Medinas *et al.*, 2019; Ramalho *et al.*, 2021). In their review, Ramalho and Aguiar (2020) found that bats tend to forage in proximity to roads in open areas, but that in woodland areas, it is the opposite, as their activity increases with greater distance from the road. Medinas *et al.* (2019) found that overall bat activity increased with greater distance from roads but was influenced by the surrounding habitat types, with the most

pronounced negative effects extending up to approximately 300 m from roads in woodland habitats and exceeding 500 m in open field environments. Also, long-range echolocator species (*Eptesicus* spp., *Nyctalus* spp., and *Tadarida teniotis*) and *Pipistrellus kuhlii* appeared more tolerant of road proximity (Medinas *et al.*, 2019). Similarly, Ramalho *et al.* (2021) reported that open-space insectivorous bats (including species like *Cynomops* spp., *Eumops* spp., *Molossus molossus*, *Molossus* spp., *Nyctinomops laticaudatus*, and *Promops* spp.) exhibited significantly higher activity along road edges than in areas located farther from roads, whereas the activity of edge-space insectivorous bats was influenced by seasonal variation only.

Road width can be related to habitat fragmentation and a barrier effect, impeding species movement. Habitat fragmentation causes immediate and long-term biodiversity loss through cascading trophic effects across multiple trophic levels (Krauss *et al.*, 2010), and excessive fragmentation can negatively affect bat activity (Russo-Petrick and Root, 2023). Road-induced habitat fragmentation is complex, involving the loss of structural connectivity, the division or reduction of larger habitat patches, and the loss of functional connectivity within metapopulations due to traffic noise and other disturbances that deter wildlife from areas near roads (Fahrig and Rytwinski, 2009). In a meta-analysis of mammals, roads of all types, from large highways to narrow roads, can impede movement for certain species (Chen and Koprowski, 2019). The barrier effect was increased by greater road width but decreased as body mass increased (Chen and Koprowski, 2019). For bat populations, habitat loss, degradation, and fragmentation caused by roads, may also act as barriers to movement, leading to avoidance behaviors and restricting access to resources due to gaps in commuting routes (Bennett and Zurcher, 2013; Fensome and Mathews, 2016). Few papers specifically test the effects of various road widths on bats. Ramalho *et al.* (2021) and Roemer *et al.* (2021) measured road width in their study sites but later excluded it for further statistical analysis. Large highways reduce bat activity (Berthinussen and Altringham, 2012; Kitzes and Merenlender, 2014; Bhardwaj *et al.*, 2021), suggesting they may cause more severe habitat degradation critical to bat populations (Kitzes and Merenlender, 2014). Pourshoushtari *et al.* (2018) found that bat activity was higher along smaller unpaved roads bordered by forest edges, whereas it was lower along larger roads, such as highways and service roads, situated in open areas. Overall, as Altringham and Kerth (2016) point out, there is a need to study the effects of road width to understand how best to mitigate their impacts.

Within our studied species, the big brown bat is a large, high-speed-flying species using lower frequency echolocation (<35 kHz) that is considered a habitat generalist and can be found in more anthropized areas

(Fabianek *et al.*, 2011; Lehrer *et al.*, 2021), and is listed as “Least Concern” by the International Union for Conservation of Nature (IUCN) (Miller *et al.*, 2016). The term “species complex” refers to a group of distinct species with similar acoustic calls, which are grouped when they cannot be reliably distinguished based on their vocalizations. The big brown bat can sometimes be difficult to differentiate by calls from the silver-haired bat. When there is a doubt in the acoustical diagnosis, these two species are grouped into the big brown bat/silver-haired bat species complex, referred to hereafter as “Eppo” (Fabianek *et al.*, 2011). On the other hand, bats of the *Myotis* genus are all small-bodied, lower and slower-flying species that use high-frequency echolocation (>32 kHz). They are also associated with forest habitats and are found flying under the canopy. All *Myotis* genus species have a legally protected status in Quebec (*Liste des espèces fauniques menacées ou vulnérables*, s.d.), and two out of three in Canada (*Registre public des espèces en péril*, 2025). Bats of the *Myotis* genus include the little brown bat, the northern long-eared bat, and the eastern small-footed bat, referred to hereafter as the “Mysp” species complex. Within this complex, the little brown bat is listed as “Endangered” (Solari, 2021), the eastern small-footed bat is listed as “Endangered” (Solari, 2018a), and the northern long-eared bat is listed as “Near Threatened” (Solari, 2018b) by the IUCN.

Regardless of wing morphology and echolocation call characteristics, both the big brown bat and the little brown bat exhibit the highest activity levels in edge environments (Jantzen and Fenton, 2013), which correspond to agricultural-forest interfaces in our research study location. However, *Myotis* species are more strongly influenced by the amount and connectivity of the remaining forest cover, reflecting their reliance on structurally complex, wooded environments (White *et al.*, 2017). It is generally expected that a bigger proportion of old forest cover at a given station should result in overall higher activity for the *Myotis* species, since some smaller bat species roost and hunt in old enclosed forests (Ford *et al.*, 2005; Henderson and Broders, 2008; Henderson *et al.*, 2008; Segers and Broders, 2014; Russo *et al.*, 2016). In comparison, the big brown bat demonstrates greater habitat flexibility and appears less dependent on forest continuity (Perry and Thill, 2008). With its larger wingspan, it prefers open-space hunting habitats such as agricultural fields because of its large foraging range (Agosta, 2002; Duchamp *et al.*, 2004; Fabianek *et al.*, 2011).

In the context of road ecology, bat species can exhibit varying responses to roads depending on their habitat preferences (Claireau *et al.*, 2019a; Ramalho *et al.*, 2021). The effects on bat activity are strongly influenced by surrounding landscape features: in open habitats, bats tend to forage near roads, whereas

in woodland areas, bat activity increases with distance from roads (Ramalho and Aguiar, 2020). Fensome and Mathews (2016) found that the extent to which roads act as barriers to bat movement is determined by a complex interplay between habitat characteristics and species-specific behavioral traits. *Myotis* species are particularly sensitive to anthropogenic changes to their natural environments (Fabianek *et al.*, 2011; Gili *et al.*, 2020), and members of this species complex are expected to avoid roads and have overall reduced activity since low-flying species are more affected by roads than high-flying species (Fensome and Mathews, 2016; Claireau *et al.*, 2019a). We expect bat activity to vary by species, as each has different preferences and adaptations for its hunting habitat (Fenton, 1990; Threlfall *et al.*, 2011; Russo-Petrick and Root, 2023).

Other factors influencing variations in bat activity are meteorological and habitat characteristics rather than road-associated variables. A bigger proportion of water cover at a given station is expected to result in overall higher bat activity since the presence of a water source nearby affects bat presence and activity (Lisón and Calvo, 2011; Straka *et al.*, 2016; Lehrer *et al.*, 2021; Legros *et al.*, 2024). Meteorological variables can also influence bat activity (O'Donnell, 2000; Kohyt *et al.*, 2021), particularly night temperatures and relative humidity (Erickson and West, 2002; Appel *et al.*, 2019; Gorman *et al.*, 2021). Nights with cooler temperatures or higher humidity levels are generally associated with reduced activity in insectivorous bats (Straka *et al.*, 2016). Higher temperatures can also confound effects as they are associated with increased overall bat activity and a diminished contrast between near-road and far-road activity (Kitzes and Merenlender, 2014).

Although a growing body of evidence indicates that roads affect bats, significant gaps remain in our understanding of the specific effects of road characteristics and how they affect bats (Berthinussen and Altringham, 2012; Lehrer *et al.*, 2021). Earlier research evaluated the effects of traffic noise on bats in controlled laboratory environments (Schaub *et al.*, 2008; Siemers and Schaub, 2011; Bunkley and Barber, 2015; Finch *et al.*, 2020; Yantén *et al.*, 2022), and some studies tested the relative effects of traffic noise directly in the field (Sołowczuk, 2019; Borkin *et al.*, 2020; Claireau *et al.*, 2021). Also, some have tested the effect of different types of roads on bats (Pourshoushtari *et al.*, 2018; Ramalho *et al.*, 2021) or gap size between commuting roads as a proxy for road width (Bennett and Zurcher, 2013) without testing road width directly. Finally, as a majority of studies on the impacts of roads were conducted in Europe (67.44%) (Ramalho and Aguiar, 2020), it is imperative to identify the respective influences of various road

characteristics on bats in North America to orient conservation interventions better, since they are the most threatened group of vertebrates in North America (U.S. Fish and Wildlife Service, 2020).

Among the studies examining the effects of roads on bats, few provide a temporal and spatial analysis of how various roads and their characteristics influence bat activity and use. We conducted a field study to determine which road features influence the activity of insectivorous bats while accounting for other environmental factors that could affect their behavior. We hypothesized that roads disrupt bats and that they would adopt road-avoidance behavior depending on traffic noise, proximity of roads to the bat recording stations, and road width (Sołowczuk, 2019; Ramalho *et al.*, 2021). According to this hypothesis, we made the following predictions:

- 1 Bat activity will decrease in response to increased traffic noise, increased road proximity, and increased road width.** We expect that bats will actively avoid hunting in noisy environments (Schaub *et al.*, 2008), and thus their nocturnal activity should be negatively correlated with traffic noise, as bats avoid noisier environments (Ramalho *et al.*, 2021). Bat activity should be reduced closer to roads (Berthinussen and Altringham, 2012; Kitzes and Merenlender, 2014; Claireau *et al.*, 2019a). The effect of habitat fragmentation caused by roads has been reported to have a non-negligible negative impact on bats (Claireau *et al.*, 2019b; Sołowczuk, 2019; Damásio *et al.*, 2021; Laforge *et al.*, 2022), so we expect larger road widths to be associated with a decrease in bat activity as they are typically associated with a decrease in forest cover due to habitat fragmentation (Kitzes and Merenlender, 2014). A decline in bat activity near large roads with increased road width could also be attributed to higher traffic levels and elevated traffic noise (Berthinussen and Altringham, 2012).
- 2 The activity of the big brown bat, an urban-tolerant species, will be less negatively impacted (fewer negative correlations between bat activity and road-related effects) by the road than that of the urban-sensitive *Myotis* species.** We expect the latter to be more vulnerable to habitat fragmentation because their foraging habitat preferences consist of connected closed forests and thus will be more sensitive to the various negative effects of roads (Schaub *et al.*, 2008).

2.3 Material and Methods

Our study aimed to test whether variations in nocturnal bat activity were consistent with our hypothesis. To do this, we divided the research into three main steps:

First, we conducted *in situ* field recordings at 14 recording stations across three summers (2021, 2022, and 2023) using ultrasound and acoustic recorders along gradients of road distance and road width to capture bat calls and the ambient nocturnal soundscape. Second, we conducted an acoustic analysis to quantify bat activity using automated and manual identification, and filtered our soundscape recordings to quantify traffic noise levels. Third, we examined the influence of the tested variables (traffic noise, distance to road, and road width) and the meteorological and spatial variables on the nocturnal activity of bat species.

2.3.1 Field Recording

2.3.1.1 Study Site

Our study was conducted near the Brome-Missisquoi Municipal County Region in southeastern Quebec, Canada. The border with the U.S.A. was approximately 6 km from the centroid of all recording stations. We chose these locations as study sites for both the presence of different habitat-preferring insectivorous bat species and various types of roads, to perform a field study evaluating the activity of multiple insectivorous bat species in a habitat anthropized by roads (Jones, 2008). Before the research began, the presence of different bat species was confirmed through mobile surveys completed in 2018 (Lazure, 2018).

2.3.1.2 Sampling Effort

We installed 14 recording stations along three types of roads during each sampling summer (Figure 2.1). We installed five recording stations along a two-lane road (recording stations starting with “SCR”), five along a one-lane road (recording stations starting with “SCT”), and four along regional and collector roads (recording stations starting with “SCC”).

We initially installed 15 recording stations, but after soundscape analysis, one of the recording stations (“SCC2” in Figure 2.1) was removed from the dataset because it was too close to a water treatment plant where water occasionally flowed noisily, which could bias soundscape recordings. Measurements from the remaining 14 recording stations were used in the research, located at various fixed distances ranging from 60 m to 1400 m from the nearest operational road to create a gradient of traffic noise (Berthinussen

and Altringham, 2012; Claireau *et al.*, 2019b; Ramalho *et al.*, 2021). Recording stations were placed near different types of roads to create a gradient of road widths that varied with road type (Ramalho *et al.*, 2021). The characteristics of the nearest road to each recording station are summarized in APPENDIX A. Also, two recording stations were not accessible due to denied access during the summer of 2023 only (as indicated by crossing out “2023” in Figure 2.1).

We collected data using a stationary survey method, in which the recording devices were deployed at the beginning of July and remained in place until the beginning of September to capture the reproductive and autumnal migratory periods of bats (MELCCFP, s.d.). The stationary survey allowed us to detect physical and temporal variation in bat activity at multiple stations simultaneously, while also offering a higher probability of detecting bat activity than a mobile survey (Charbonneau *et al.*, 2023). The stationary survey method provided a representative picture of the species present at a given habitat over a well-defined period (Brigham *et al.*, 2004; Kunz and Parsons, 2009).

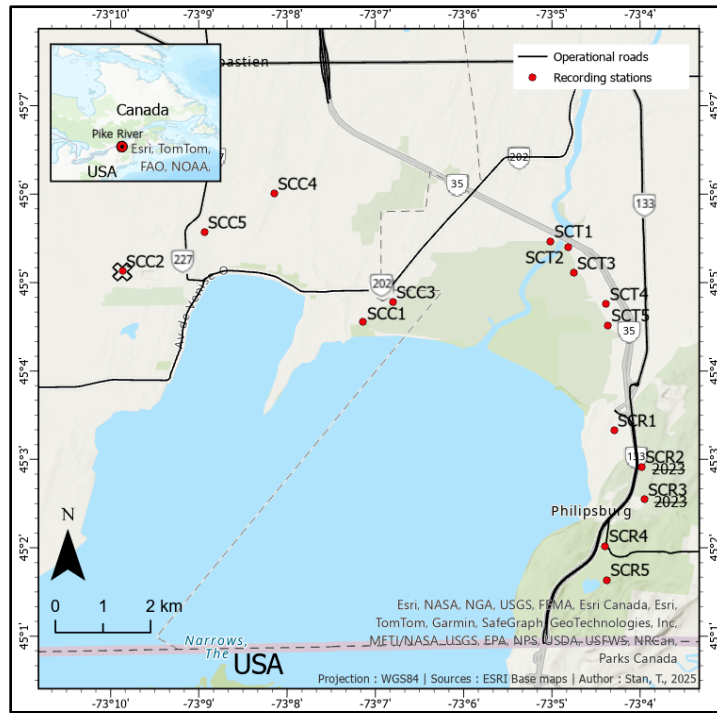


Figure 2.1 Map of the 14 sound recording stations, located near the Brome-Missisquoi Municipal County Region in southeastern Quebec (Canada). Note that two recording stations were not accessible due to denied access during the summer of 2023 only (this is represented by crossing out “2023”). The SCC2 station was removed from the dataset (cross on “SCC2”), as noise at this station was influenced by a nearby water treatment facility.



Figure 2.2 Picture of a recording station at the forest’s edge. The Anabat® was installed at a 45° angle pointing upward, and at a 2-meter height from its microphone to the ground. The SM4® was positioned below the Anabat®, at 50 cm between the microphones of each recording device (Grinfeder *et al.*, 2022; Charbonneau *et al.*, 2023).

2.3.1.3 Recording Stations

Each recording station consisted of two recording devices installed on the same tree to identify bat activity and ambient noise, particularly from road traffic. We used an Anabat Swift® acoustic detector (Anabat®) (Anabat Swift®, Titley Scientific, USA) to record bat calls and a Song Meter SM4® (SM4®) (Song Meter SM4®, Wildlife Acoustics, USA) to record ambient noise levels (Figure 2.2). We positioned each recorder perpendicular to the forest's edge by attaching the Anabat® with a Camo Strap® (Titley Scientific, USA) and the SM4® with an adjustable locking cable (Python®, Master Lock®, USA). All recorders were time-calibrated to ensure they showed the same timestamp in the data (audio recordings and bat passes). We later used that information to associate bat passes and audio recordings, and to ensure the data were compatible between measurements made at different recording stations (Sołowczuk, 2019). A maintenance visit every two weeks was essential to replace the batteries, verify the performance of all microphones used, and replace the memory cards of the SM4®, necessitating more data than their Anabat® counterparts.

