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Mechanisms and Mitigation: Effects of Light Pollution on West Nile Virus Dynamics

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Mechanisms and Mitigation: Effects of Light Pollution on West Nile Virus Dynamics

by

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DEDICATION

First, I would like to dedicate this dissertation to my parents, Michael Kernbach and Nancy Harmon, who encouraged me to pursue my dreams and go wherever they may take me. Without their support from the beginning, none of this would have been possible. They always listened to my morbid sparrow stories and consoled me when I got into fights with security guards over bird nets or despaired over computer troubles. I would also like to dedicate this dissertation to my siblings, Gretchen and August Kernbach, who may not have a clue what any of this says but were always there to keep things light and give me a laugh when I didn't feel like smiling. I would not have been able to get through any of this work without the everlasting support and love from my labmate and best friend, Haley Hanson. Even though I often distracted Haley from the million things that she had to do, she managed to give me the brightest and most thoughtful advice and wisdom I could ever ask for (and still accomplish an insane amount of amazing research… animal!). My fondest memories at USF (and in life) will always include spinning you around the lab in chairs that are falling apart, getting pooped on by birds together, whipping the golf cart to places that we definitely could have walked to, slam dunking heads (don't ask), spitting up carrots because you made me laugh too hard, and lighting the murder candle to ~calm the senses~. I would also like to dedicate this dissertation to my other labmates (and the real brains behind this whole document) Emily Ruhs, Cedric Zimmer, Jeanette Miller, and countless others I did not have room to thank here. This work was impossible without the help of an army, and I thank all of those who helped, even if it was taking down a net one day! Additionally, I want to dedicate this dissertation to my best friends who from day one were cheering me on and

celebrated my accomplishments like they were their own. Even if I did not want to go out and celebrate a publication, they pushed me to bask in the temporary glory! Last, but certainly not least, I would like to dedicate this to my future husband, Ryan DeWitt, for his unconditional love and unparalleled compassion through all my successes and setbacks. I apologize for screaming at my computer in the other room constantly while we worked from home during quarantine, but I swear your patience was worth it! After all, our mail will immortalize this experience forever as Dr. -then Mr.- DeWitt… You were always the first person to celebrate with me during the good times and sit with me during the hard times. I could not have finished this dissertation without you and the joy you brought into my life. Your authenticity and optimism will always inspire me to trust in God, have faith in humanity, and have hope for the future.

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ABSTRACT

Light pollution, or the presence of unnatural light at night, is a pervasive and growing problem across the globe. While often pictured in urban centers, light pollution is far reaching and can affect seemingly safe and minimally developed environments. For example, agricultural communities with artificial lighting near facilities can generate such light pollution in rural areas. Further, streetlights and illuminated billboards along roads and highways can generate light pollution far from cities. Given how pervasive this anthropogenic stressor is, it is surprising that not much is known about how artificial light at night, or ALAN, affects humans or wildlife, especially those that harbor infectious diseases.

Previously, the biomedical field uncovered many negative effects of ALAN exposure on the immune system. Laboratory rodents experience exaggerated fever responses and decreases in bacterial killing ability, among other consequences. This is altogether not surprising as components of vertebrate immune systems possess circadian rhythms. Additionally, other studies have found that exposure to ALAN induces hormonal dysregulation, leading to a mismatch in circadian and circannual timing. Ultimately, the consensus among the research community is that ALAN generates a myriad of negative effects on immunity and other components of organismal physiology. However, it is yet to be uncovered how ALAN may affect infectious disease dynamics.

Here, I investigated for the first time how light pollution might affect infectious disease dynamics. To do so, I considered the mosquito-transmitted flavivirus, West Nile virus (WNV). WNV is among one of the most important arboviruses worldwide, and continues to affect

human, horse, and bird populations in the United States following its introduction in 1999. WNV has also been described as a "peri-urban" disease-causing agent, as it often emerges in suburbs and other built environments. Lastly, as WNV is harbored by common and urban-residing avian reservoirs, light pollution has the potential to affect transmission through effects on these amplifying hosts. I used the ubiquitous house sparrow as the study species here because they are competent WNV reservoirs in nature, reside in almost all part of the continental United States, and are residents of light polluted areas. Overall, I asked how exposure to low-intensity light pollution may affect the host competence (i.e., ability to generate new infections) of house sparrows to WNV.

