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Research Report

Trick of the Light: An Investigation into the Impact of Artificial Light at Night (ALAN) on Immune System Function and Space-Use by Irish Bats

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1. Abstract

Artificial light at night (ALAN) is a major anthropogenic stressor, with a particularly dramatic effect on nocturnal wildlife, such as bats. ALAN can change where and how bats move, and may be also altering their immune systems. This project set out to measure both of these effects — changes in space use and changes in immune activation along a gradient of artificial light. For this purpose, 5 roosts of different levels of light exposure were identified. Static recorders were used to measure bat activity at these sites. To assess immune health, faecal samples were collected, and analysed for two different markers - neopterin, and helminth load. The results for both immune markers were inconclusive, highlighting the need for further development of non-invasive and bat-specific techniques in this area.

2. Introduction

2.1 Introduction to Project

Human fingerprints are woven into all earth systems, from the geology beneath our feet, to changing climates and ocean currents. Zoonoses - infectious diseases that can spread between animals and humans - have not escaped this effect. Diseases spilling over into humans are now being largely exacerbated by anthropogenic factors, such as climate change[1], habitat fragmentation[2], and potentially, artificial light.[3] This study intends to gauge the two-fold response of Irish bats to artificial light at night. Firstly, their activity, and secondly their immune response, by assessing both variables at 5 roosts along a gradient of artificial light.

The aim of this research is to investigate the vulnerability that ALAN may be imparting onto the Irish bat population, either by energetic costs of immune activation, or by altered space use.

2.2 Bat Ecology and Zoonotic Risk

Bats are a prolific reservoir of zoonotic disease, hosting three known virus groups of pandemic concern.[4] Awareness of viral spillover from bats into the human population has grown in recent years, owing to the COVID-19 pandemic, widely considered to have its origin in Rhinolophus bats in central China.[5]

Bats are highly social and vagile animals. Living in populous colonies, often in enclosed spaces, creates opportunities for viral transmission, as does pro-social behaviour such as trophallaxis in *Desmodontinae.*[6] Echolocation may allow viral particles to become aerosolised, which again contributes to transmission.[7] Certain bats, such as *Pteropodidae spp.* ingest and spit out fruit as they fly, which may be consumed by an intermediate host, and later transmitted to humans.[8]

Bats also have unique immune systems. They have an unusually high tolerance for viruses, with an ability to host highly pathological viruses, such as the ebola virus, asymptomatically. The reasons for this are "likely multifaceted, perhaps involving sophisticated immune adaptations and controlled inflammatory responses".[9] Their unique adaptations to inflammatory pathways, and potential adaptations to cell-death regulating proteins, may prevent tissue damage during infection,[9] minimising the impact on the health of the bat. This presents a risk of transmission, as an infectious bat can fly and hunt as normal, spreading infection to a human or intermediate animal.

Spillover is defined as the transmission of a pathogen (e.g. a virus) from one species, into another species which did not previously host that pathogen[10]. If this pathogen can pass between animals and humans, it is known as a zoonosis. Spillover from bats into the human

population has been suggested as the origin of several highly virulent diseases, such as Ebola virus disease[11], Marburg disease[12], and COVID-19[13]. This may occur directly from the original species into humans, as may have been the case for Ebola virus[11], or through an intermediate, such as MERS-CoV, which is believed to have originated from bats, and passed to humans through the camel population.[14]

Determinants of spillover are somewhat poorly understood, but are known to include a host ecological, genetic, and anthropological factors. Olival et al. proposed a model suggesting that mammalian spillover risk to humans can be predicted using a combination of "phylogenetic distance from humans, the ratio of urban to rural human population across a species range, host order, whether or not a species is hunted, disease-related research effort, and total viral richness". With a relatively short phylogenetic distance from humans, an almost global range, and unusually high tolerance for pathogens, bats represent the highest spillover risk of any mammal.[15]

Furthermore, anthropogenic encroachment on bat habitats has forced many bats to live in closer proximity to human populations, exposing them to more artificial light. Light represents an "invisible barrier" which further fragments the already shrinking habitats of bats worldwide by increasing exposure to predators. In this way, it alters the space-use of bats. ALAN also threatens biodiversity, as different species have varying levels of light tolerance.[16] Some agile insectivorous species, e.g. *pipistrellus spp*. are attracted to ALAN due to the increased presence of insects, while others avoid it entirely.[16] This leads to an overrepresentation of the former in ALAN-polluted areas, reducing biodiversity.[17] Diminished biodiversity reduces ecosystem function, as well as increasing the vulnerability of the species to population decline.