2.3.1.4 Recording of Bat Echolocation

The acoustic inventory of bats within the framework of this research conforms with the Collection of standardized protocols for acoustic bat surveys in Quebec (Charbonneau *et al.*, 2023). We used Anabats® to passively monitor bat calls (Bat Conservation and Management, Inc., 2020). We installed them at the forest's edge in a forest-agricultural field interface, an optimal location for recording bats (Jantzen and Fenton, 2013). The directional ultrasonic microphones (US-D, Titley Scientific, USA) (Blumstein *et al.*, 2011) on each Anabat® were located 2 m above the ground, pointing toward the opening of the canopy (Jantzen and Fenton, 2013), and angled at 45° upward (Wickramasinghe *et al.*, 2003). The Anabat® we placed in the field did not record continuously. They operate using a decibel (dB) trigger mechanism and remain mostly in standby mode, activating only when the sound pressure level at the microphone exceeds the background noise by a specified signal-to-noise ratio (SNR) set in the recording settings in APPENDIX B.

For the Anabat® recorder settings, we selected a trigger sensitivity threshold (signal-to-noise ratio) of 14 kHz to reduce sensitivity to ambient noise (Charbonneau *et al.*, 2023). To ensure the detection of all bat species present in Quebec, we specified minimum and maximum trigger frequencies based on their echolocation call characteristics. The minimum frequency was set to 16 kHz (Charbonneau *et al.*, 2023), slightly below the expected minimum call frequency of the hoary bat, which echolocates at approximately 20 ± 3 kHz in open-space environments based on the reference acoustic database of bats in Quebec

(Fabianek, 2015). This minimum frequency setting ensured that all species with low-frequency calls would be detected, as it was the lowest echolocation frequency we expected to record. The maximum frequency was set to the default 250 kHz to allow detection of the northern long-eared bat, the only bat species in Quebec with echolocation calls that exceed 100 kHz (Fabianek, 2015). Transect mode was disabled, as we used a stationary survey method and did not require GPS data logging at 1-second intervals, thereby conserving battery life. The minimum event time was set to 1 millisecond, representing the minimum bat pulse duration required to trigger a recording (Charbonneau *et al.*, 2023). The recording window was set to 5 seconds, meaning that recording would continue for 5 seconds after a trigger event, which is longer than the shortest time between bat pulses (Charbonneau *et al.*, 2023). This setting prevents splitting a single bat pass across multiple files. Recordings were saved in ZC format, which used less memory than the WAV format and was therefore more suitable for extended deployment. The sample rate was set to 320 kHz, exceeding the highest frequency bat of interest by at least twice (i.e., $120 \text{ kHz} \times 2 = 240 \text{ kHz}$). According to the Nyquist-Shannon sampling theorem (Shannon, 1949), the sampling rate must be at least twice the highest frequency of interest to avoid aliasing in that range. Therefore, a sampling rate of 320 kHz was selected to capture bat echolocation calls accurately up to 120 kHz (Blumstein *et al.*, 2011; Charbonneau *et al.*, 2023). A bat pass corresponds to a sequence of multiple bat calls emitted by a bat flying past the microphone. The maximum file length was limited to 15 seconds of recording multiple bat pulses, after which a new file would be created if bat activity continued to trigger the microphones. We used a division ratio of 8 to maintain sufficient recorded resolution across a wide frequency range without favoring low-frequency calls (Charbonneau *et al.*, 2023). The filename prefix option was enabled to include the Anabat® serial number in each file name, facilitating data organization and traceability. Lastly, the analog high-pass filter was activated to attenuate signals below 10 kHz, since sub-10 kHz frequencies were not relevant to this study. This setting reduced the number of non-target noise recordings and further helped to conserve storage and battery life. These chosen parameters for the Anabats® are listed in APPENDIX B. (Titley Scientific, 2020)

Before installing, we tested the Anabat® microphones and checked each device for malfunctions. Detector parameters were chosen with consideration of the acoustic properties of bats native to Quebec. The selected “zero crossing” recording mode, while less data-rich than a “full spectrum” mode, enabled more efficient field data collection due to its lower storage requirements. (Titley Scientific, 2020)

2.3.1.5 Recording of Soundscape

At each recording station, we used the SM4® to record soundscapes in the audible range, which is most often used for studies of anthrophony (Grinfeder *et al.*, 2022). We programmed SM4® to record continuously, making 60-minute audio recordings all night, starting 30 minutes before sunset and ending 30 minutes after sunrise, based on Eastern Standard Time, during each entire summer.

For the SM4®, we selected the default stereo channel mode to enable both left and right microphones. The audio gain for both channels was set to 12 dB, which is lower than the default 16 dB setting appropriate for high-noise environments to prevent clipping. The preamplifier gain for both internal microphones was maintained at the default value of 26 dB. We left the filters for both channels disabled to retain all ambient and anthropogenic noise (such as traffic noise). A sample rate of 44,100 Hz was selected, exceeding twice the highest frequency within the human audible range (~20,000 Hz), in accordance with the Nyquist-Shannon sampling theorem (Shannon, 1949), to ensure accurate representation of the full range of audible frequencies, which included the frequencies related to traffic noise. Recordings were segmented at the default 1-hour mark; after this duration, the recording ended, and a new recording file was automatically created. We turned off audio compression to retain full waveform fidelity, saving all files in uncompressed WAV format. GPS coordinates (latitude and longitude) were adjusted according to the location of each recording station. The UTC offset was set to -4, aligning with the local time zone of southeastern Quebec. The sunrise and sunset timing method was configured to solar civil, which defines twilight as when the sun is 6 degrees below the horizon, ensuring that recordings began during nighttime hours. Finally, the LED indicator was set to display only for the first 5 minutes of each recording session to minimize the device's visibility in the field, helping maintain camouflage and reduce the risk of tampering or theft. These chosen parameters for the SM4® are listed in APPENDIX C. (Wildlife Acoustics, 2024)

Before each recording season, we calibrated the microphone of each SM4® with a Sound level calibrator (Model R8090, REED Instruments, USA). Calibration was also verified during maintenance visits to ensure that the data collection was not affected by a defective microphone.

2.3.1.6 Meteorological Data

We extracted online weather data from the Government of Canada website¹. We chose the Acadie weather station (climatic ID: 702LED4), located 32 km away from the centroid of all recording stations. It was the closest weather station to provide all necessary hourly nocturnal climate data for the research period: wind speed (km/h), temperature (°C), relative humidity (%), and precipitation (mm). Because sunset and sunrise times vary throughout the summer months, we standardized the sampling period by retaining only data collected between 8:00 pm and 4:00 am the following day. This fixed interval was consistently defined as “nighttime” across all months, enabling temporal standardization and facilitating comparisons across sampling dates. We then calculated the mean wind speed, mean temperature, mean relative humidity, and total precipitation for each night from 8:00 pm to 4:00 am the next day.

2.3.2 Spatial Analysis

For habitat variables, we extracted forest and water cover data within multiple buffer radii (100 m, 200 m, 400 m, 800 m, and 1600 m), around each recording station using the Fifth Ecoforestry Inventory Database provided by the Government of Quebec². We chose a 400-meter radius buffer for water and forest cover after analyzing the optimal radius that would explain bat activity in our models using the *hier.part* function [R package *hier.part* v.1.06 (Nally and Walsh, 2004)]. Larger radii would cause spatial autocorrelations, while lower radii didn’t sufficiently explain bat activity and were thus avoided. For forest cover, we initially extracted data on forests ranging from 10 to over 80 years of age, but ultimately included only forests older than 80 years in our analyses. Older forests, characterized by large, decaying trees with wide trunk diameters, are more likely to provide suitable roosting habitat and resources for bats (Tremblay and Jutras, 2010; Fabianek *et al.*, 2015; Lang *et al.*, 2015). Finally, we calculated the percentages of forest and water cover within a 400 m buffer radius around each recording station. Previous studies have shown that forest cover and water bodies influence site use due to the need for drinking water and for roosting and feeding sites (Kurta, 2001; Fabianek *et al.*, 2011). We calculated the shortest distance between each recording station to the water surface (Ramalho *et al.*, 2021) and to major roads. We calculated our habitat variables (proportion of forest and water cover, and the shortest distance to a water source) and road variables in

1 <https://changements-climatiques.canada.ca/donnees-climatiques/#/donnees-climatiques-horaire>

2 <https://www.donneesquebec.ca/recherche/dataset/carte-ecoforestiere-avec-perturbations>

ArcGIS Pro (v3.0.0, Esri, USA). Road data were extracted from the Government of Quebec's road geobase³. Road width, a tested explanatory variable defined as the distance between the outer edge lines of the roadway (Roemer *et al.*, 2021), was measured using the ruler tool in Google Maps. Road width served as a proxy for habitat fragmentation rather than traffic intensity. Although average annual daily traffic data from the Ministère des Transports et de la Mobilité durable (MTMD) were initially considered, incomplete records for certain roads and years limited their reliability for our analyses. Consequently, nocturnal traffic intensity was estimated from acoustic recordings as an indirect proxy for vehicle passages, assuming that higher traffic volumes generate higher overall noise levels, even without precise counts of individual vehicles.

2.3.3 Acoustic Analysis

2.3.3.1 Bat Activity

Since the stationary survey method generates a large volume of data, it requires substantial time for acoustic analysis (Charbonneau *et al.*, 2023). We analyzed Anabat[®]'s bat recordings using both automated identification (auto-ID) and manual identification (manual-ID) of bat species.

We used the randomForest (RF) package in R (Liaw and Wiener, 2002) to build the bat call classification model. This automated technique for bat call identification showed good performance in bat acoustic analyses when trained on specific acoustic data (Zamora-Gutiérrez *et al.*, 2016; Lausen *et al.*, 2023). Our RF classification model was previously trained with recorded calls from a manually identified sound library composed of thousands of sonograms of bat calls from Quebec (Fabianek F., pers. comm.). Species or complexes were identified by statistically comparing the extracted acoustic parameters of the recorded sonograms with reference parameters from the above-mentioned sound library using the Extremely Randomized Trees classification algorithm available in R [package *ExtraTrees* v1.0.5 (Geurts *et al.*, 2006)]. Before extracting the acoustic parameters, we applied a filtering process (filter integrated into AnalookW[®]) to remove background noise and echoes from bat calls (Clement *et al.*, 2014). The chosen acoustic parameters for the RF classification model are summarized in APPENDIX D. The statistical comparison of

3 <https://www.donneesquebec.ca/recherche/dataset/debit-de-circulation/resource/9de14998-2e3b-4936-a587-2da4f3ddd3af>

sonograms generated a classification confidence index estimate (0.0 - 1.0) for each bat call classification; this was used as a basis for manually validating the reliability of the identifications (Russo and Voigt, 2016).

To determine an appropriate FR confidence index threshold to use for manual validation, we created a subset out of the auto ID call dataset based on the confidence index, ranging from 0.0 to 1.0, to obtain 10 randomly selected calls per 0.1 increment of confidence level, resulting in a total of 100 calls per species or complex of species to validate manually, yielding a total of 1,000 calls for the subsample. We considered satisfying a minimum threshold of 8 out of 10 calls correctly auto-ID (80% true positives or 20% false positives) for each increment of 0.1 in confidence level. False positives were mainly due to overlapping call characteristics among species. Confidence intervals below this 80% accuracy threshold were deemed unreliable, and all corresponding calls from the original dataset at those confidence levels were subsequently manually validated. The chosen range of confidence levels for which data were manually identified for each species or complex is shown in APPENDIX E.

Manual-ID was conducted by visually analyzing one-by-one recordings containing bat calls using Analoow[®]. When in doubt between a specific *Myotis* genus species, or between the big brown bat and the silver-haired bat, we grouped them respectively in the “Mysp” or “Epno” complexes to ensure scientific identification, parsimony, and accuracy. If a sonogram contained three bat calls or fewer, or the calls couldn’t be associated with any specific species, it was categorized as “Spp”. A total of 39,151 bat passes (37,886 bat files) were manually identified out of the complete set of 200,459 automatically generated bat files, 18.90% out of the full dataset (APPENDIX F). Manually validated calls were then reintegrated into the full dataset, replacing the corresponding low-confidence auto-ID calls identified as below the acceptable confidence threshold.

2.3.3.2 Traffic Noise

To characterize and isolate traffic noise within our acoustic dataset, we first selected a random subsample of recordings from multiple stations representing a range of acoustic environments. Using Audacity[®] v3.4.2 (Audacity, 2024), we manually listened to and identified all distinct noise types within these recordings. Once all major noise sources in the dataset were classified, we obtained a representative subsample totaling 6.93 GB, including examples of anthropophony, biophony, wind, rain, and silence. In the context of our acoustic analysis, we defined true positives as traffic-related sounds, including tire-road friction, engine noise, and associated vehicle-generated sounds. Conversely, false positives referred to any non-

traffic noise sources (i.e., wind or rain) that overlapped with traffic noise frequency ranges and could therefore be mistakenly detected as traffic noise during further analysis, where manual verification was not used.

We then performed Sound Pressure Level (SPL) analyses to determine the frequency bands most indicative of traffic noise, aiming to maximize true-positive detection while minimizing spectral confusion with non-traffic sounds that overlap in frequency, such as wind and rain. Visual examples of the two main types of traffic noise (cars and trucks), represented in spectrograms, and their respective peak amplitudes from frequency analysis, compared with other sources of noise with overlapping frequency bands (rain and wind), are provided in APPENDIX G.

Initially, we measured traffic noise levels using SPL analysis at 630.0 Hz, 793.7 Hz, and 1000.0 Hz. The literature supported this choice: Yang *et al.* (2023) found traffic noise spectra often exhibit peaks within the range of 50-80 Hz, 200-315 Hz, and 800-1250 Hz attributable to tire-road friction and related sources, and Saliunas and Volkovas (2014) identified dual SPL peaks in heavy-traffic environments at approximately 63 Hz and between 800-1600 Hz. However, due to high false-positive rates caused by overlapping geophonic noise (such as wind and rain), we refined our analysis to focus on a single frequency (793.7 Hz), which more reliably isolated traffic noise signals in our dataset than the previously selected multi-frequency approach. Although restricting road noise detection to a single frequency (793.7 Hz) was a reductive approach that excluded harmonics and transient acoustic peaks typically associated with other traffic-related sounds, such as honking or braking, this limitation did not affect our objectives. Our analysis specifically targeted tire-road friction noise as a proxy for traffic levels. Unlike horns or braking sounds, which occurred sporadically and did not systematically accompany vehicle passages, tire-road friction noise was consistently produced by moving vehicles and is a primary source of traffic noise (Chen *et al.*, 2021), which constituted a more reliable and representative indicator of traffic presence and noise intensity. The SPL analysis was linked to traffic noise and was considered a proxy for road traffic. This interpretation was supported by the observed linear relationship between traffic noise levels and the distance of each recording station from the nearest road, with higher traffic noise levels measured at shorter distances from roads (Figure 2.3).

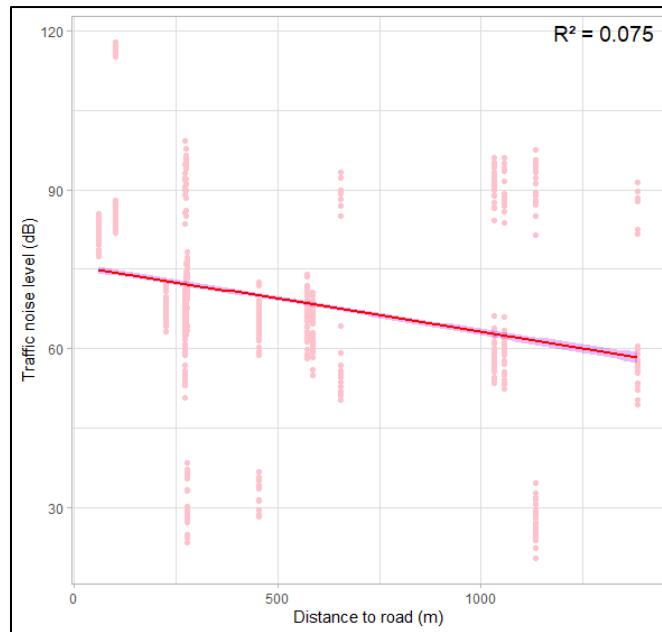


Figure 2.3 Linear relationship between distance to the nearest road and ambient noise, with a 95% confidence interval shaded in purple around the red regression line. The raw data is represented in pink points.