In my first chapter, I considered how light pollution might affect multiple aspects of WNV dynamics in house sparrows, from molecular mechanisms to outbreak potential. I first discussed the circadian nature of immunity and viral defenses and outline how these may become dysregulated by exposure to light at night and other downstream effects. I then discussed the effects on host competence including infectious period, mortality, and vector-associated behavior. Lastly, I walked through multiple components of the R_0 equation (i.e., the basic reproductive number) which essentially determines how many new infections one host can generate based on infectious period, probability of being infected after a bite (in hosts and vectors), biting rate, background mortality, disease-induced mortality, and other relevant parameters. Combined, this chapter outlined how ALAN might be altering vector-transmitted diseases.

In my second chapter, I experimentally tested whether low-intensity ALAN affected WNV responses in House sparrows. My results first revealed that exposure to ALAN did not affect glucocorticoid regulation in sparrows, indicating that any such immune dysregulation

would not have been mediated by this stress hormone. Importantly, I found that exposure to ALAN, no matter the duration (ranging from 7 to 21 days), allowed sparrows to maintain infectious levels of WNV for twice as long as controls. Although ALAN-exposed sparrows lost a significant amount of body mass, there were no differences in mortality rate among the groups. Additionally, transcriptomic analyses revealed that while ALAN-exposed birds upregulated WNV immune defenses earlier than controls, they displayed signs of immunopathology. After considering all of this, I used a simple single-host, single-vector R_0 model, and found that extending the infectious period by two days increased outbreak potential by ~41%.

In my third chapter, I explored whether spectral composition of ALAN affects host competence to WNV in house sparrows. I repeated a similar WNV infection experiment but substituted out an incandescent light for 3 different types of LED lights: cool white, warm white, and amber-hued (which are marketed as "wildlife-safe"). First, I found that exposure to warm white light at night significantly suppressed the circadian hormone, melatonin. Melatonin is known for regulating many circadian functions, but also plays a role in mediating immune responses and attenuating infection-induced damage. Additionally, I found that exposure to broad-spectrum (both cool and warm white) ALAN did not affect viremia (i.e., amount of virus in circulation), but interestingly, exposure to amber-hue ALAN marginally but significantly increased WNV resistance (i.e., decreases viremia). Alternatively, birds exposed to broad-spectrum ALAN did experience higher WNV-induced mortality and tended to die at lower viremias than control birds. Altogether, altering spectral composition of light at night has the potential to alleviate negative effects on wildlife.

In my fourth and last chapter, I investigated whether these effects of ALAN observed in the lab manifest ecologically using Florida Department of Health (FDOH) sentinel chicken

surveillance data. The FDOH monitors environmental circulation of vector-transmitted diseases by surveying chickens throughout the state weekly for the presence of WNV antibodies, indicating that they have been exposed to the virus. I extracted ALAN intensities from the World Atlas of Artificial Sky Brightness at these surveillance sites while accounting for weather and urbanization variables. Mixed effect model selection revealed that ALAN as a polynomial term and temperature of the month prior were the best predictors of WNV exposure risk across Florida. Exposure risk was lowest in non-light polluted areas, peaked in areas of low light pollution, and then tapered off in areas of moderate to high light pollution. Unlike previous results, I did not find support for parameters of urbanization here, but more work needs to be done to uncover what about ALAN exposure at ground-level may be driving WNV exposure risk.