2.3 Measuring Immune Activation

The immune system is constantly active, surveilling for threats, clearing waste, and monitoring our microbiome. What is meant by "immune activation" in this context is an acute response to a perceived threat, such as a virus, when the immune system uses specialised cells and proteins to fight it off.

Neopterin (NEO) is a marker of immune activation, as immune cells such as monocytes, macrophages, and dendritic cells, release NEO upon stimulation by IFN-γ.[18] This is an important signalling molecule in the immune system, which activates pathways that "switch on" the immune system response. Cells differentiate, division slows down, and a cascade response enables the immune system to fight off a threat. All of this is highly energetically costly, meaning that less energy is available for other physiological processes.

NEO in the circulatory system is secreted via bile into the digestive tract, where it is largely resistant to enzymatic breakdown, and is secreted into stool.This means that faecal NEO concentration can be seen as a marker of the degree of immune activation.[19] Therefore, in a bat population where infections are prevalent, we would expect a higher NEO concentration in faeces.

Helminths are endoparasitic worms which colonise the digestive tract of many animals, including bats. These parasites are largely regulated by Th2 cells, which maintain the immune response against extracellular threats.[20] Therefore, a higher helminth load indicates reduced effectiveness of this immune response. Th1 immune activity controls the response against intracellular threats, such as bacteria and viruses.[21] There is an antagonistic relationship between the two, meaning that when a Th1 immune response is mounted, Th2 effectiveness could be expected to decrease.[22] This would lead to a greater helminth load within the digestive system. This study is non-invasive, and therefore will not be directly measuring helminths within the digestive tract, instead using the number of eggs found in guano as a proxy for the population size.

It should be noted that helminth load and NEO concentration can vary according to age, species, and sex of the individual, among other factors. This would ideally be considered within the study, but due to time and resource constraints, it is beyond the scope of this project.

3. Materials and Methods

3.1 Fieldwork

5 individual roosts were surveyed, containing a mix of native Irish bat species. The exact species were unconfirmed, but the species that had previously been confirmed at these sites were; *pipistrellus pipistrellus, Plecotus auritus*, and *Pipistrellus pygmaeus.* The roosts were subject to different levels of ALAN-exposure, and were located in Dublin, Meath, and Waterford.

Site Number	Name	Latitude	Longitude	County
1	Brú na Bóinne	53.705417	-6.439972	Meath
2	Mellifont Park, Tullyallen	53.735207	-6.475563	Meath
3	Kilternan	53.232373	-6.189202	Dublin
4	The Anchorage	52.232318	-7.067726	Waterford
5	Nelluvelli House	53.2734	-7.77832	Waterford

Faecal samples were collected non-invasively from each roost. These were collected by placing muslin cloth down in an area where guano had been previously spotted. This was done after dusk, when the bats of the roost had emerged to hunt. At the same time, a static Apodemus PippyG acoustic recorder was placed at the entrance of the roost. This was placed in such a way that it did not impede access to the roost. These were left in dry areas which were out of direct sunlight.

After 48 hours, the recorder and muslin were collected from the roosts. Soiled areas of the muslin were identified and cut to be placed in a labelled sterile plastic bag. These were placed in a coolbox, and were returned to the lab. The time elapsed between collection and storage of the samples was noted. At the lab, the guano/urine collected from each roost was pooled, and split. Half of the samples were kept at 4℃ (suitable for parasitology), and -20℃ (suitable for neopterin ELISA).

3.2 Parasitology

The helminth eggs were recovered from refrigerated guano using a saturated salt solution. The eggs floated in the salt solution, while the faecal matter sank, allowing the two to be separated.

After collection, the samples were stored in resealable plastic bags, and transported to the lab in a chilled container. The samples were then stored at 4℃ for 2-4 days. The samples were first weighed. The faeces were then homogenized with a known volume of distilled water (approximately 10ml/gram of faeces). The muslin was left to soak in the water until the guano was visibly detached. The muslin was then kneaded and squeezed repeatedly until all faecal matter had detached from the cloth, adding additional water as needed. The resultant wash water constituted the faecal suspension. The separated muslin was dried fully and weighed again. This mass was subtracted from the pre-extraction mass, to give an estimated mass of the faecal material extracted.