We extracted traffic noise sound levels from the soundscape recorded with the SM4[®], using the SPL analysis in Kaleidoscope Pro[®] version 5.6.4 (Wildlife Acoustics, Maynard, MA, USA). Acoustic analysis was conducted in “non-bat analysis mode” on a batch of .wav recordings, processing both input channels (left and right), corresponding to the left and right microphones on the SM4[®] recorders.

Signal parameters were configured so that only signals meeting the specified criteria of traffic noise were designated as detected and subjected to further analysis. We defined an acceptable frequency range for detection between 600 Hz and 1100 Hz, which included the previously identified target frequency of 793.7 Hz. The minimum length of detection was set to 6 - 60 seconds, corresponding to the typical time window of a passing car or truck. The maximum inter-syllable gap (i.e., the maximum allowable silent interval between parts of a detected signal) was set to 0 seconds, accounting for successive vehicle passages with no pause in between. We opted not to remove the DC offset, as it primarily affects power measurements at very low frequencies, and it should not affect analysis under normal conditions. The SPL analysis was carried out using a weighted band centered at 793.7 Hz, as previously justified. We selected the maximum SPL measurement using a 60-minute sampling period for each 1-hour .wav recording. To calibrate SPL

values relative to the standard reference pressure of 20 μPa , we applied a 94.0 dB adjustment to all level measurements. The SEL peak threshold (12) and SEL off threshold (3) above the mean were left at default settings. These chosen parameters for the SPL analysis are listed in APPENDIX H. (Wildlife Acoustics, 2018)

The resulting maximum SPL values were then aggregated across the night to produce a single mean SPL per night, an average of the highest traffic noise level recorded each hour, which was used in subsequent statistical analyses. We excluded all nights with recorded rainfall (> 0 mm) and wind speeds exceeding 5 km/h from the dataset before statistical analysis to reduce the number of low bat activity nights (Kunz, 1973; Fenton *et al.*, 1977; Fabianek *et al.*, 2011; Moore and Best, 2018; Charbonneau *et al.*, 2023), and minimize the risk of false positives due to high dB levels triggered by sounds such as heavy rainfall or windy nights in the SPL analysis. This filtering ensured that the resulting dB values were not confounded with loud geophonic noise such as rain or wind.

2.3.4 Statistical Analysis

For the statistical analysis, we first synchronized bat activity data with the corresponding traffic noise levels using the embedded timestamps from both Anabat[®] and SM4[®] recording units. The response variable was the bat activity characterized by the total number of bat passes per night detected by the recording device and subsequently identified through either auto-ID or manual-ID per bat species in each of the 14 recording stations across all 3 years of temporal replicates, resulting in 42 sampling events in total (14 recording stations \times 3 years = 42).

Several factors led to the exclusion of specific recording nights from the full dataset, resulting in a reduced dataset comprising 90 nights and 27,951 bat passes (APPENDIX F). Wind speed and precipitation levels were not included as variables in the model, as both were controlled for by removing windy and rainy nights from the reduced dataset (APPENDIX F). Other removed raw data also included recordings from dates associated with firework displays. Specifically, St. John the Baptist Day (June 24, year 2022 only), Canada Day (July 1, year 2022 only), U.S.A. Independence Day (July 4, years 2022 and 2023 only) and Bastille Day (July 14, year 2022 only) were associated with fireworks that produced clipping patterns capable of triggering false positives in the recordings when listened to manually. We removed all data from recording stations with night recordings that had a recording defect in which the SM4 recorded only 1 hour during the entire night (from 2023-07-30 to 2023-08-02, and 2023-07-05 to 2023-07-24), to avoid risking a false dB approximation during those nights. We removed one recording station (SCC2) from the

analysis because it was too close to a water purification plant (Figure 2.1), as periodic flow into a river near the microphones mimicked rain and overlapped with traffic noise.

We also limited our statistical comparisons to the big brown bat and the *Myotis* species nocturnal activity from our reduced dataset (APPENDIX F). These two groups of bat species represented ideal examples of contrasting ecological strategies: the highly urban-tolerant big brown bat lacks protection status, while the more sensitive and threatened *Myotis* species complex is more influenced by an anthropized habitat. We selected the big brown bat specifically, rather than the big brown bat/silver-haired bat complex, to isolate activity to a single species, as despite similar call structures, the latter differ in habitat preferences, potentially confounding results. In contrast, *Myotis* species were analyzed together because of their comparable habitat preferences. Thus, the big brown bat model was comprised of 6,528 bat calls. For the *Myotis* species model, a total of 2,597 bat calls were analyzed, corresponding to the combined number of calls from the little brown bat (494) and *Myotis* species complex (2,103) within the reduced dataset presented in APPENDIX F.

We evaluated the effects of traffic noise on the big brown bat and *Myotis* species activity using two generalized linear mixed-effect models (GLMM) with binomial negative distribution (`nbinom2`) and a log link function using the `glmmTMB` function [R package *glmmTMB* v1.1.11 (Brooks *et al.*, 2017)]. This choice was due to overdispersion in the initial model when fitting a basic GLMM with a Poisson distribution, a suitable solution to better accommodate highly overdispersed bat count data showing zero-inflated counts (Brooks *et al.*, 2017). To assess model fit, we calculated the marginal and conditional R^2 values for each model in the framework using the `r2_nakagawa` function, which is ideal for mixed-effects models with complex random-effect structures [R package *performance* v0.14.0 (Nakagawa *et al.*, 2017)].

The different tested models contained various fixed effects and random cross-effect variables that weren't all included in the final models (APPENDIX I). Each variable is listed and explained more extensively in Table 2.1 below.

Table 2.1 Tested fixed and random cross-effect variables in multiple models.

Variable	Description	Unit
Fixed effect		
Bat Activity	Nightly total bat passes per species. Varied between recording stations, species, and in time.	Bat passes
Traffic Noise	Nightly mean of maximum hourly traffic noise levels. Varied between recording stations and in time.	dB
Distance to Road	Distance to the nearest road for each recording station. Varied between recording stations but remained constant over time.	m
Road Width	Width (distance between both outer edge lines of the roadway) of the nearest road to each recording station. Varied between recording stations but remained constant over time.	m
Proportion of Forest	Proportion of ≥ 80 -year-old forest cover within a 400-m radius of each recording station. Varied between recording stations but remained constant over time.	%
Proportion of Water	Proportion of water cover within a 400-meter radius of each recording station. Varied between recording stations but remained constant over time.	%
Proportion of Habitat	Composite variable created by summing the proportions of forest and water cover within a 400-meter radius around each recording station. Varied between recording stations but remained constant over time.	%
Distance to Water	Distance to the nearest water body for each recording station. Varied between recording stations but remained constant over time.	m
Temperature	Nightly mean temperature. Varied over time, but identical spatially across all recording stations.	$^{\circ}\text{C}$
Relative Humidity Level	Nightly mean relative humidity level. Varied over time, but identical spatially across all recording stations.	%
Random cross-effect		
Station	Recording stations. A total of 14 recording stations were used in the study.	
Site	Each of the three study sites is associated with a different road type and includes 4 or 5 recording stations. The site with recording stations, starting with "SCR", located along the two-lane road. The site with recording stations, starting with "SCT", located along the one-lane road. The site with recording stations, starting with "SCC", located along the regional and collector roads.	
Date	Recording night.	
Device Installation Date	Night when the two recording devices were installed in the field and initiated recording at a specific recording station. Varied over time and between recording stations.	

We created a composite variable “Proportion of Habitat”, the result of the “Proportion of Forest” and “Proportion of Water” (APPENDIX J), to limit the number of spatial variables, since the small number of recording stations limited the number of spatial variables we could include. The proportions of forest, water, and habitat cover were used to assess the effectiveness of our study design, as we wanted to control for the effects of other landscape parameters to focus on roads. All fixed effects were scaled from 0 to 1 (except for the “Proportion of Habitat” variable, which was already between 0 and 1) so that their effects were comparable. “Station” and “Date” random cross-effects variables were chosen to optimize the reality of the experimental design to consider the non-independence between observations (Meyer *et al.*, 2024). Sampling dates were converted to the Date class using the `as.Date()` function.

Our dataset contained a strong temporal component due to an unbalanced experimental design in which installation dates and monitoring durations varied across recording stations, recording periods, and years. To reduce the risk of Type I errors, we accounted for the temporal structure of the data in the statistical model. Two random cross-effects, “Device Installation Date” and the interaction between “Device Installation Date” and “Date” (Device Installation Date: Date), were added to better capture the temporal variability in our data. Linking the installation date to the recording date created a unique temporal identifier for each recording period. This approach allowed the model to assume that recordings from the same period are more likely to be correlated with each other than with recordings from different periods. Using only the recording date as a random effect would not have adequately captured this dependence structure.

Given our limited number of fixed-effect variables, including Julian date or year as a fixed effect could cause overfitting. Rather than using the Julian date to test for seasonal patterns, by including temporal variables (Date, Device Installation Date, and Device Installation Date: Date) as random effects, the model captured nightly variation across recording stations, improving the estimation of fixed effects without consuming additional degrees of freedom. Year was not included as a factor in the statistical models, since we did not aim to quantify interannual variation, and because bat populations can vary between years due to natality and winter survival rates. The individuals present in a given year are not necessarily the same as those present in subsequent years.

We verified that explanatory variables were not strongly autocorrelated. Simple pairwise correlations were assessed using Pearson correlation tests using the function `cor()` from base R package, and multicollinearity was evaluated using Variance Inflation Factors (VIF) using the function `check_collinearity()`, suggesting no concern for collinearity in the model; diagnostics were computed (APPENDIX K). The tests showed no correlation between the variables. Pairwise correlations among predictors were generally low to moderate ($|r| \leq 0.51$), indicating no strong bivariate collinearity. Multicollinearity diagnostics indicated low correlation among predictors (all VIF < 2.5); hence, all variables were retained and included simultaneously in the same multivariable model.

Multiple models were tested and passed through a filtering decision process. First, we constructed full models that included variations in fixed and random effects for the big brown bat and *Myotis* species (APPENDIX I). We tested each model to verify whether it met statistical diagnostics using *post hoc* tests in the performance and Dharma packages (`check-overdispersion()`, `check_collinearity()`, `testZeroInflation()`, `testDispersion()`, `testUniformity()`). We also tested all models for temporal autocorrelation with `testTemporalAutocorrelation()`, and spatial autocorrelation with `testSpatialAutocorrelation()` (APPENDIX I). Then, we selected a single model that satisfied all diagnostic criteria and was consistently applied to both bat groups to assess differences in bat activity in response to road characteristics. The final model structure applied to the reduced dataset of 760 observations (n=760), which comprised 90 recorded nights during the summers of 2021, 2022, and 2023, and was implemented separately for the big brown bat and for *Myotis* species, was as follows: ***Bat Activity ~ Traffic Noise + Distance to Road + Road Width + Temperature + Relative Humidity Level + Proportion of Forest + Proportion of Water + (1|Station) + (1|Date) + (1|Device Installation Date) + (1|Device Installation Date: Date)***

We did residual graphs for each model with the `simulateResiduals()` function to ensure statistical diagnostics were respected within each model and are available in APPENDIX L and APPENDIX M. We constructed the models, and figures, and performed model diagnostics in R 4.5.1 (R Core Team, 2025), using the functions `nlme` [package `nlme` v3.1.168 (Pinheiro *et al.*, 2025)], `lme4` [package `lme4` v1.1-37 (Bates *et al.*, 2015)], `performance` [package `performance` v0.14.0 (Lüdtke *et al.*, 2021)], `magrittr` [package `magrittr` v2.0.3 (Bache and Wickham, 2014)], `dplyr` [package `dplyr` v1.1.4 (Wickham *et al.*, 2014)], `ggplot2` [package `ggplot2` v3.5.2 (Wickham, 2016)], `DHARMA` [package `DHARMA` v0.4.7 (Hartig, 2016)], and `knitr` [package `knitr` v1.50 (Xie, 2016)].

2.4 Results

2.4.1 Overall Insectivorous Bat Activity

We recorded a total of 27,951 bat passes at the 14 sampled recording stations during 90 nights of recording under good recording and weather conditions in the summers of 2021, 2022, and 2023, within the reduced dataset (APPENDIX F). The highest number of bat passes was made by the big brown bat/silver-haired bat species complex (n = 14,175; 50.71%), followed by the big brown bat (n = 6,528; 23.35%). The lowest number of bat passes was made by the hoary bat (n = 102; 0.36%), the eastern red bat (n = 115; 0.41%), and the tri-coloured bat (n = 67; 0.24%). We were unable to identify bat species for 2,258 passes (8.08%). The northern long-eared bat and the eastern small-footed bat were present in the entire dataset but were categorized as *Myotis* species complex due to few or no occurrences. The number of bat passes for each species in the reduced dataset is summarized in Table 2.2 below.

Table 2.2 Results of acoustic inventories of chiropterans conducted during summers 2021 to 2023 at 14 recording stations near the Brome-Missisquoi Municipal County Region in southeastern Quebec (Canada). The results (i.e., number of bat passes / recording station) were presented by species or species complex per recording station and then added together in the last column.

Species or complex of species recorded			Recording station code														Total bat passes
Scientific name	Common name	Code	SCC1	SCC3	SCC4	SCC5	SCR1	SCR2	SCR3	SCR4	SCR5	SCT1	SCT2	SCT3	SCT4	SCT5	
<i>Eptesicus fuscus</i>	Big brown bat	Epfu	40	269	275	1386	128	49	64	343	2265	31	131	52	14	1481	6528
<i>Lasionycteris noctivagans</i>	Silver-haired bat	Lano	16	98	26	107	24	27	151	99	1431	11	5	6	3	105	2109
<i>Lasiurus cinereus</i>	Hoary bat	Laci	0	4	4	10	2	4	9	3	35	0	0	2	14	15	102
<i>Lasiurus borealis</i>	Eastern red bat	Labo	4	9	1	14	1	1	2	16	52	0	2	1	0	12	115
<i>Perimyotis subflavus</i>	Tri-colored bat	Pesu	1	0	0	3	1	1	1	26	28	0	0	0	0	6	67
<i>Myotis lucifugus</i>	Little brown bat	MyLu	35	51	9	21	143	7	6	75	80	0	5	4	0	58	494
<i>E. fuscus / L. noctivagans</i>	Big brown or silver-haired bat	Epno	200	1119	443	2781	311	149	351	2524	3682	111	60	305	17	2122	14175
<i>Myotis</i> spp.	Bats of the <i>Myotis</i> genus	MySp	75	239	35	72	548	59	23	227	458	16	78	60	66	147	2103
Spp.	Unidentifiable bat species	Spp	44	222	102	212	85	46	45	354	847	31	27	46	12	185	2258
Total			415	2011	895	4606	1243	343	652	3667	8878	200	308	476	126	4131	27951

* = Bat species complex.

In each study year, the big brown bat and the big brown bat/silver-haired species complex activity were much greater than that of *Myotis* species and other species and complexes (Figure 2.4). The big brown bat had a mean of 7.05 (95% CI: [4.70, 9.41]) bat passes nightly in 2021, 7.0 (95% CI: [5.15, 8.78]) in 2022, and 13.91 (95% CI: [7.91, 19.91]) in 2023, while *Myotis* species were not even half as frequent with a mean of 3.50 (95% CI: [1.24, 5.77]) passes in 2021, 1.97 (95% CI: [1.60, 2.33]) in 2022, and 2.89 (95% CI: [1.93, 3.84]) in 2023 (Figure 2.4).