INTRODUCTION:

DIM LIGHT AT NIGHT: PHYSIOLOGICAL EFFECTS AND ECOLOGICAL CONSEQUENCES FOR INFECTIOUS DISEASE

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Abstract:

Light pollution has emerged as a pervasive component of land development over the past century. Several detrimental impacts of this anthropogenic influence have been identified in night shift workers, laboratory rodents, and a plethora of wildlife species. Circadian, or daily, patterns are interrupted by the presence of light at night and have the capacity to alter rhythmic physiological or behavioral characteristics. Indeed, biorhythm disruption can lead to metabolic, reproductive, and immunological dysfunction depending on the intensity, timing, duration, and wavelength of light exposure. Light pollution, in many forms and by many pathways, is thus apt to affect the nature of host-pathogen interactions. However, no research has yet investigated this possibility. The goal of this manuscript is to outline how dim light at night (dLAN), a relevant and common form of light pollution, may affect disease dynamics by interrupting circadian rhythms and regulation of immune responses as well as opportunities for host-parasite interactions and subsequent transmission risk including spillover into humans. We close by proposing some promising interventions including alternative lighting methods or vector control efforts.

Introduction:

Urbanization and other anthropogenic changes can impact humans and wildlife in many ways. Among the many changes that co-occur with land development (i.e., noise pollution, toxic pollution, habitat loss), light pollution is one of the most influential and widespread. Light pollution, or the presence of unnatural levels of light at night, now covers 18.7% of the United States land mass at intensities that can be detected from space, and its spatial extent grew at a rate of 2.2% per year between 2012 and 2016 (Cinzano et al. 2001; Kyba, Kuester, et al. 2017). It

has been estimated that 80.7% of the U.S. population lives in urban areas; however, 99% of the U.S. population lives under light polluted skies (Cinzano et al. 2001; United States Census Bureau 2016).

Several organizations, such as the Fatal Light Awareness Program (FLAP) and The International Dark-Sky Association (IDSA), have begun to draw attention to this 'barely-addressed' conservation problem, which has permeated even our most protected and precious natural sites (Aubrecht, C., Jaiteh, M., & De Sherbinin 2010). Many forms of light pollution have been described by the international astronomical union (IAU), such as skyglow (i.e., the illumination of the atmosphere surrounding intensely illuminated regions) and glare (i.e., the presence of light at night that disrupts object perception) (International Dark-Sky Association 2009). Over-illumination (i.e. the excessive use of nighttime illumination to highlight memorials or buildings) at the 9/11 memorial, "A Tribute in Light", in New York City was recently highlighted as a major disruption of navigation to migrating birds, which caused individuals to become entrapped in the light (Van Doren et al. 2017). Our focus in the present manuscript, dim light at night (dLAN), is one of the most widespread forms of light pollution (International Dark-Sky Association 2009) with little sign of being moderated in spite of its effects on human and wildlife health (Falchi et al. 2011; Gaston et al. 2013a).

dLAN is emitted from street and billboard lights, vehicle headlights, and other sources that vary in duration, intensity, and wavelength. Typical natural light intensity ranges from 103,000 lux (sunny day) to 0.1 lux (moon on cloudy night), but levels of dLAN as low as 5 lux can have biological effects (Longcore and Rich 2004; Navara and Nelson 2007; Gaston et al. 2013a). Spectral composition of light differs across nighttime lighting regimes (Boyce 2003). Historically, incandescent and halogen lighting were favored, but there has been a recent switch

to energy efficient cool-white LEDs, which pose new risks to humans and wildlife (Frank 1988). Whereas orange hues emitted from traditional lighting are not harmless, broad spectrum LEDs predominantly emit short wavelengths, posing a greater threat to wildlife. Wildlife are particularly sensitive to these light forms due to the ecological relevance of blue light cues (Elvidge et al. 2010). Despite interannual consistency in light pollution, temporal heterogeneity exists throughout the night due to social and economic demands (Gaston et al. 2013b). Artificial lighting is critical to facilitate the flow of commerce, safe transportation networks, and public confidence in security of otherwise threatening areas. As the majority of life on earth evolved in the absence of nighttime illumination (with the exception of occasionally bright moonlit nights), light pollution poses a novel and spatiotemporally extensive threat to a wide variety of organisms (Verheijen 1985).