Centrifugation was then used to separate the denser faecal matter and the less dense helminths. The suspension was mixed thoroughly, and transferred to a centrifuge tube. This tube was centrifuged at 1500 rpm for 3 minutes, until a pellet formed. The overlaying supernatant was carefully decanted, leaving a pellet of extracted guano.

A saturated NaCl solution was added to the pellet, and this was centrifuged again at 1500 rpm for 3 minutes. A blunt forceps was clamped just below the meniscus, allowing the surface fraction to be removed and poured into a lidded cuvette. The cuvette was slightly overfilled before fitting the lid, to avoid trapping air bubbles.

This cuvette was examined under a compound microscope at 4x, 10x, and 40x magnification.

3.3 Neopterin ELISA

After collection, the samples were stored in individual disposable bags, at -18℃ The samples were removed from the freezer and allowed to come to room temperature. 0.1g of guano was mixed with 0.5 ml of 0.9% saline solution, and the mixture was manually homogenized using a pipette tip, before being vortexed for 30 minutes. Once visibly homogenized, the

samples were centrifuged at 3500 rpm for 20 minutes. The supernatant was decanted, and used for analysis.

Fecal neopterin levels were measured using commercial ELISA kits (#RE59355D, IBL International GmbH, Germany) as per the manufacturer's instructions. Standards, controls, and fecal supernatant samples were each pipetted in duplicate (20 µL per well) into microtiter plate wells pre-coated with goat anti-rabbit antibody. 100 µL of enzyme conjugate and 50 µL of neopterin antiserum was then added to each well. The plate was sealed with black adhesive foil to block light and incubated for 90 minutes with orbital shaking. The foil was then removed and the plate washed four times using 300 µL of wash buffer per wash. Substrate solution (150 µL) was added to each well and left to incubate for 10 minutes, after which the reaction was terminated by adding 150 µL of stop solution to each well.

4. Results

4.1 Helminth Counts

Faecal samples were recovered from 4 sites, and stored for analysis. No helminth eggs were recovered from any samples.

4.2 Neopterin ELISA

A clear result was not obtained for the neopterin ELISA - a strong colour was obtained in all of the wells, including the standards.

4.3 Audio Data

No data was recovered due to improper calibration of the equipment.

4.4 Artificial Light Data

The following maps show artificial light around the 5 bat habitats used in this study, using satellite data collected and compiled by Dr. Brian Espey of Dark Sky Ireland. The yellow circles represent bat roosts used in the study.