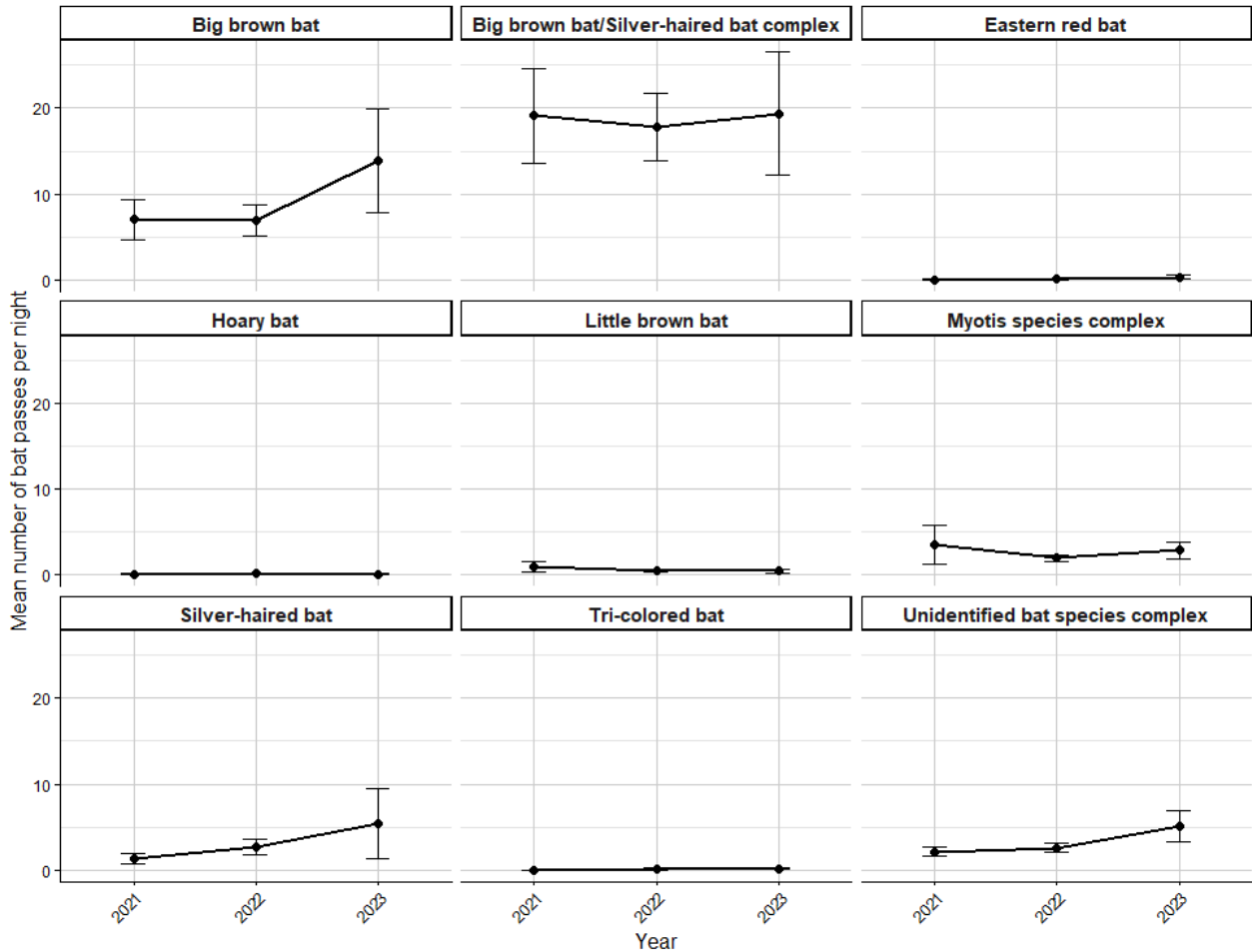


Figure 2.4 Mean bat activity (number of bat passes per night) per species and species complex of bats by year with 95% confidence intervals.

Spatially, the mean number of big brown bat/silver-haired bat species complex passes per night was also higher than that of the other bat species and complexes for most recording stations (Figure 2.5). Most recording stations positioned along the one-lane road (SCT1, SCT2, SCT3, and SCT4) and one recording station along the two-lane road (SCR2) showed a lower overall bat activity (Figure 2.5). The big brown bat had a higher mean activity at the recording station SCR5, followed by SCT5 and SCC5, and the least activity at the recording stations SCT4, SCT1, SCT3, SCR2, and SCR3 (Figure 2.5). *Myotis* species exhibited the highest mean activity at recording stations SCR1 and SCR5 and the lowest at SCR3, SCT1, and SCC4 (Figure 2.5). To better visualize each species separately, see APPENDIX N. The recording station with the highest bat activity for all studied bats was SCR5, while the one with the lowest activity was SCT4. Multiple recording stations starting with “SCT”, all located close to the one-lane road, had lower overall bat activity.

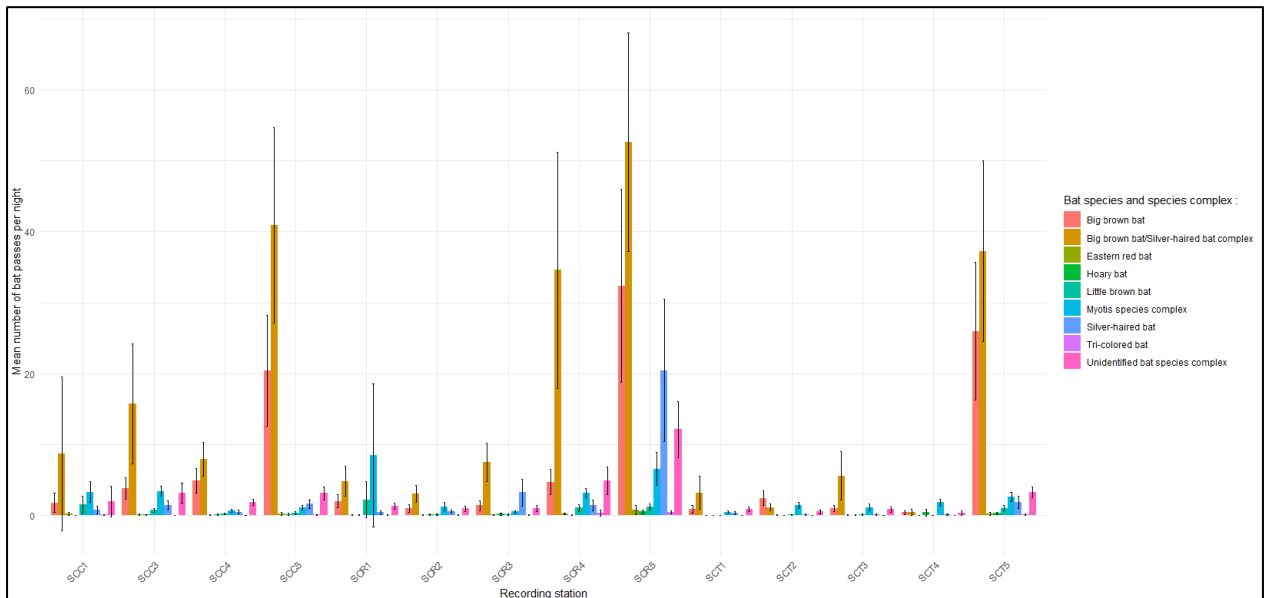


Figure 2.5 Mean bat activity (number of bat passes) per night per bat species and species complex by recording station from 2021 to 2023 with 95% confidence intervals.

Throughout each recording season, when comparing with mean traffic noise levels, we see that mean bat activity for all species combined seemed overall higher in the beginning of the season and diminished towards the end (Figure 2.6).

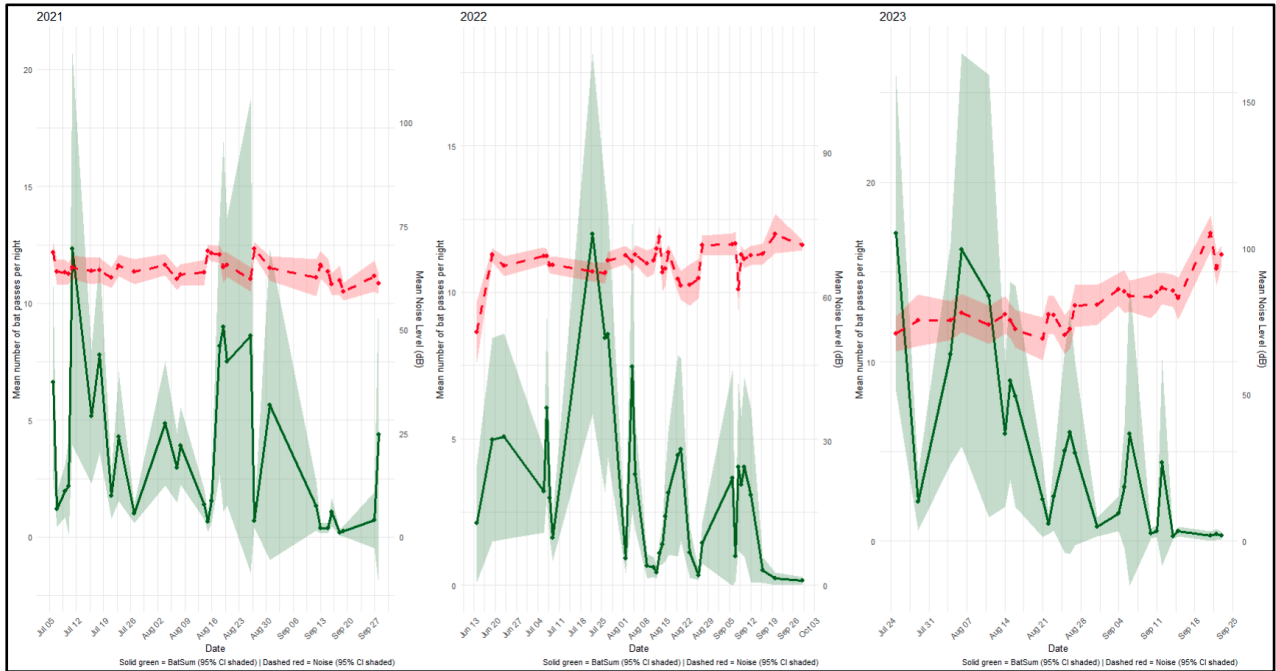


Figure 2.6 Bat activity (mean number of bat passes per night), represented by a solid green line, and mean traffic noise level (dB), represented by a dashed red line, in time during the years 2021, 2022, and 2023. Solid or dashed lines represent tendency lines, and shaded ribbons around them represent 95% confidence intervals.

2.4.2 Road Effects on Studied Species Activity

Our best models explaining the activity of the big brown bat and *Myotis* species (Table 2.3) accounted for spatial and temporal variation. For the big brown bat model, the marginal R^2 was 0.33, and the conditional R^2 was 0.89. For the *Myotis* species model, the marginal R^2 was 0.24, while the conditional R^2 was 0.76. This suggests that while the fixed effects accounted for a moderate proportion of the variability in the response variable, the inclusion of random effects substantially improved the explanatory power of both models. We defined statistical significance as $p < 0.05$ and considered results with $0.05 < p < 0.10$ to be marginally significant.

Table 2.3 Results of the final chosen generalized linear mixed-effects model (GLMM) with negative binomial distribution testing the effect of the road on the total number of big brown bats and *Myotis* species bat passes nightly in all recording stations. Sample sizes were $n = 760$ for the big brown bat and for *Myotis* species models for 90 nights. Significant values are in bold characters.

Big brown bat activity ~				
Fixed effects	Estimate (± s.e.)	z value	Pr (> z)	Signif. Codes
Intercept	-0.43 (± 1.27)	-0.34	0.74	ns
Traffic Noise ^{Sc}	-2.86 (± 0.76)	-3.76	0.0001	***
Distance to Road ^{Sc}	-2.58 (± 1.44)	-1.79	0.07	.
Road Width ^{Sc}	0.30 (± 1.18)	0.25	0.80	ns
Temperature ^{Sc}	6.50 (± 0.80)	8.18	3e-16	***
Relative Humidity Level ^{Sc}	-1.67 (± 0.75)	-2.23	0.03	*
Proportion of Forest (r = 400 m) ^{Sc}	-0.61 (± 1.15)	-0.53	0.60	ns
Proportion of Water (r = 400 m) ^{Sc}	0.24 (± 0.96)	0.25	0.80	ns
Random effects	Variance (± s.d.)			
Station	1.04 (± 1.02)			
Date	1.11 (± 1.05)			
Device Installation Date	1.51 (± 1.23)			
Device Installation Date: Date	0.08 (± 0.28)			
Myotis genus bat activity ~				
Fixed effects	Estimate (± s.e.)	z value	Pr (> z)	Signif. Codes
Intercept	3.67 (± 0.78)	4.70	3e-06	***
Traffic Noise ^{Sc}	-2.25 (± 0.64)	-3.51	0.0004	***
Distance to Road ^{Sc}	-2.00 (± 0.89)	-2.25	0.02	*
Road Width ^{Sc}	0.63 (± 0.73)	0.86	0.39	ns
Temperature ^{Sc}	-0.47 (± 0.33)	-1.40	0.16	ns
Relative Humidity Level ^{Sc}	-0.83 (± 0.34)	-2.44	0.01	*
Proportion of Forest (r = 400 m) ^{Sc}	-1.46 (± 0.71)	-2.07	0.04	*
Proportion of Water (r = 400 m) ^{Sc}	0.26 (± 0.60)	0.43	0.66	ns
Random effects	Variance (± s.d.)			
Station	0.37 (± 0.61)			
Date	0.06 (± 0.24)			
Device Installation Date	0.93 (± 0.96)			
Device Installation Date: Date	0.18 (± 0.42)			

^{Sc}: Standardized variables on a scale of 0 to 1.

Signif. Codes : *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; . $p < 0.1$; ns $p \geq 0.1$.

To facilitate interpretation of model outputs, we derived predicted values using the *ggpredict* function from the *ggeffects* package in R. This function generated model-based predictions rather than descriptive statistics computed directly from the raw data. In our case, predictions were derived from a GLMM fitted with a negative binomial distribution (*nbinom2*) using the *glmmTMB* package. The reported values represent the expected number of bat passes per night, adjusted for all other road-related predictors (traffic noise, distance to road, and road width) included in the model while accounting for physical and temporal variations.

Increased traffic noise levels were associated with a significant decrease in bat activity for the big brown bat ($\beta = -2.86$, 95% CI: [-4.36, -1.37], $p = 0.0001$) and *Myotis* species ($\beta = -2.25$, 95% CI: [-3.50, -0.99], ($p = 0.0004$) in our models (Figure 2.7).