Known effects of light pollution on vertebrate traits relevant for the spread of infections:

Nearly 100 years ago, scientists observed that trees next to street lights maintain their leaves longer into autumn (Matzke 1936). Some argued the advent and widespread use of artificial light set in motion 'eternal summers', as light conditions began to lack the seasonal rhythmicity they had for millions of years (Wehr 2001). Indeed, the impact of light exposure at night became particularly apparent when scientists began to discover poor health conditions in humans performing night shift work. In addition to augmented risk of various types of cancer, metabolic syndrome, and type 2 diabetes, these individuals experiencing almost constant exposure to light suffered from higher rates of depression and insomnia than control populations (Viswanathan et al. 2007; Chepesiuk 2009; Pietroiusti et al. 2010; Pan et al. 2011). Because the hormone melatonin is regulated by light, many studies evaluated and eventually linked melatonin dysregulation to the above maladies (Lewy et al. 1980; Blask et al. 2002; Reiter et al. 2007;

Gooley et al. 2011; Niemelä and Dingemanse 2018). That research revealed that light pollution often led to arrhythmia in the suprachiasmatic nuclei (SCN), the part of the brain responsible for integrating light signals and synchronizing melatonin secretion (Brainard et al. 1988a; Reiter et al. 2007). However, other studies implicated direct effects of light pollution on circadian activities of cells in several tissues, as most tissues in the body possess molecular clocks, which are naturally synchronized by melatonin from the pineal gland (Balsalobre et al. 1998; Yamazaki et al. 2000). Receptors that detect light at night are most sensitive to peaks in the blue light part of the visible light spectrum (Elvidge et al. 2010). These findings led to the recent emphasis of limiting exposure to electronics rich in blue light at night (Figueiro et al. 2011; Wood et al. 2013). The 'night-shift' application implemented on smartphones, computers, and tablets by Apple and other manufacturers attempt to mitigate dLAN-associated disease risks by reducing blue light emissions (Nagare et al. 2019).

Similar observations as the above for humans have been made in lab rodents. Indeed, dLAN can have many physiological and behavioral consequences in these species, including altered immune function, metabolism, and behavior (Bedrosian et al. 2011; Fonken et al. 2013; Borniger et al. 2014). When exposed to light at night, nocturnal Siberian hamsters (*Phodopus sungorus)* have i) weaker ability to control bacterial infections ex vivo and ii) reduced cutaneous inflammatory responses in vivo (Bedrosian et al. 2011). Conversely, Swiss Webster mice have exaggerated febrile responses and over-express pro-inflammatory cytokines (Fonken et al. 2013). Exposure to dLAN in lab rodents also induces a dramatic, yet reversible, change in metabolism by shifting feeding patterns (Fonken et al. 2010, 2013). Knocking out genes involved in clock oscillations also induces diabetic and obesity-associated phenotypes (Fonken and Nelson 2014). Although many of these effects can be ameliorated when light pollution is ceased, exposure to

dLAN during development can sometimes have enduring effects, including the induction of anxiety-like behavior in adulthood (Borniger et al. 2014).

There is evidence that dLAN and other forms of light pollution affect wildlife too. For instance, songbirds that reside in the proximity of light pollution shift the timing of migration and reproductive behaviors ahead by several weeks (Dominoni et al. 2013a; Agarwal et al. 2015; Weishampel et al. 2016). Light pollution can also disrupt navigation ability in species that use visible light to coordinate large-scale movements (Frank 1988; Cochran et al. 2004; Wiltschko et al. 2007). As evidence, the incidence of building collision deaths is exceptionally high in light polluted cities, such as Galveston, Texas, which occur along major migration routes (Van Doren et al. 2017). Many species ranging from zooplankton to marine turtles suffer reproductive losses too and try to avoid light polluted habitats altogether (Moore et al. 2000; Weishampel et al. 2016). It is also well-known that multiple insect species display "fly to light" behavior, particularly towards sources of reflective polarized light. However, it has yet to be determined whether light pollution itself exacerbates such effects (Horváth et al. 2009; Gaston et al. 2013a; Shimoda and Honda 2013).