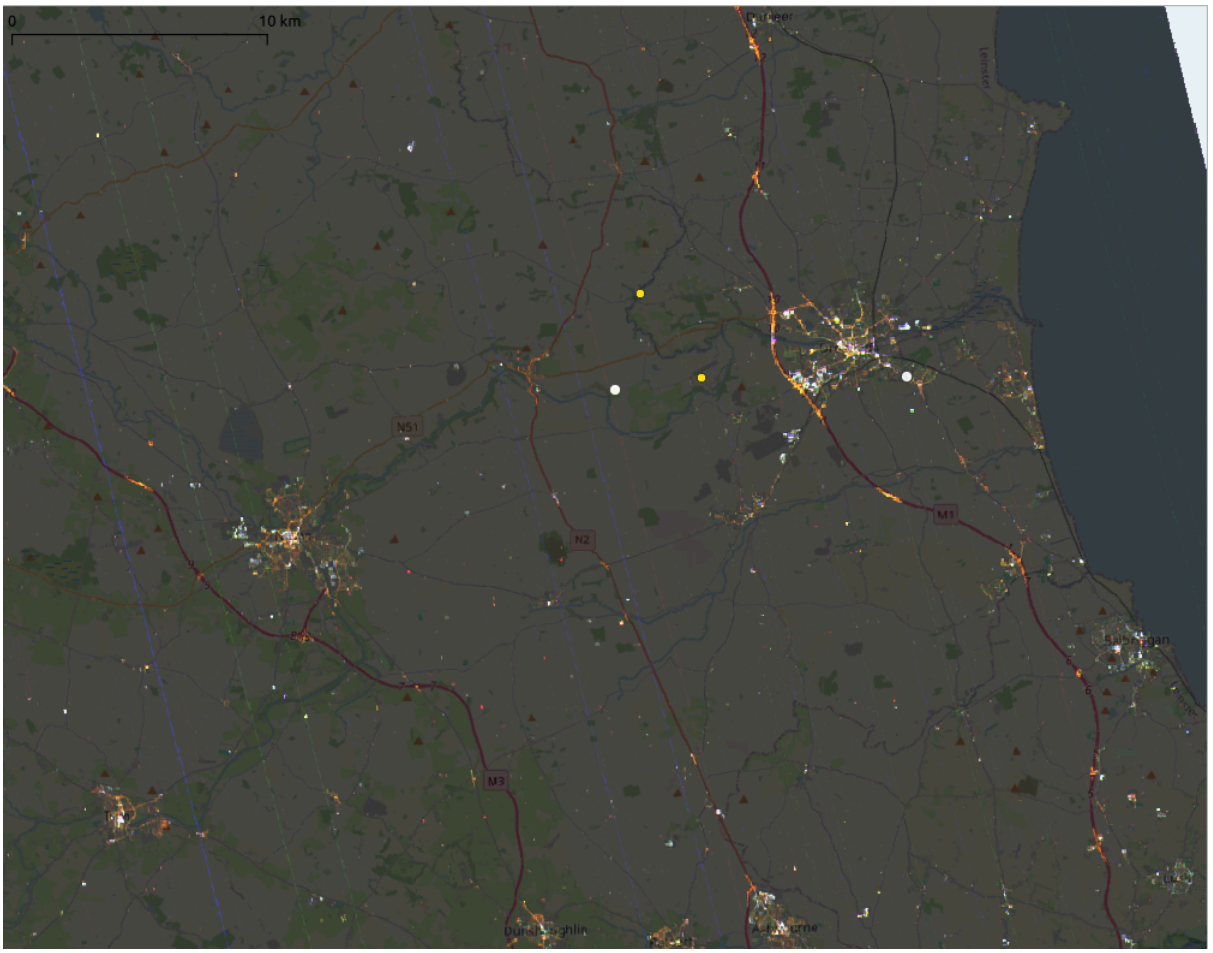

Figure 1: Artificial Light Intensity Around Sites 1 and 2, Represented as Yellow Dots