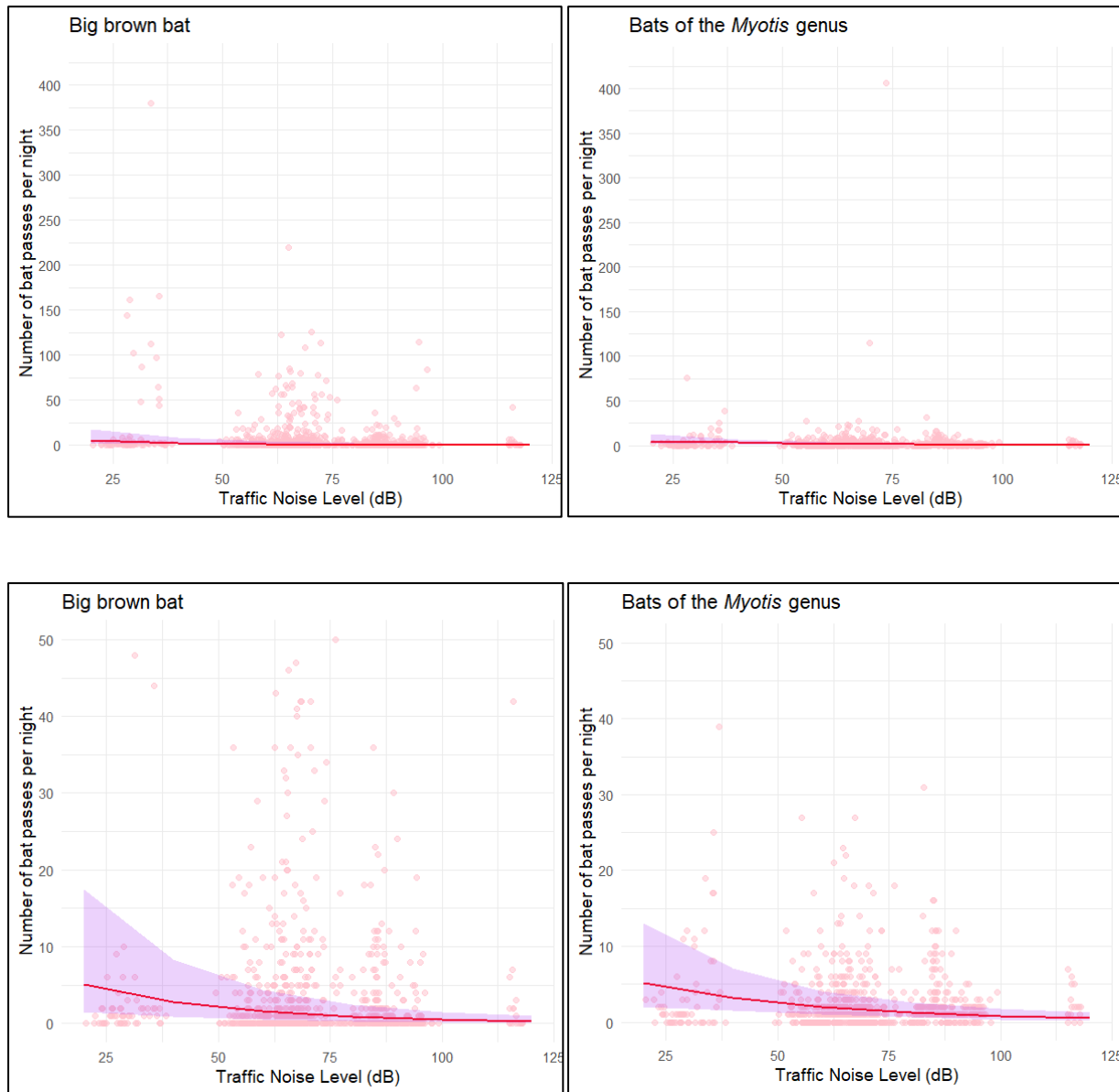


Figure 2.7 Model prediction of the effect of traffic noise on the nocturnal activity of the big brown bat and bats of the *Myotis* genus in southeastern Quebec ($n = 760$). The figures were truncated at a y-axis value of 50 to improve visualization of the regression curve.

The effect of distance from the recording station to the closest road was marginally significant for the big brown bat ($\beta = -2.58$, 95% CI: [-5.41, 0.25], $p = 0.07$) and significant for *Myotis* species ($\beta = -2.00$, 95% CI: [-3.74, -0.26], $p = 0.02$), with lower bat activity farther away from the road for both species.

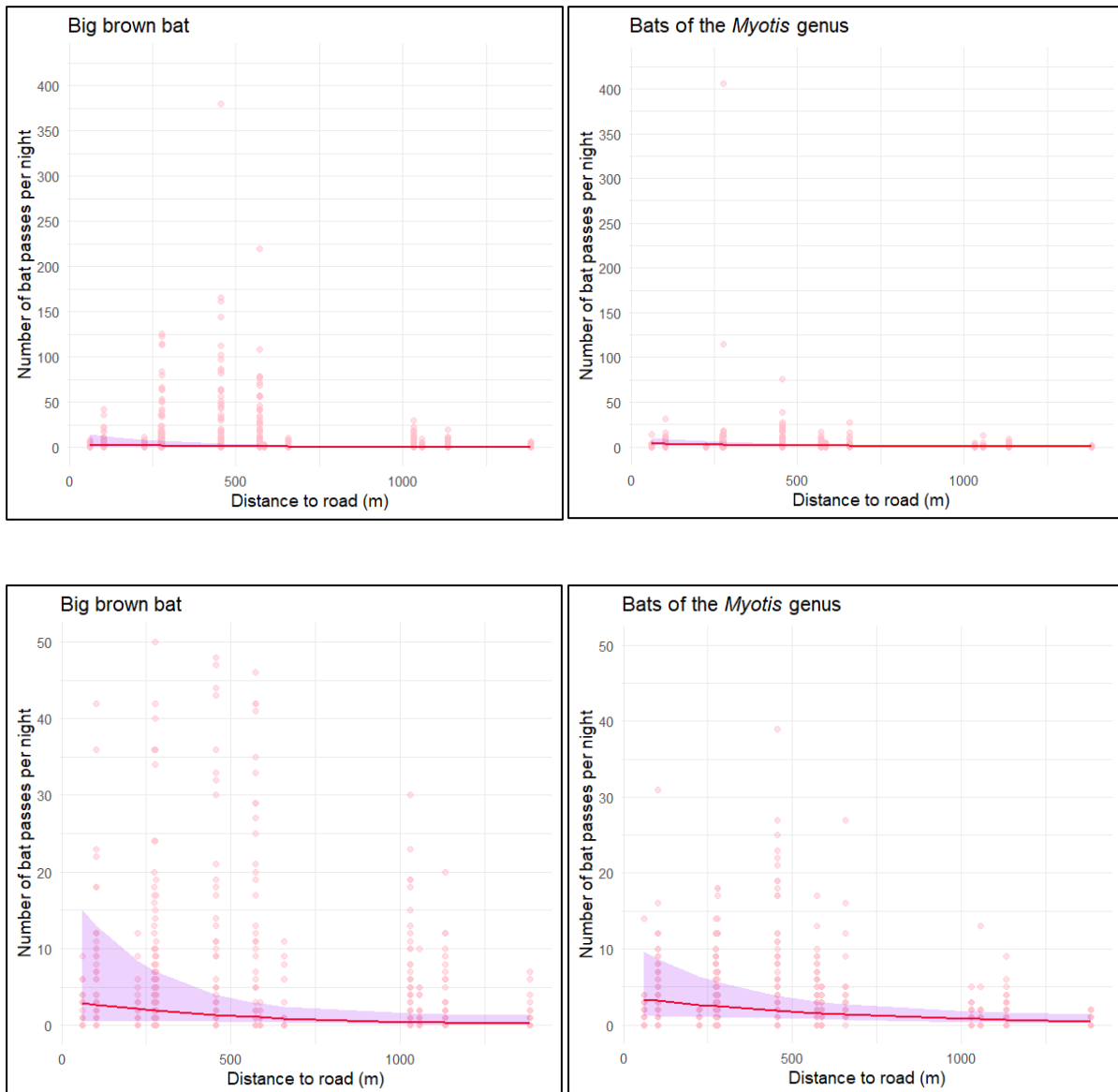


Figure 2.8 Model prediction of the effect of distance to nearest road on the nocturnal activity of the big brown bat and bats of the *Myotis* genus in southeastern Quebec ($n = 760$). The figures were truncated at a y-axis value of 50 to improve visualization of the regression curve.

There were no correlations between road width and bat activity for both the big brown bat ($\beta = 0.30$, 95% CI: [-2.01, 2.61], $p = 0.80$) or *Myotis* species ($\beta = 0.63$, 95% CI: [-0.81, 2.06], $p = 0.39$).

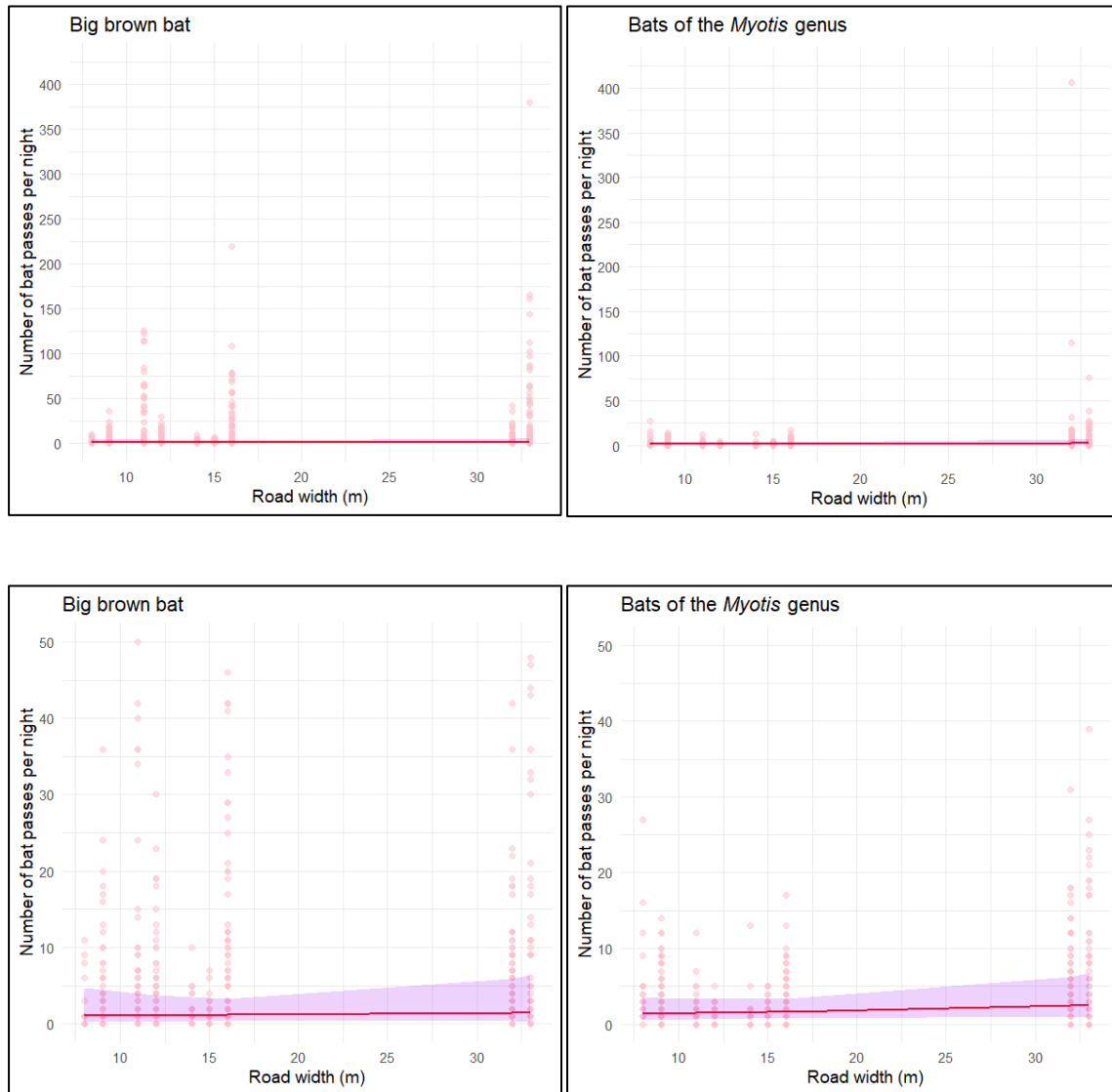


Figure 2.9 Model prediction of the effect of road width on the nocturnal activity of the big brown bat and bats of the *Myotis* genus in southeastern Quebec ($n = 760$). The figures were truncated at a y-axis value of 50 to improve visualization of the regression curve.

2.4.3 Habitat and Meteorological Effects on Studied Species

For the big brown bat, temperature was the most significant meteorological variable influencing bat activity, with a positive effect estimate of 6.50 ($\beta = 6.50$, 95% CI: [4.94, 8.06], $p < 0.0001$), indicating higher activity levels at elevated temperatures (Table 2.3). Both the big brown bat ($\beta = -1.67$, 95% CI: [-3.14, -0.20], $p = 0.03$) and *Myotis* species ($\beta = -0.83$, 95% CI: [-1.49, -0.16], $p = 0.01$) exhibited significantly reduced activity at higher humidity levels (Table 2.3). The big brown bat ($\beta = 0.24$, 95% CI: [-1.65, 2.12], $p = 0.80$) and the *Myotis* genus bat ($\beta = 0.26$, 95% CI: [-0.91, 1.43], $p = 0.66$) activity didn't change with the proportion of water cover. The proportion of forest cover had no significant effect on big brown bat activity ($\beta = -0.61$, 95% CI: [-2.87, 1.66], $p = 0.60$), but it was significantly negatively associated with *Myotis* species activity ($\beta = -1.46$, 95% CI: [-2.85, -0.07], $p = 0.04$).

2.5 Discussion

2.5.1 Effect of Traffic Noise

We predicted that bat activity would decrease with increasing traffic noise (Schaub *et al.*, 2008). Consistent with our hypothesis, our results show a significant negative correlation between bat activity and traffic noise for both the big brown bat and *Myotis* species (Table 2.3), with bat activity decreasing with increasing noise levels (Figure 2.7). It has been suggested that the effect of road noise on bats is not due to echolocation masking but rather to an aversive stimulus that leads to an avoidance response (Luo *et al.*, 2015). An earlier study also found that search time when hunting for prey increased by a factor of five in noisy conditions near a highway (Siemers and Schaub, 2011). This avoidance may thus be due to reduced foraging efficiency, as traffic noise interferes with their passive listening to their environment (The California Department of Transportation, 2016).

Our study suggests that nights with higher noise intensity are correlated with reduced bat activity. However, it remains unclear whether bats exhibited immediate avoidance behavior at the precise moment a vehicle passes by. Although avoidance behavior by commuting bats in response to vehicles has been observed (Zurcher *et al.*, 2010), integrating behavioral observations with long-term field-based studies like ours is needed. It could be achieved by using thermal video recording to characterize bat flight paths (Claireau *et al.*, 2021). Overall activity for all bat species was lower at the recording stations near the one-lane road, likely because this road was the main transportation route to the U.S.A. before a new highway was built, resulting in higher traffic and traffic noise levels. The highway construction project in the study area began in the summer of 2020 and ended in the summer of 2025, when it became operational (MTMD, 2024). We did not consider traffic noise along the constructed highway in our study, since the highway became operational after our study ended (MTMD, 2024). However, the highway construction may have influenced bats at nearby recording stations by generating various construction-related noises, which could potentially disrupt their daytime rest (The California Department of Transportation, 2016).

2.5.2 Effect of Road Proximity

Our results on the effect of road proximity did not support our second hypothesis, as both bat species groups showed higher activity near roads. We predicted that bat activity would decrease with road proximity (Kitzes and Merenlender, 2014; Claireau *et al.*, 2019a). Our results, however, show the opposite effect: a significant negative correlation between *Myotis* species activity and distance to roads, and a marginally significant negative correlation for the big brown bat (Table 2.3). Although road noise was

generally higher at recording stations located near roads (Figure 2.3), based on our results, the effect of noise on bats did not appear to be linked to distance from the road, since bat activity was negatively correlated with both noise and distance from the road (Figure 2.8).

We suggest that a distinction between spatial and temporal scales may explain these seemingly contradictory behaviors. Higher bat activity near roads during calm nights may reflect habitat preferences, as bats may use roads as flyways. However, bat activity decreases with increasing noise intensity, indicating a temporal avoidance response on nights with higher noise levels. In the literature, Ramalho *et al.* (2021) found similar results for open-space aerial insectivorous bats, observing a significant negative correlation between distance from roads and activity across the three road types they tested. They suggested that open-space foraging bats used roads as flyways to reach other hunting sites (Ramalho *et al.*, 2021). This may explain the foraging behavior of the big brown bat in our study, an open-space aerial insectivorous bat (Ford *et al.*, 2005). However, it seems unlikely that this explanation would apply to the *Myotis* species, as they prefer to fly and hunt in closed forests (Charbonneau *et al.*, 2023). Future studies and compensation measures should separate temporal and spatial factors to better understand the underlying interactions between bat activity, noise, and distance from the road.