Reproductively, light pollution can work through many pathways to jeopardize wildlife prospects. Organisms that reproduce seasonally shift the timing of their gonadal development and courtship behavior nearly a month in advance. These changes occur because light at night is mistaken for extensions of daylength, which many species use to time gonadal recrudescence or maturation (Nelson 2011; Dominoni et al. 2013a). When given a choice, frugivorous bats prefer to forage in completely dark habitats, rather than those that are dimly lit, which indirectly shifts habitat preference in those reservoir species (Lewanzik and Voigt 2014). Even in dimly lit

habitats (i.e., 8 lux), European blackbirds intentionally avoid light exposure and roost under levels of light as low as 0.2 lux (Dominoni et al. 2014).

Given the many known effects of light pollution, it is surprising that effects of dLAN on host-pathogen interactions have largely yet to be investigated. Our main goal in this manuscript is to summarize the many paths by which light pollution could induce circadian or circannual rhythm disruption (figure 1.1a), and affect infection coping mechanisms at the individual level (figure 1.1b). A complementary goal is to summarize how such individual level effects could scale-up to alter disease dynamics at the population and community levels including spillover of zoonoses to humans (figure 1.1c).

dLAN effects on circadian clocks and melatonin:

Most biological processes within individuals follow a circadian (meaning 'about a day') rhythm. Blood pressure and heart rate, physical activity and food intake, hormone regulation, and immune system traits such as T cells concentrations in the blood and macrophage tissue migration are just a few examples (Aschoff 1966; Martí et al. 1993; ADAMOPOULOS et al. 1995; Keller et al. 2009; Arble et al. 2012). Most organisms show some form of circadian biorhythms mediated by autonomous intra-cellular circadian oscillators that function on an approximate 24-hour feedback loop (Bell-Pedersen et al. 2005a). A peak in copies of an RNA transcription factor, such as Per and Cry (Kornmann et al. 2007) and CLOCK and BMAL1 (Shearman et al. 2000), work together to allow organisms and their various organ systems to time events precisely (Albrecht et al. 1997; Reppert and Weaver 2002). CLOCK:BMAL1 heterodimers interact with Per and Cry to control the expression of timekeeping genes, processes that are independent of exogenous environmental changes (e.g., ambient temperature; (Maes et al. 1994)). Light pollution can disrupt clock actions in multiple ways. The absence of the

transcription factor BMAL1 leads to the depletion of Per gene expression and causes arrhythmia in the SCN can be generated; BMAL1 absence leads to a depletion of mPer1 and mPer2 (Bunger et al. 2000), compromising circadian rhythmicity. Circadian feedback loops were first described in retinal base neurons of the snail (*Bulla gouldiana*), but since then have been observed in multiple species (Binkley et al. 1978; Aronson et al. 1993; Welsh et al. 1995; Brandstätter et al. 2001). Intrinsic rhythms can be extrinsically entrained by zeitgebers, a German word meaning "time giver" (Pevet and Challet 2011). For example, rats possess a free-running endogenous rhythm of 24.25-hour days but synchronize to a 24-hour day by zeitgeber-induced melatonin secretion (Redman et al. 1983). Light, or photoperiod, typically serves as the circadian zeitgeber because it is the most reliable environmental cue an organism can use to predict future conditions.

Although climate has changed vastly since the origination of life on Earth, photoperiod has remained consistent (Crowley and North 1988). The integral role that light information plays in synchronizing physiological functions across taxa confirms that individuals have placed a heavy reliance on light cues for millions of years. Organisms developed these sensitive light reception and synchronization mechanisms long before light pollution appeared, which probably explains why light exposure at night has many negative effects. For example, in the presence of light pollution, Per and Cry genes could be expressed sporadically throughout the day, thus cells would not express oscillating genes in synchrony.