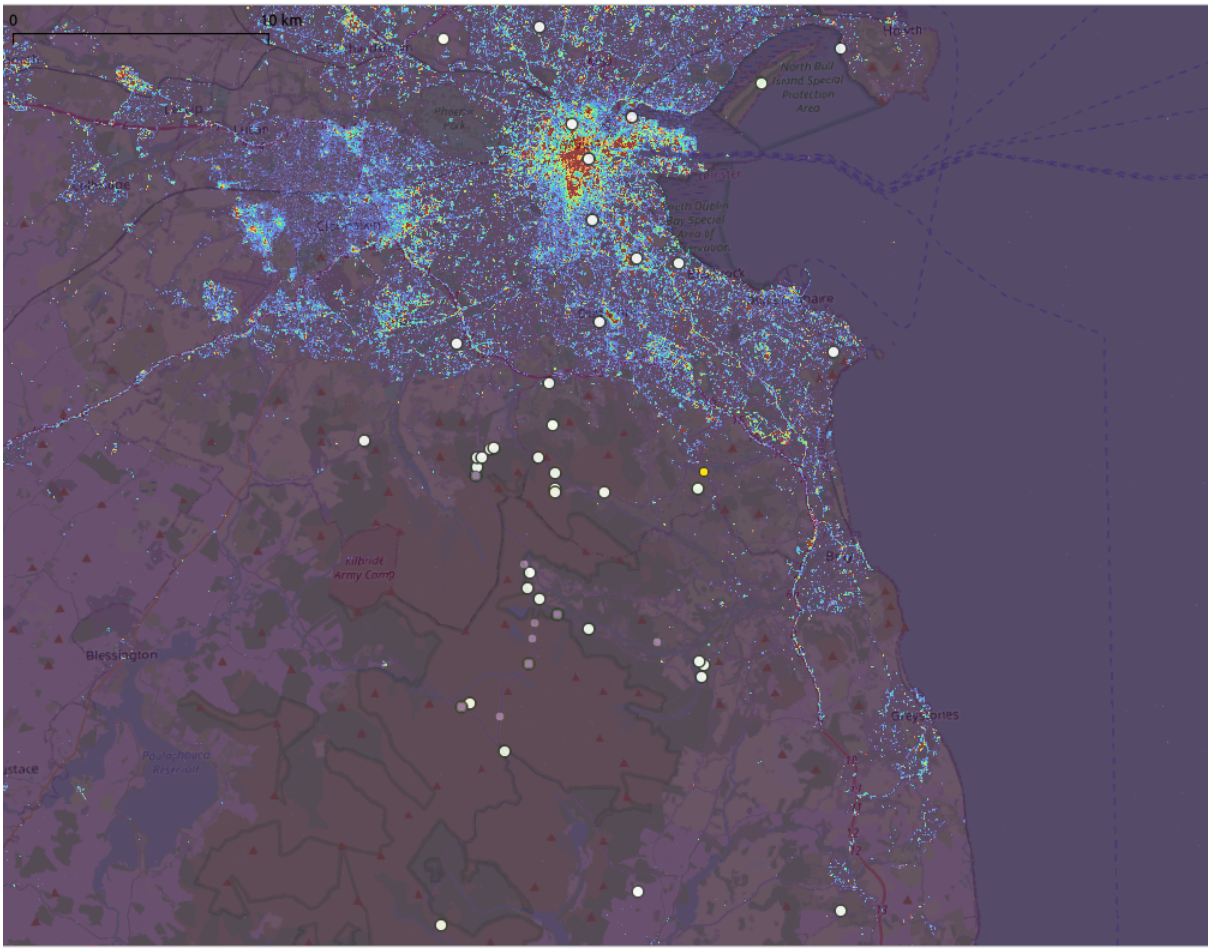

Figure 2: Artificial Light Intensity Around Site 3, Represented as a Yellow Dot

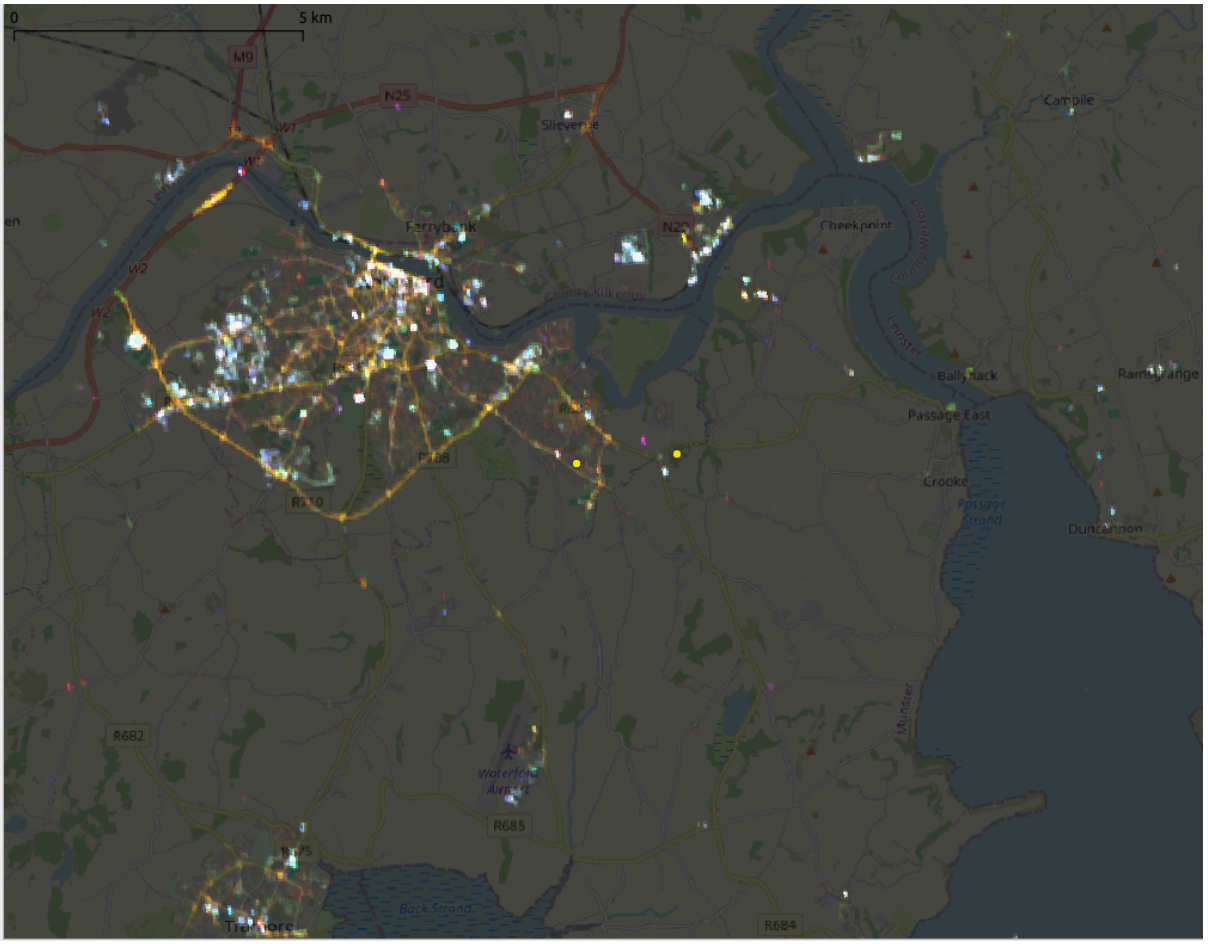

Figure 3: Artificial Light Intensity Around Sites 4 and 5 Represented as Yellow Dots

5. Discussion

The intention of this study was to assess the immune activation, and space-use of Irish bats along a gradient of artificial light. Faecal samples were collected, and analysed for NEO concentration, and helminth eggs, as a proxy of immune activation. Acoustic data was collected to assess space-use. Clear data was not obtained from any of these methods, highlighting the need for elaboration of non-invasive techniques for monitoring bat populations.

As for the helminth counts, there were no eggs identified in any of the samples collected. There is limited up-to-date research relating to helmintheases in Irish bats, with the most recent identified publication dating April 1977, making it difficult to know the modern state

of bat endoparasites in Ireland.[23] This study, based also on a colony of pipistrelles in Galway, found trematodes present in 43.2% of adult hosts, but only 6.9% of juveniles. The authors posited that this difference was due to the juveniles being only recently weaned from milk, meaning that they had only been feeding off of the intermediate hosts (insects) for a short time. Irish bat pups are typically born from late June to early July, continuing to feed on milk for 3-4 weeks. With our samples having been collected in early August, it is possible that the roosts surveyed contained juveniles who had not yet been infected by helminths. As mentioned previously, helminth populations can vary by age of the individual, seasonality, etc, however it was not within the scope of this study to consider these variations. Another reason for this may have been the quantity collected. Less than 1g of faecal matter was recovered from each roost, which limits the availability of helminths for analysis. For future studies, a greater amount of faecal matter should be collected to rule this issue out. The issue may also be the methodology itself. We intended to employ non-invasive methods for the analysis of helminths in bats, which has been successfully employed for insectivorous bats by Adhikari et. al. in Nepalese bats.[24] In their method, the guano was collected within 24 hours, which, due to limited resources, was not possible in our case.