2.5.3 Effect of Road Width

We predicted that nocturnal bat activity should decrease as road width increased (Berthinussen and Altringham, 2012). However, our results were also inconsistent with this hypothesis, as there were no correlations between road width and bat activity for either the big brown bat ($p = 0.80$) or the *Myotis* species ($p = 0.39$) (Table 2.3 and Figure 2.9). This result might be explained by missing data from two key stations (SCR2 and SCR3) that couldn't be included in the third year (2023) since planned access was not permitted by the construction authority (Figure 2.1). Both stations were located along the two-lane road, which represented the widest road category in the study area (APPENDIX A). Notably, 2023 was characterized by particularly high activity of the big brown bat as seen in Figure 2.4. However, given $p = 0.80$, it seems very unlikely that adding two stations would make the results significant for the big brown bat, or even for the *Myotis* species ($p = 0.39$). The absence of these stations in 2023 resulted in an unbalanced representation of the widest road category across years, thereby introducing potential bias in interannual comparisons; comparisons among years should be interpreted with caution. The reduced representation of the widest road category in 2023 likely decreased the statistical power to detect an effect of road width on bat activity. We therefore used a GLMM to accommodate the unbalanced datasets;

however, even after adding specific time-related variables (Device Installation Date: Date) to our models to better capture the temporal variability of our data, we still did not observe an effect.

2.5.4 Difference of Nocturnal Bat Activity Between Studied Species

We expect bat activity to vary between the two chosen bat groups, as they differ in their hunting habitat preferences (Fenton, 1990; Threlfall *et al.*, 2011; Russo-Petrick and Root, 2023). Thus, we predicted the average total nocturnal activity of the big brown bat would be higher than that for *Myotis* species, since the latter group is more vulnerable to the negative effects of roads and habitat fragmentation (Schaub *et al.*, 2008), and more sensitive to environmental variation (Gili *et al.*, 2020). Overall, there was a higher total number of bat passes of big brown bats (n = 6,528; 23.36%) than *Myotis* species (n = 2,597; 9.29%) (Table 2.2). The stationary survey method is more sensitive to small bat species when placed in a forested habitat (Charbonneau *et al.*, 2023), and even though every recording station was placed at forest edge, the proximity of urban, peri-urban habitats, agricultural fields and roads could explain why there was higher big brown bat activity (Fabianek *et al.*, 2011; Lehrer *et al.*, 2021). Another study in Quebec by Faure-Lacroix *et al.* (2019) found decreased detection of *Myotis* species, which had likely declined after white-nose syndrome, notably the little brown bat (Frick *et al.*, 2010; Dzal *et al.*, 2011). Throughout all three years of the study (Figure 2.4), and across the recording stations (Figure 2.5), we also see that the big brown bat had a generally higher mean activity than the *Myotis* species. One recording station (SCR5), with consistently high bat activity for both the big brown bat and *Myotis* species (APPENDIX N), was located near the Philipsburg Bird Sanctuary. Sanctuaries and protected areas have previously been associated with a higher bat activity (Tena and Tellería, 2022), even near road edges (Ramalho *et al.*, 2021).

2.5.5 Landscape and Meteorological Conditions

The proportion of forest and water cover didn't significantly affect big brown bat activity, which may be understandable, since big brown bats tend to prefer open-space habitats (Fabianek *et al.*, 2011; Lehrer *et al.*, 2021), so their activity was not influenced much by the forest cover component of the habitat variable. There are, however, surprising results for the *Myotis* species, as their activity seemed also significantly negatively correlated with the proportion of forest cover, with a negative effect estimate of -1.46 ($\beta = -1.46$, 95% CI: [-2.85, -0.07], $p = 0.04$) (Table 2.3). This result should not be interpreted as meaning that habitat features are not important. Indeed, maintaining connectivity within farmland landscapes plays a critical role in supporting bat activity, as both short- and long-range echolocating bats exhibited higher activity levels in well-connected landscapes (Frey-Ehrenbold *et al.*, 2013).

More importantly, the model for the big brown bat had a marginal R^2 of 0.33 and a conditional R^2 of 0.89. In contrast, the model for *Myotis* species yielded a marginal R^2 of 0.24 and a conditional R^2 of 0.76. These results suggest that, for both species groups, the fixed effects associated with road variables explained only a moderate portion of the variability in bat activity. In contrast, a large share of the variance was attributable to site- and time-related random effects, highlighting their strong influence and the importance of accounting for them in the models. The weak correlations with landscape variables and distance to the road suggest that important explanatory variables may be missing from the analysis. The study's scale may explain our results on bat activity related to road and forest cover variables. Russo-Petrick and Root (2023) found that, at the local scale, bat activity was positively associated with canopy cover, but at the landscape scale, it was positively associated with roads and open natural habitats. More specifically, some variables might be strongly associated with bat activity at 100 m but not at 500 m, and the opposite for other variables. Maybe the road and landscape variables in our study would have had different effects on bat activity at different scales; hence, it is important to use multiple spatial scales (Gallo *et al.*, 2018; Russo-Petrick and Root, 2023). The decrease in activity observed with increasing distance from roads may also have been an effect of the study site, since the dominance of the surrounding agricultural landscape might influence bat activity, as Ramalho and Aguiar (2020) found that, in open habitats, bats tend to forage near roads. The forest-agricultural field interfaces in the study area may have increased bat use of roads, as bats are known to preferentially utilize open habitat types of this kind (Jantzen and Fenton, 2013). Finally, the limited number of recording stations constrained the number of spatial variables we could include, as we wanted to avoid overfitting, which could accentuate the effect of the study site.

Temperature had a significant positive effect on big brown bat activity ($\beta = 6.50$, $p < 0.0001$), which is consistent with the literature, which states that bats exhibit higher overall activity on warm nights (Johnson *et al.*, 2011; Ramos Pereira *et al.*, 2022). This result could also be explained by the fact that high temperatures, coupled with light reflected from asphalt, can attract insects to roads (Muñoz *et al.*, 2015). This would then increase the activity of bats that hunt in open habitats, which could already be flying near roads (Ramalho *et al.*, 2021), such as the big brown bat.

Relative humidity also had a significant negative effect on big brown bat activity ($p = 0.03$) as well as on bats of the *Myotis* genus ($p = 0.01$). Again, this is consistent with the literature, as insectivorous bats tend to have lower activity levels in higher humidity (Rodríguez-San Pedro *et al.*, 2024). This could be because,

as humidity increases, bats tend to lower the frequency and lengthen the duration of their echolocation calls (Chaverri and Quiros, 2017); hence, there were fewer bat calls and bat activity. However, this might also be due to the 400 m scale we used in our research model, as the humidity variable at a different scale might have had a positive effect on bat activity (Russo-Petrick and Root, 2023).

Also, the construction highway, located between the recording stations and the tested one-lane road (Figure 2.1), caused habitat destruction, as the agricultural fields near the recording stations were excavated. Newly constructed highways significantly impact the environment, leading to reduced vegetation and moisture and increased land surface temperatures (Feng *et al.*, 2021). This unused highway during the sampling period might have affected bat activity results. However, we don't know whether it caused habitat fragmentation or any barrier effect, since there was no night traffic.

2.5.6 Limitations

This study had certain limitations that point to valuable avenues for future research.

First, a larger number of recording stations would have been necessary to develop a more robust spatial model with fewer site effects, since the limited sample size, combined with strong site-level dependencies, affected the observed outcomes. This limited number of recording stations constrained the number of variables that could be included in the model, with two recording stations (SCR2 and SCR3) missing during the 2023 sampling period. This limitation restricted our ability to incorporate additional spatial variables within the model. Furthermore, all recording stations were located at forest edges, which prevents our study from disentangling the effects of road proximity from those associated with edge habitats. As forest edges represent favorable foraging habitats for bats (Jantzen and Fenton, 2013), part of the observed activity patterns may therefore be related to edge characteristics rather than to roads themselves. Thus, increasing the sampling effort in future studies to address this limitation would not only strengthen the spatial modeling framework but also allow the use of additional spatial variables, such as Enhanced Vegetation Index, Leaf Area Index, canopy cover or vegetation height (Ramalho and Aguiar, 2020), to better assess the potential influence of edge effects on bat activity, as road impacts on bat activity are strongly influenced by landscape features (Ramalho and Aguiar, 2020).

Second, the SPL analysis wasn't efficient at analyzing traffic noise on windy and stormy nights, but this should not be an issue, since bats aren't active on nights with severe weather (Fabianek *et al.*, 2011; Moore

and Best, 2018). Nonetheless, employing an artificial neural network to automatically detect and classify distinct types of anthropogenic noise (Grinfeder *et al.*, 2022) associated with road use, such as engine humming, acceleration on access roads, motor noise, honking, and tire screeching, would improve future acoustic analyses. This approach would help us retain recordings collected during rainy or windy conditions by separating traffic-related signals from geophonic noise, while also testing whether bats respond differently to specific categories of road-related noise. In addition, it would enable the inclusion of nights following rainfall events, during which road traffic noise exhibits altered spectral characteristics. On wet road surfaces, rolling noise is amplified, and its spectral signature is modified (Alonso Fernández *et al.*, 2015), representing a distinct category of road-related noise that should also be specifically identified and classified. We tried using the open-source BirdNET classification model to do that (Kahl *et al.*, 2021). However, it proved ineffective at correctly classifying road noise, as this type of noise was likely underrepresented in their training database, which was mainly designed to identify bird sounds. We would need to build our own model or find an open-source model for identifying road noise specifically, as this strategy could effectively exclude other sources of noise from the acoustic dataset, retaining only traffic-related sounds, and advancing future research on the effects of traffic noise on bats.

Third, a further study on the long-term effects at the same recording stations should include an assessment of the buzz ratio (i.e., the number of feeding buzzes, specific echolocation calls associated with prey capture, per bat pass), to evaluate foraging activity (Vaughan *et al.*, 1996; Yantén *et al.*, 2022). This approach could provide insights into the influence of roads on bats' preferred foraging habitats (Ramalho *et al.*, 2021).

Fourth, human land use (including road construction) has been implicated in arthropod declines across both grassland and forest ecosystems (Seibold *et al.*, 2019), which could then negatively impact insectivorous bats, as species such as the big brown bat and the little brown bat, who rely on a seasonally diverse assemblage of arthropod prey (Maslo *et al.*, 2022). However, a study by Meyer *et al.* (2024), also conducted in southeastern Quebec, found that differences in arthropod abundance did not necessarily affect bat activity. Therefore, we suggest incorporating analyses of arthropod abundance and diversity into field bat studies to provide deeper insight into the mechanisms underlying road-related ecological cascades.

Fifth, light pollution should be considered in future studies, as some bat species in Quebec are known to forage for insects near artificial light sources such as lamp posts (Grindal *et al.*, 1999; Ford *et al.*, 2005; Menzel *et al.*, 2005; Prescott and Richard, 2013). Therefore, light from nearby houses, streetlights near certain recording stations, or overall light pollution may influence bat activity and should be considered in future road research.

2.6 Conclusion

Our study examined the impact of road characteristics to determine whether bats exhibit road-avoidance behavior that depends on traffic noise, road proximity, or road width. Our results on the influence of traffic noise on the big brown bat and on *Myotis* species were consistent with our hypotheses, such that bat activity decreases with traffic noise. However, the studied species did not respond as expected to distance from roads and road width, as they showed slightly higher activity closer to roads, and road width had no effect on their activity. Our study shows the need to mitigate the impact of roads to protect insectivorous bat habitat (Jaureguiberry *et al.*, 2022; Richardson *et al.*, 2023). Among the studies examining the effects of roads on bats, to our knowledge, none have yet provided a temporal and spatial analysis of how various road characteristics influence bat activity and their use of newly anthropogenic habitats in North America. The unique experimental conditions of this study offer valuable insight for future road development projects. Incorporating mitigation measures into road construction is necessary to mitigate negative impacts and support conservation efforts for these vulnerable species.

2.7 Credit Authorship Contribution Statement

Teodora Stan: Conceptualization, Data curation and analysis, Methodology, Visualization, Writing – original draft, Review & editing. **François Fabianek:** Conceptualization, Data curation and analysis, Supervision, Validation, Writing – Review & editing. **Daniel Kneeshaw:** Funding acquisition, Project administration, Resources, Supervision, Validation, Writing – Review & editing.

2.8 Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

2.9 Data Availability

Data will be made available on request.

CHAPITRE 3

CONCLUSION GÉNÉRALE

Afin d'identifier quelles caractéristiques de la route ont un effet sur les populations de chauves-souris insectivores, nous avons effectué une étude de terrain dans la région du sud-est du Québec en utilisant une approche à l'échelle du paysage sur plusieurs tronçons routiers par l'acquisition de données empiriques sur trois étés (2021-2023). Les résultats de cette recherche ont confirmé l'hypothèse de départ disant que l'activité des chauves-souris diminuerait avec l'augmentation du bruit routier. Il était aussi attendu que l'activité des chauves-souris diminue à proximité des routes (Kitzes et Merenlender, 2014 ; Claireau *et al.*, 2019a). Pourtant, nos résultats ont montré l'existence d'une corrélation négative significative entre la distance à la route et l'activité des chauves-souris du genre *Myotis* et légèrement significative pour les grandes chauves-souris brunes. De plus, nous n'avons pas trouvé de corrélation entre la largeur de la route et l'activité des chauves-souris. La proportion de couverts forestiers n'avait pas d'effet sur la grande chauve-souris brune ($\beta = -0.61$, 95% CI: [-2.87, 1.66], $p = 0.60$), mais un effet significatif négatif sur l'activité des chauves-souris du genre *Myotis* ($\beta = -1.46$, 95% CI: [-2.85, -0.07], $p = 0.04$). Les températures nocturnes élevées avaient un effet positif sur l'activité de la grande chauve-souris brune, mais pas pour les chauves-souris du genre *Myotis*. Ceci est conforme à des corrélations déjà connues sur l'augmentation de l'activité des chauves-souris avec la température nocturne (Ruczyński and Bartoń, 2020). Notre étude établit un état de référence initial (« avant ») en vue d'un suivi approfondi à long terme de type « *Before-After-Control-Impact* » des effets de la construction d'une nouvelle autoroute sur la biodiversité locale (MTMD, 2024). Les travaux de construction sur le site à l'étude ont débuté à l'été 2020 et l'autoroute est devenue opérationnelle à l'été 2025, soit après la fin de notre étude (MTMD, 2024). Les stations d'enregistrement ont été positionnées en fonction de cet objectif de suivi à long terme. Ce dispositif permettra d'évaluer l'évolution de l'activité des chauves-souris jusqu'en 2033, à la suite de la mise en service de l'autoroute (MTMD, 2024). Avec le développement continu du réseau routier mondial (IEA, 2013) et une majorité des espèces de chauves-souris (53%) en Amérique du Nord qui font face à un risque d'extinction modéré à très élevé dans les années à venir (Adams *et al.*, 2024), il devient urgent de répondre à la question des effets de la route sur l'activité des chauves-souris avant et après la construction routière (Fensome and Mathews, 2016). Les conditions expérimentales uniques de cette étude pourraient servir de modèle pour l'élaboration de projets d'aménagement routier ayant un impact minimal sur les chauves-souris (Fensome and Mathews, 2016). En effet, une solution potentielle qui sera testée et surveillée dans le cadre de cette étude à long terme consiste à revégétaliser une zone définie à proximité

de l'autoroute nouvellement construite afin d'améliorer la connectivité avec les zones boisées adjacentes (MTMD, 2024). Cela pourrait orienter les efforts des projets de restauration, par exemple, par la réduction de la pollution sonore résultant de la construction et de l'utilisation routière. Il sera aussi possible de tester si l'utilisation des chauves-souris en tant qu'espèces bio-indicatrices est un bon indicateur sur l'efficacité des mesures de protection de l'habitat pour le reste de la biodiversité locale (Russo *et al.*, 2021). Ainsi, parmi les recherches sur les effets de la route sur les chauves-souris qu'il existe, aucune, à notre connaissance, ne permet d'analyser dans le temps et l'espace l'influence des diverses caractéristiques de plusieurs routes sur l'activité des chiroptères et sur leur utilisation d'un nouvel habitat aménagé.