The indoleamine hormone, melatonin, the "chemical expression of darkness," is secreted into circulation to synchronize the aforementioned intracellular oscillators and thus coordinate daily rhythms within the body (Klein and Moore 1979; Reiter 1991). Melatonin is synthesized from tryptophan and serotonin precursors in the pineal gland prior to rapid release into

circulation (Reiter 1993; Tricoire et al. 2003). Although melatonin is primarily known for its role in maintaining biological rhythms, melatonin affects a variety of other physiological functions. Melatonin receptors are expressed with a daily rhythm in the coronary arteries of healthy individuals (Ekmekcioglu et al. 2001) and assist in thermoregulation during torpor or hibernation periods (Saarela and Reiter 1994). Melatonin is also intricately intertwined with metabolic processes exhibiting circadian fluctuations (Fonken and Nelson 2011). In light of the focus in the present manuscript, the extensive and diverse effects of melatonin on the immune system are of particular relevance. Melatonin enhances T cell proliferation and differentiation, antigen presentation by MHCII molecules, and cytotoxic activity upon antibody activation (Pioli et al. 1993; Esquifino et al. 2004; Konakchieva et al. 2018). It also amplifies in vivo production of IL-1, IL-6, and IL-12 (i.e., pro-inflammatory cytokines) secreted from helper T cells, which increases various immune activities and protects against encephalitis (i.e., inflammation of the brain), bacterial infection, and septic shock (Maestroni et al. 1988). It can also restore immune functions and reduce mortality following physiological trauma such as septic or hemorrhagic shock (Wichmann et al. 2018). One of the most potent effects of melatonin is to reduce collateral damage from inflammation and free radicals (Sasaki et al. 2002; Vijayalaxmi et al. 2004; Hardeland et al. 2012). In rats faced with spinal cord injury, heat stroke, acute pancreatitis, and diabetic pathologies, melatonin reduced inflammatory cytokine production and plasma lipid peroxidation levels (Esposito et al. 2008; Gülben et al. 2010; Lin et al. 2011; Agil et al. 2012). Indeed, genomic expression of the melatonin-induced anti-inflammatory profile has been confirmed in Raw cell (clonal stem cell) lines, which typically exhibit the common response of cells in the body (Ban et al. 2011). The combination of these melatonin-mediated immune modifications has implications for infection resistance (Boga et al. 2012). Evidence suggests that

melatonin can also delay the onset of viral infection and reduce viral-induced mortality (Maestroni et al. 1988; Ben-Nathan et al. 1995). Stimulation of the early-phase interferon responses (i.e., IFN-gamma) by melatonin also appears to enhance anti-viral efficacy of various leukocytes (Bonilla et al. 2004a). Apoptosis is also moderated by melatonin (Sainz et al. 2003). Some studies have noted higher infection resistance in human patients who received melatonin (Reiter et al. 2002).

Glucocorticoids, such as corticosterone in birds and cortisol in mammals, follow daily rhythms as well (Nelson 2011). Nadirs can be around sunrise in diurnal species, such as house sparrows, (*Passer domesticus*) or around sunset in nocturnal species, such as fruit bats (*Pteropus vampyrus*) (Widmaier and Kunz 1993; Breuner et al. 1999). Much as with metabolic incoordination described above, glucocorticoid dysfunction can influence variety of functions in the body including metabolism, mobilization and transport of resources, and, importantly, immune function. Indeed, acute elevation of glucocorticoids in response to a short-term stress event enhances immune function, whereas chronic elevation of glucocorticoids dampens immune function (Rich and Romero 2005). Great tits (*Parus major*) exposed to white light at night had significantly higher baseline corticosterone than those exposed to red or no light at night (Ouyang, de Jong, et al. 2015).

Effects of light pollution on multi-level disease dynamics:

There are many ways by which dLAN could affect the success and movement of parasites within and among hosts. At the organismal level, the ability to avoid infection outright (exposure), prevent infection upon exposure (susceptibility), reduce parasite burden once infected (resistance), and maintain fitness throughout the course of infection (tolerance) partly determines the probability of transmission to another host or vector (figure 1.1b), which are

collectively termed host competence (Gervasi et al. 2015). Much heterogeneity in competence exists within and across hosts, contingent on individual health and environmental context (figure 1.1b). Individuals with high competence, namely superspreaders, maintain high fitness when infected (figure 1.1b). Variation in aspects of competence ultimately describes how individuals cope with infection, including morbidity (sickness) and mortality (fatality), and how they contribute to disease spread within communities. An explicit focus on competence allows us to investigate multiple facets of physiology and behavior, including those with circadian patterns detailed above, and determine how pathogens move among individuals in populations (figure 1.1c). Below, we focus on West Nile virus (WNV) infection as an example of how dLAN might affect disease dynamics in nature.