It is clear that there is a lack of non-invasive investigation of helmintheses in Ireland and Britain. Lord et. al, and Dodd et. al. have published reviews of helmintheases in British bats, however, these rely on invasive methods which select bats which have died of natural causes, or have been recently euthanised.[25] This introduces selection bias, and also makes it difficult to replicate due to the protected status of bats in Ireland and the UK. This highlights the need for improved non-invasive methods for investigating helmintheases in bats. If these could be developed, it would open the door to continuous monitoring of these endoparasites, which would shed light on the health of the population.

As for the ELISA, a strong colour was obtained in all of the wells, including the standards. This is an unusual result, which may have resulted from the vigorous oscillation that was required to homogenise the guano. The kit also did not use antibodies specific to bats, as these are not available. This again highlights the need for non-invasive testing for immune activation in bats. Similar studies using blood and bone marrow suffer from the lack of

bat-specific reagents. These again rely on invasive methods, which presents logistical and ethical issues when working with a protected species.

The relationship between artificial light at night (ALAN) and bat activity could not be directly assessed in this study due to the loss of the acoustic data resulting from incorrect calibration of the recording equipment. As such, conclusions cannot be made about changing bat activity along a gradient of artificial light. Nevertheless, the literature indicates that ALAN does have an effect on the activity of bats in an area, with varying light tolerance among species.[16] More research is needed to elaborate on the relationship between ALAN and bat activity in Ireland.

6. Conclusion

The results of this study highlight the need for non-invasive, bat-specific methods for monitoring immune activation and space use. Bat population health is integral to both ecosystem function and human health, and artificial light is a growing anthropogenic pressure that will increasingly shape bat populations. Minimising its effects, for their health and ours, requires continuous monitoring of space use and immunological function. This study sheds light on the need for bat specific reagents and methodologies for this purpose. Future research should prioritise developing these tools, so that continuous, non-invasive monitoring becomes achievable.

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