Il existe d'autres solutions pour limiter les impacts négatifs causés par la construction d'autoroutes, telles que la création de zones tampons boisées le long des routes, la mise en place de passages pour les chauves-souris par le contact de canopée forestière le long d'un bord et de l'autre de la route (« *hop-overs* ») ou de ponts à chauves-souris (« *bat gantries* ») afin d'améliorer les paysages dominés par les routes pour les chauves-souris (Limpens *et al.*, 2005; Bennett et Zurcher, 2013 ; Medinas *et al.*, 2019). Les (« *bat overpasses* ») permettraient aux chauves-souris de voler plus haut en évitant les collisions avec les véhicules (Claireau *et al.*, 2021). Ramalho *et al.* (2021) ont suggéré que les chauves-souris ne se nourrissent pas à proximité immédiate des routes, mais utilisent plutôt celles-ci comme corridors de déplacement ou les traversent pour accéder aux habitats où elles se nourrissent. Végétaliser l'abord des routes permettrait de transformer un obstacle à la dispersion des espèces en un corridor écologique fonctionnel afin d'accroître la connectivité entre les habitats existants (Bennett et Zurcher, 2013). D'ailleurs, une végétation plus dense et plus large le long des routes diminue l'intensité du bruit de la circulation (Kalansuriya *et al.*, 2009 ; Tyagi *et al.*, 2013). Cette gestion du bruit pourrait renforcer la résilience écologique et améliorer la qualité des habitats (Lawler *et al.*, 2002). L'ajout de zones tampons boisées atténuant la pollution sonore et lumineuse le long des routes pourrait aussi augmenter les chances de succès d'éventuels ponts pour chauves-souris, afin de maintenir la connectivité à travers le paysage (Sołowczuk, 2019). Outre les avantages écologiques, de tels aménagements pourraient également préserver et renforcer les avantages sociaux et économiques locaux, en particulier dans les paysages agricoles autour de la région étudiée, en contribuant aux services écosystémiques de lutte contre les ravageurs, car les chauves-souris insectivores se nourrissent de nombreux insectes nuisibles aux cultures agricoles (Whitaker, 1995 ; Maslo *et al.*, 2022).

Cependant, la littérature n'est pas unanime quant à l'efficacité des stratégies actuelles d'atténuation des effets néfastes de la route. Roemer *et al.* (2021) ont trouvé que les « *hop-overs* » peuvent augmenter le risque de collision plutôt que de l'atténuer, car selon leurs résultats, la plantation d'arbres à proximité immédiate des routes pourrait créer de nouvelles opportunités de recherche de nourriture, augmentant ainsi la densité locale de chauves-souris et attirant paradoxalement des individus vers des zones à haut risque de collision. Roemer *et al.* (2021) affirment donc que la mise en place de « *hop-overs* » sans mesures complémentaires de mitigation des risques de collisions, telles que la réduction de la vitesse, devrait être évitée jusqu'à ce que leur efficacité soit démontrée. De même, Fensome et Mathews (2016) ont constaté que la présence d'un habitat forestier favorable aux chauves-souris était associée à une réduction significative des effets de barrière de la route, mais à un risque accru de collision. Une autre solution consiste à installer des écrans de chaque côté des routes afin de dissuader les chauves-souris de la traverser, mais Christensen *et al.* (2016) ont constaté que cette mesure s'avérait inefficace, car de nombreux individus contournent souvent les écrans et traversent la route malgré tout. Claireau *et al.* (2019b) affirment que les ponts pour chauves-souris ne rétablissent pas la connectivité de l'habitat, car la proportion de chauves-souris traversant le long de leur trajet habituel n'a pas changé, indépendamment de la présence du pont. Afin de combler les lacunes actuelles concernant l'efficacité des mesures d'atténuation des effets de la route (Spellerberg, 2002), nous proposons pour la suite une évaluation comparative sur le terrain de diverses stratégies d'atténuation afin de bonifier l'évaluation continue de l'impact écologique de la nouvelle autoroute.

ANNEXE A

CHARACTERISTICS OF THE NEAREST ROAD TO EACH RECORDING STATION. DATA FOR THE SCC2 RECORDING STATION WERE REMOVED DUE TO ITS PROXIMITY TO A NOISY WATER PURIFICATION PLANT

Recording station	Distance to road (m)	Width (m)	Type
SCC1	656	8	Regional
SCC2	NA	NA	NA
SCC3	273	9	Regional
SCC4	1031	12	Collector
SCC5	276	11	Collector
SCR1	277	32	Two-lane
SCR2	61	32	Two-lane
SCR3	225	32	Two-lane
SCR4	102	32	Two-lane
SCR5	455	33	Two-lane
SCT1	1384	15	One-lane
SCT2	1133	16	One-lane
SCT3	1056	14	One-lane
SCT4	585	15	One-lane
SCT5	571	16	One-lane

ANNEXE B
ANABAT® PARAMETERS

Anabat® parameters	Value	Unity
Trigger sensibility (Signal to Noise Ratio)	14	kHz
Minimum frequency	16	kHz
Maximum frequency	250	kHz
Transect mode	OFF	
Minimum event	1	ms
Recording window	5	sec
File type	ZC	
Sample rate	320	kHz
Maximum file length	15	sec
Div ratio	8	
Filename prefix	ON	
Analog filter	ON	

ANNEXE C
SM4® PARAMETERS

SM4® parameters	Value	Unity
Channel	stereo	
Left gain	12	dB
Right gain	12	dB
Left preamp	26	dB
Right preamp	26	dB
Left filter	OFF	
Right filter	OFF	
Sample rate	44100	kHz
Maximum length	1h00	
Compression	none	
Latitude	To adjust	DD
Longitude	To adjust	DD
Timezone UTC	-4	
Sunrise/set type	Solar civil	
LED indicator	5 minutes only	

ANNEXE D

ACOUSTIC PARAMETERS OF BAT CALLS IN SONOGRAMS USED IN THE RANDOM FOREST CLASSIFICATION MODEL

Acoustic parameters
Signal duration
Maximum frequency
Minimum frequency
Frequency band
Frequency band over duration
Maximum inflection frequency
Minimum inflection frequency
Maximum inflection time
Minimum inflection time
Slope in octaves per second
Distance between maximum frequency and maximum inflection frequency
Distance between minimum frequency and minimum inflection frequency
Distance between maximum and minimum inflection frequency
Distance between maximum and minimum inflection time
Distance between signal duration and maximum inflection time
Distance between signal duration and minimum inflection time
Difference between frequency band and frequency of maximum inflection
Difference between frequency band and frequency of minimum inflection

ANNEXE E

RANGE OF CONFIDENCE LEVELS AT WHICH DATA WOULD BE MANUALLY IDENTIFIED FOR EACH SPECIES OR COMPLEX

Species or complex	Confidence levels [min, max]
<i>Eptesicus fuscus</i> (Epfu)	NA*
<i>Lasionycteris noctivagans</i> (Lano)	[0.1, 0.6]
Complex (Epno)	[0.1, 0.4]
<i>Lasiurus cinereus</i> (Laci)	[0.1, 0.8]
<i>Lasiurus borealis</i> (Labo)	[0.1, 0.8]
<i>Perimyotis subflavus</i> (Pesu)	[0.1, 0.7]
<i>Myotis lucifugus</i> (Mylu)	[0.1, 0.5]
<i>Myotis leibii</i> (Myle)	[0.1, 1.0]
<i>Myotis septentrionalis</i> (Myse)	[0.1, 1.0]
Complex (Mysp)	[0.1, 0.4]

*No manual identification needed for this species

ANNEXE F

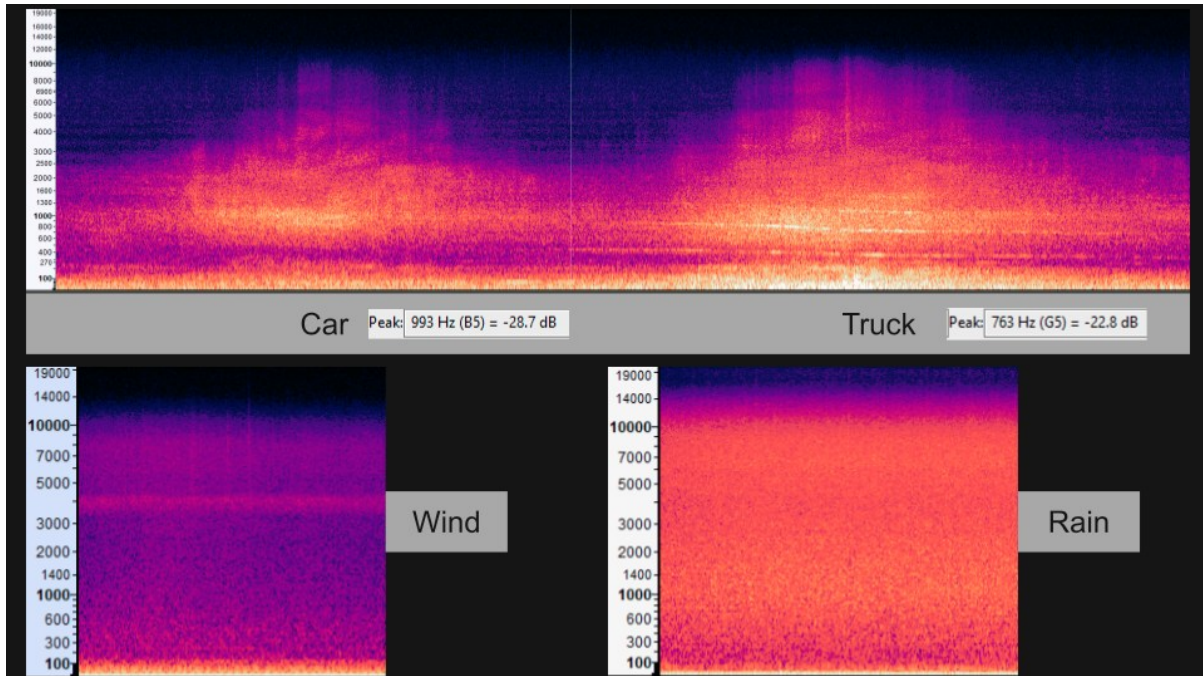
NUMBER OF BAT PASSES AUTOMATICALLY AND MANUALLY IDENTIFIED FOR EACH BAT SPECIES AND COMPLEX OF SPECIES OVER THE YEARS 2021 TO 2023 INCLUSIVELY IN THE FULL AND REDUCED DATASETS

Species or complex	Full dataset		Reduced dataset
	Automated id	Manual id	
<i>Eptesicus fuscus</i> (Epfu)	28,726	3,390	6,528
<i>Lasionycteris noctivagans</i> (Lano)	13,722	1,602	2,109
Complex (Epno)	86,371	15,582	14,175
<i>Lasiurus cinereus</i> (Laci)	728	115	102
<i>Lasiurus borealis</i> (Labo)	3,619	162	115
<i>Perimyotis subflavus</i> (Pesu)	4,802	1,075	67
<i>Myotis lucifugus</i> (Mylu)	7,947	5,100	494
<i>Myotis leibii</i> (Myle)	1,204	0	NA*
<i>Myotis septentrionalis</i> (Myse)	33	7	NA*
Complex (Mysp)	44,629	4,157	2,103
Unidentifiable bat species (Spp)	8,678	7,961	2,258
Total	200,459	39,151	27,951

*Myle and Myse were categorized in the Mysp complex in the reduced dataset.

ANNEXE G

VISUAL EXAMPLES OF MAIN TYPES OF TRAFFIC NOISES (CAR AND TRUCK) AND THEIR RESPECTIVE FREQUENCY ANALYSIS (PEAK AMPLITUDE) COMPARED TO OTHER SOURCES OF NOISE (WIND AND RAIN) WITH OVERLAPPING FREQUENCIES, ALL REPRESENTED IN SPECTROGRAMS (FREQUENCY IN HZ BY TIME) WITH AUDACITY®



ANNEXE H

KALEIDOSCOPE PRO® SOUND PRESSURE LEVEL ANALYSIS PARAMETERS

Kaleidoscope Pro® parameters			Value	Unity
Mode			Non-bat Analysis mode	
Batch	INPUTS	Include subdirectories	yes	
		WAC files	no	
		WAV (and W4V) files	yes	
	OUTPUTS	Channel selection	Process all input channels	
		Create subdirectories	None	
		WAV (and W4V) files	no	
Signal Params	Minimum and Maximum Frequency Range		600-1100	Hz
	Minimum and Maximum Length of Detection		6-60	s
	Maximum inter-syllable gap		0	s
	Remove DC offset		no	
SPL Analysis	SOUND LEVEL ANALYSIS		ENABLED	
	Select weighted bands		793.7	Hz
	Select columns per band		Max SPL	
	Sample period		60	minutes
	dB adjustment		94.0	dB
	SEL peak threshold above mean		12.0	
	SEL off threshold above mean		3.0	

ANNEXE I

RESULTS OF MODEL SELECTION FOR BAT ACTIVITY NEAR THE STUDY REGION IN SOUTHEASTERN QUEBEC (CANADA) FROM SUMMERS 2021 TO 2023. MODELS INCLUDE THE FIXED AND RANDOM EFFECTS. TEST = IF MODEL DIAGNOSTICS ARE RESPECTED "YES". K = NUMBER OF ESTIMATED PARAMETERS. AICC = CORRECTED AKAIKE INFORMATION CRITERION VALUE. THE SELECTED MODELS THAT RESPECT THE MODEL DIAGNOSTICS IN BOTH THE BIG BROWN BAT AND *MYOTIS* SPECIES ARE HIGHLIGHTED IN BOLD. TABLE DESIGN INSPIRED BY RAMALHO *ET AL.* (2021)

Model		Test	K	AICc
Big brown bat activity ~				
1	Traffic Noise + Distance to Road + Road Width + Temperature + Relative Humidity Level + Proportion of Forest + Proportion of Water + Distance to Water + (1 Station) + (1 Date) + (1 Device installation date) + (1 Device installation date : Date)	Yes	14	3384.34
2	Traffic Noise + Distance to Road + Road Width + Temperature + Relative Humidity Level + Proportion of Forest + Distance to Water + (1 Station) + (1 Date) + (1 Device installation date) + (1 Device installation date : Date)	Yes	13	3382.33
3	Traffic Noise + Distance to Road + Road Width + Temperature + Relative Humidity Level + Proportion of Forest + Proportion of Water + (1 Station) + (1 Date) + (1 Device installation date) + (1 Device installation date : Date)	Yes	13	3382.27
4	Traffic Noise + Distance to Road + Temperature + Relative Humidity Level + Proportion of Forest + Distance to Water + (1 Station) + (1 Date) + (1 Device installation date) + (1 Device installation date : Date)	Yes	12	3380.40
5	Traffic Noise + Distance to Road + Temperature + Relative Humidity Level + Proportion of Forest + Proportion of Water + (1 Station) + (1 Date) + (1 Device installation date) + (1 Device installation date : Date)	Uniformity test failed	12	3380.27
6	Traffic Noise + Distance to Road + Road Width + Temperature + Relative Humidity Level + Proportion of Habitat + Distance to Water + (1 Station) + (1 Date) + (1 Device installation date) + (1 Device installation date : Date)	Yes	13	3382.38
7	Traffic Noise + Distance to Road + Road Width + Temperature + Relative Humidity Level + Proportion of Habitat + (1 Station) + (1 Date) + (1 Device installation date) + (1 Device installation date : Date)	Yes	12	3380.32
8	Traffic Noise + Distance to Road + Temperature + Relative Humidity Level + Proportion of Habitat + Distance to Water + (1 Station) + (1 Date) + (1 Device installation date) + (1 Device installation date : Date)	Uniformity test failed	12	3380.45
9	Traffic Noise + Distance to Road + Temperature + Relative Humidity Level + Proportion of Habitat + (1 Station) + (1 Date) + (1 Device installation date) + (1 Device installation date : Date)	Yes	11	3378.41
10	Traffic Noise + Distance to Road + Road Width + Temperature + Relative Humidity Level + Proportion of Habitat + (1 Station) + (1 Station : Site) + (1 Date) + (1 Device installation date) + (1 Device installation date : Date)	Yes	13	3382.39
11	Traffic Noise + Distance to Road + Temperature + Relative Humidity Level + Proportion of Habitat + (1 Station) + (1 Station : Site) + (1 Date) + (1 Device installation date) + (1 Device installation date : Date)	Convergence problem	12	NA
12	Traffic Noise + Distance to Road + Road Width + Temperature + Relative Humidity Level + Proportion of Habitat + (1 Station) + (1 Site) + (1 Station : Site) + (1 Date) + (1 Device installation date) + (1 Device installation date : Date)	Convergence problem	14	NA