dLAN effects on infectious disease dynamics in wildlife:

The intricate balance among natural biorhythms could be disrupted when individuals are exposed to dLAN. The perception of day time would likely become skewed when individuals receive light cues sporadically or consistently throughout the night, but there have been few studies that have explicitly related dLAN exposure to circadian disruption on a molecular or physiological level. It is known that almost every function in organisms operate with a daily rhythm, so it is likely that in the event that circadian disruption occurs, the immune functions responsible for coordinating and executing pathogen control will be affected as well (Reppert and Weaver 2002). Many studies have found organismal consequences that implicate clock and melatonin disruption. For example, great tits (*Parus major*) exposed to white light at night maintain higher activity levels than controls, and American robins (*Turdus migratorius*) exposed to light pollution sing earlier in the morning (Miller 2006a; Ouyang et al. 2017).

Contradictory cues induced by light pollution can impact seasonal timing of activities by individuals. In European blackbirds, individuals exposed to light pollution elevated testosterone levels and accelerated testicular development by nearly a month earlier in the season (Dominoni et al. 2013b). Additionally, dLAN exposed birds began to sing both earlier in the season and earlier in the morning than control individuals (Dominoni et al. 2013b). Redheaded buntings (*Emberiza bruniceps*) exposed to light pollution have altered migrational timing, which can affect reproductive success upon arrival (Agarwal et al. 2015). Although the seasonal effects of light pollution on host immunity are not yet clear, the shift towards reproductive and migratory life histories may indicate a shift away from investment in immune defenses. It has been well observed in nature that during reproduction, individuals sacrifice immune function and invest in courtship behavior, gonadal development, and provisioning their young (Sheldon and Verhulst 1996).

West Nile virus:

Not long after its initial introduction to the United States, WNV became widespread and influential. Since WNV is a vector-transmitted multi-host pathogen, there are many facets of the transmission cycle that can be impacted by light pollution. This zoonotic arbovirus was introduced to New York in 1999 (Campbell et al. 2002) and is now found in 326 bird species (Kilpatrick et al. 2006), which vary in competence. WNV has been isolated from 65 mosquito species, but mosquito species also differ in their competence for WNV (Marra et al. 2004). While Culex spp. are the most competent vectors, WNV has been found in Anopheles spp. and Aedes spp. as well (Apperson et al. 2002). WNV regularly spills over from reservoir species to hosts that do not promote further transmission of pathogen such as horses, other mammals, humans, and rarely reptilian species (Miller et al. 2003; Marra et al. 2004). With over 2.5 million

West Nile infections between its introduction in 1999 and 2011, WNV remains a threat to both wildlife and humans (Petersen et al. 2013).

Putative effects of dLAN on host competence for WNV:

After a vertebrate is infected by WNV, early viral replication occurs in a variety of local cells when virions bind to an unknown membrane receptor, enter the cytoplasm, and reassemble the capsid in the endoplasmic reticulum to promote replication. Eventually, virions are re-released into circulation via exocytosis (Suthar et al. 2013). The RIG-I-like receptor (part of the RLR pathway), combined with MDA5 and LGP2, is activated upon detection of WNV RNA (Suthar et al. 2013). Zebra finches inoculated with WNV upregulate the transcription of several genes including DDx58 and IFIH1 (which control RIG-1 and MDA5) to promote the production of RLR throughout the course of infection (Newhouse et al. 2017). Upon activation, the RLR cascade stimulates production of interferons (IFN), which coordinate subsequent antiviral immune effectors (Loo and Gale 2011). MAVS, a signaling molecule in the RLR pathway, has been implicated as the most important aspect of the anti-WNV defense cascade. Mice with MAVS-deficiency have an exaggerated inflammatory response and dysregulated T-cell responses and humoral responses to WNV (Suthar et al. 2013). Type-1 IFN signaling and NOD-like receptor activation also play a role in control of WNV.