Model		Test	K	AICc
Myotis genus bat species activity ~				
1	Traffic Noise + Distance to Road + Road Width + Temperature + Relative Humidity Level + Proportion of Forest + Proportion of Water + Distance to Water + (1 Station) + (1 Date) + (1 Device installation date) + (1 Device installation date : Date)	Uniformity test failed	14	3107.90
2	Traffic Noise + Distance to Road + Road Width + Temperature + Relative Humidity Level + Proportion of Forest + Distance to Water + (1 Station) + (1 Date) + (1 Device installation date) + (1 Device installation date : Date)	Uniformity test failed	13	3109.24
3	Traffic Noise + Distance to Road + Road Width + Temperature + Relative Humidity Level + Proportion of Forest + Proportion of Water + (1 Station) + (1 Date) + (1 Device installation date) + (1 Device installation date : Date)	Yes	13	3113.50
4	Traffic Noise + Distance to Road + Temperature + Relative Humidity Level + Proportion of Forest + Distance to Water + (1 Station) + (1 Date) + (1 Device installation date) + (1 Device installation date : Date)	Uniformity test failed	12	3110.37
5	Traffic Noise + Distance to Road + Temperature + Relative Humidity Level + Proportion of Forest + Proportion of Water + (1 Station) + (1 Date) + (1 Device installation date) + (1 Device installation date : Date)	Uniformity test failed	12	3112.15
6	Traffic Noise + Distance to Road + Road Width + Temperature + Relative Humidity Level + Proportion of Habitat + Distance to Water + (1 Station) + (1 Date) + (1 Device installation date) + (1 Device installation date : Date)	Uniformity test failed	13	3110.61
7	Traffic Noise + Distance to Road + Road Width + Temperature + Relative Humidity Level + Proportion of Habitat + (1 Station) + (1 Date) + (1 Device installation date) + (1 Device installation date : Date)	Uniformity test failed	12	3112.03
8	Traffic Noise + Distance to Road + Temperature + Relative Humidity Level + Proportion of Habitat + Distance to Water + (1 Station) + (1 Date) + (1 Device installation date) + (1 Device installation date : Date)	Uniformity test failed	12	3111.17
9	Traffic Noise + Distance to Road + Temperature + Relative Humidity Level + Proportion of Habitat + (1 Station) + (1 Date) + (1 Device installation date) + (1 Device installation date : Date)	Uniformity test failed	11	3111.31
10	Traffic Noise + Distance to Road + Road Width + Temperature + Relative Humidity Level + Proportion of Habitat + (1 Station) + (1 Station : Site) + (1 Date) + (1 Device installation date) + (1 Device installation date : Date)	Convergence problem	13	NA
11	Traffic Noise + Distance to Road + Temperature + Relative Humidity Level + Proportion of Habitat + (1 Station) + (1 Station : Site) + (1 Date) + (1 Device installation date) + (1 Device installation date : Date)	Convergence problem	12	NA
12	Traffic Noise + Distance to Road + Road Width + Temperature + Relative Humidity Level + Proportion of Habitat + (1 Station) + (1 Site) + (1 Station : Site) + (1 Date) + (1 Device installation date) + (1 Device installation date : Date)	Uniformity test failed	14	3116.18

ANNEXE J

HABITAT CHARACTERISTICS WITHIN A 400 M RADIUS OF EACH RECORDING STATION. DATA FOR THE SCC2 RECORDING STATION WERE REMOVED DUE TO ITS PROXIMITY TO A WATER PURIFICATION PLANT. THE COMPOSITE VARIABLE "PROPORTION OF HABITAT" WAS CALCULATED BY SUMMING THE "PROPORTION OF FOREST COVER" AND THE "PROPORTION OF WATER COVER"

Recording station	Proportion of forest (%)	Proportion of water (%)	Proportion of habitat (%)
SCC1	12	4	16
SCC2	NA	NA	NA
SCC3	19	0	19
SCC4	18	1	19
SCC5	27	0	27
SCR1	8	0	8
SCR2	62	3	65
SCR3	73	0	73
SCR4	56	0	56
SCR5	48	11	59
SCT1	40	11	51
SCT2	40	10	50
SCT3	56	0	56
SCT4	24	0	24
SCT5	37	0	37

ANNEXE K

DIAGNOSTIC RESULTS FOR COLLINEARITY PREDICTORS FOR THE BIG BROWN BAT AND MYOTIS SPECIES GLMM MODELS (N = 760): PEARSON CORRELATIONS AND VARIANCE INFLATION FACTORS (VIF) ESTIMATES. VIF < 5 INDICATES LOW MULTICOLLINEARITY.

Predictors	1	2	3	4	5	6	7
Big brown bat model							
1. Traffic Noise ^{Sc}	1.00						
2. Distance to Road ^{Sc}	-0.27	1.00					
3. Road Width ^{Sc}	0.08	-0.51	1.00				
4. Temperature ^{Sc}	-0.04	-0.02	0.05	1.00			
5. Relative Humidity Level ^{Sc}	0.14	0.06	-0.03	0.07	1.00		
6. Proportion of Forest (r = 400 m) ^{Sc}	0.20	-0.12	0.50	0.03	-0.05	1.00	
7. Proportion of Water (r = 400 m) ^{Sc}	-0.30	0.45	0.16	-0.0007	0.03	0.17	1.00
Myotis species model							
1. Traffic Noise ^{Sc}	1.00						
2. Distance to Road ^{Sc}	-0.27	1.00					
3. Road Width ^{Sc}	0.08	-0.51	1.00				
4. Temperature ^{Sc}	-0.04	-0.02	0.05	1.00			
5. Relative Humidity Level ^{Sc}	0.14	0.06	-0.03	0.07	1.00		
6. Proportion of Forest (r = 400 m) ^{Sc}	0.20	-0.12	0.50	0.03	-0.05	1.00	
7. Proportion of Water (r = 400 m) ^{Sc}	-0.30	0.45	0.16	-0.0007	0.03	0.17	1.00

^{Sc}: Standardized variables on a scale of 0 to 1.

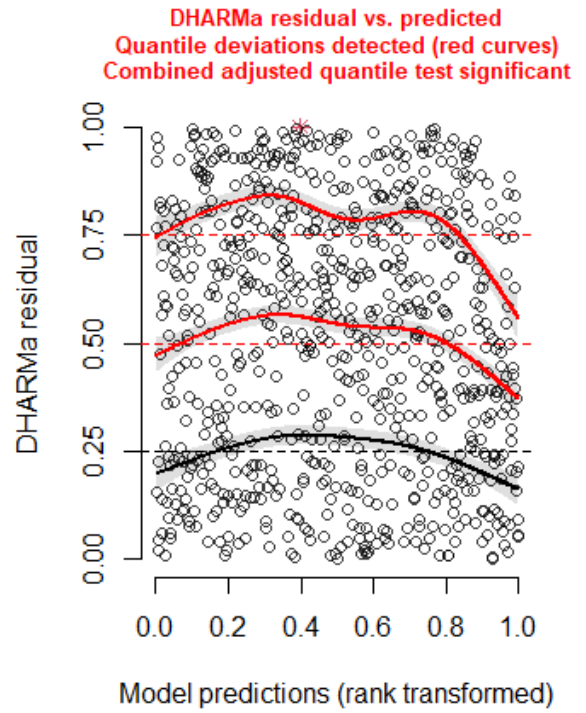
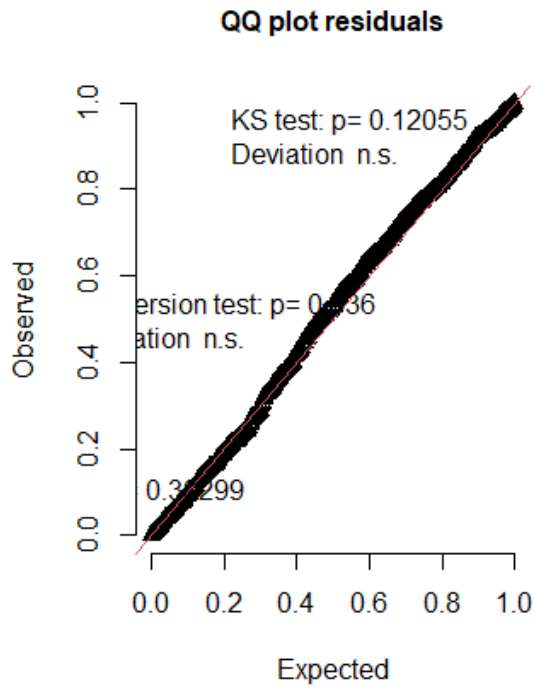
Predictor	VIF	95% CI (VIF)	Adjusted VIF	Tolerance	95% CI (Tolerance)
Big brown bat model					
Traffic Noise ^{Sc}	1.13	[1.07, 1.26]	1.06	0.88	[0.79, 0.94]
Distance to Road ^{Sc}	2.33	[2.10, 2.62]	1.53	0.43	[0.38, 0.48]
Road Width ^{Sc}	2.41	[2.16, 2.70]	1.55	0.42	[0.37, 0.46]
Temperature ^{Sc}	1.01	[1.00, 7.17]	1.01	0.99	[0.14, 1.00]
Relative Humidity Level ^{Sc}	1.01	[1.00, 6.63]	1.01	0.99	[0.15, 1.00]
Proportion of Forest (r = 400 m) ^{Sc}	1.47	[1.35, 1.63]	1.21	0.68	[0.61, 0.74]
Proportion of Water (r = 400 m) ^{Sc}	1.72	[1.57, 1.92]	1.31	0.58	[0.52, 0.64]
Myotis species model					
Traffic Noise ^{Sc}	1.25	[1.17, 1.38]	1.12	0.80	[0.72, 0.86]
Distance to Road ^{Sc}	2.13	[1.92, 2.38]	1.46	0.47	[0.42, 0.52]
Road Width ^{Sc}	2.32	[2.09, 2.60]	1.52	0.43	[0.38, 0.48]
Temperature ^{Sc}	1.01	[1.00, 1336.31]	1.00	0.99	[0.00, 1.00]
Relative Humidity Level ^{Sc}	1.00	[1.00, 4.79 × 10 ¹¹]	1.00	1.00	[0.00, 1.00]
Proportion of Forest (r = 400 m) ^{Sc}	1.44	[1.33, 1.60]	1.20	0.69	[0.63, 0.75]
Proportion of Water (r = 400 m) ^{Sc}	1.70	[1.55, 1.89]	1.30	0.59	[0.53, 0.65]

^{Sc}: Standardized variables on a scale of 0 to 1.

ANNEXE L

GRAPHS OF THE RESIDUALS OF THE CALCULATED GLMM FOR THE BIG BROWN BAT

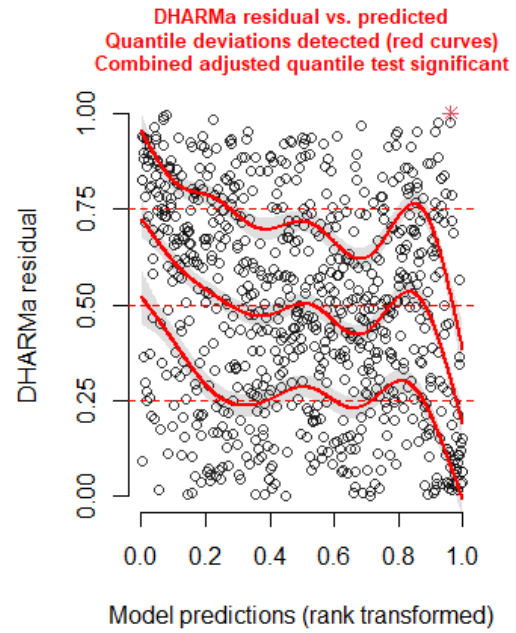
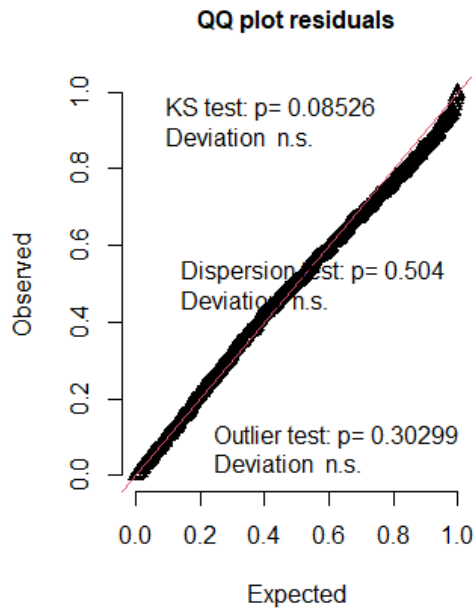
DHARMa residual



ANNEXE M

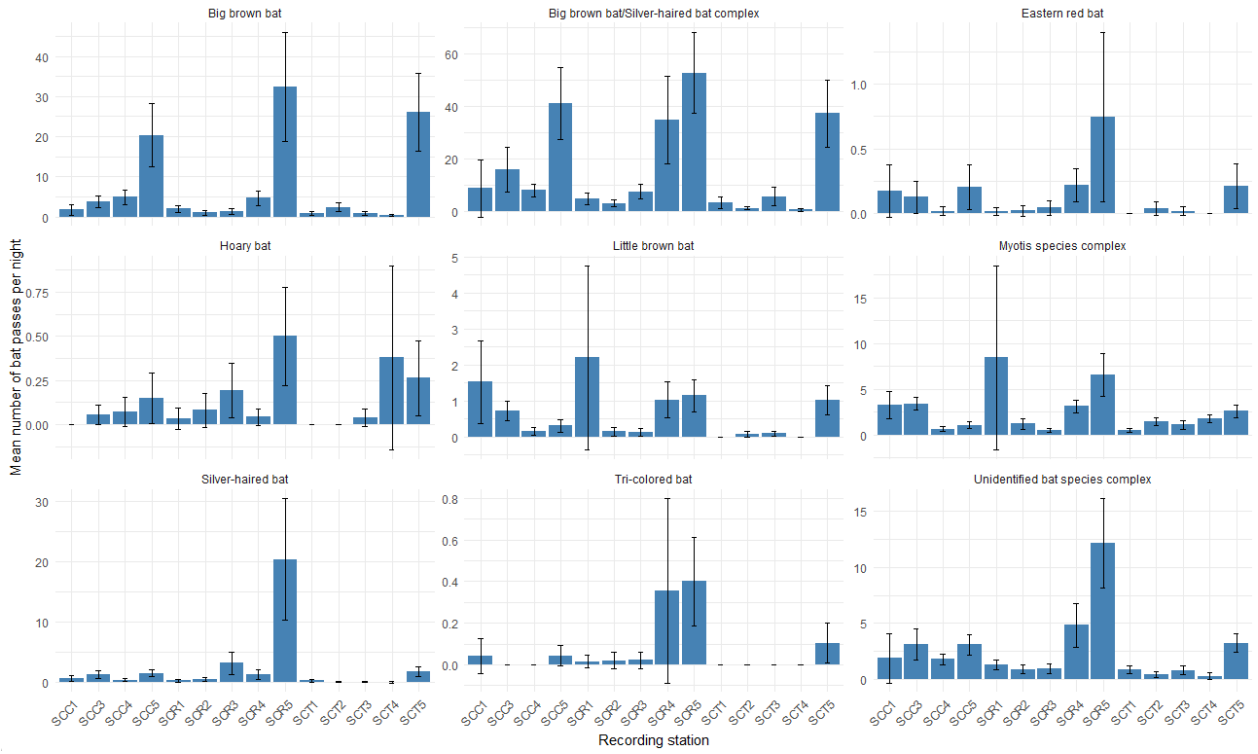
GRAPHS OF THE RESIDUALS OF THE CALCULATED GLMM FOR *MYOTIS* SPECIES

DHARMA residual



ANNEXE N

MEAN BAT ACTIVITY PER RECORDING STATION (FACETED BY SPECIES) WITH 95% CONFIDENCE INTERVALS



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