There is also circadian variation in the propensity of host immune defenses relevant to WNV. Tissue emigration of cellular immune defenses and phagocytic ability of macrophages required for the initial control of WNV infection in Langerhans cells are robust during the active period of the host (Scheiermann et al. 2013). Some T cell types peak in circulation during the day as well (Bollinger et al. 2010).

Unlike mediators of WNV resistance and susceptibility, there has been almost no work to elucidate how hosts tolerate WNV infections. Experiments on laboratory rodents revealed that dLAN exposure might exaggerate pro-inflammatory cytokine signaling and fever responses (Fonken et al. 2013). Over-exuberant inflammation has recurrently been associated with low tolerance (Råberg et al. 2009; Sears et al. 2011). Several studies in humans have also found that night-shift work increases the incidence of autoimmune disorders (Boscolo et al. 2008), further implicating self-inflicted damage as a common outcome of dLAN exposure (Warkany 1986). Given that WNV infection can lead to West Nile fever, extreme cases of which include lethal encephalitis, it remains a possibility that dLAN will generally compromise vertebrate host health when infected.

In spite of reasons for concern, the direct effects of dLAN on melatonin and viral resistance are largely unknown. dLAN clearly has the capacity to alter within-host viral replication, but to what extent these effects are mediated by changes in the regulation of melatonin are as yet obscure (Carrillo-Vico et al. 2006; Navara and Nelson 2007). As emphasized earlier, melatonin levels increase in the dark, and are suppressed in the presence of light, including that originating from light pollution sources. A direct relationship between melatonin and WNV resistance pathways has not yet been established, but it is plausible. For example, individuals exposed to dLAN would not secrete natural levels of melatonin at night, which may lead them to produce an exaggerated and damaging inflammatory response and/or inappropriate T-cell mediated responses to WNV (Carrillo-Vico et al. 2005). Individuals who do not possess natural levels of melatonin at night, subsequently, may be more competent for WNV, either by being disproportionately susceptible, remaining infective for longer, and/or experiencing different mortality rates than individuals exposed to natural photoperiods. Some

individuals might even be able to tolerate higher viral titers for longer, behaving normally but with higher burdens than unpolluted conspecifics (Gervasi et al. 2017). Individuals might also be more attractive or conspicuous to vectors of WNV.

Indeed, many forms of tolerance are apt to be affected in dLAN, particularly when they involve behaviors regulated by melatonin (e.g., timing and duration of arousals, selection of roosting sites, rates of interspecific encounters). In the context of WNV, mosquito-directed behaviors are particularly important to investigate. There is clear evidence that dLAN extends nighttime activity in birds by several hours earlier in the morning and throughout the course of the entire night (Miller 2006b; Ouyang et al. 2017). This extension of activity may increase interactions among species, especially the crepuscular and nocturnal feeding vectors of WNV. On the one hand, non-roosting hosts may be easier blood-meal targets for mosquitoes as they often forage in the vicinity of mosquito breeding habitat (i.e., small bodies of still water), but on the other, active hosts might be better able to avoid or defend against vectors.

Consequences of light pollution exposure for population and community level disease dynamics:

Heterogeneity in WNV competence within and among hosts will affect the probability of transmission among hosts (Martin et al. 2016a; VanderWaal and Ezenwa 2016). Demographic rates such as fecundity and mortality combined with host competence determine the potential for emergence and maintenance of WNV epidemics at the population-level. Once we have data on how dLAN affects aspects of competence at the individual-level, we can link within-organism processes to population-level transmission dynamics using compartmental epidemiological models (e.g., SEIR; Susceptible, Exposed, Infectious and Recovered). A useful metric that summarizes the potential of a pathogen to cause an outbreak when introduced to a wholly

susceptible population is the pathogen basic reproductive number, R_0. This term aggregates model parameters relating to transmission and recovery processes and demographic parameters determining population size in hosts and vectors (box 1.1). For a pathogen to cause an outbreak, R_0 must be greater than one (i.e., the index case must generate at least one new infection during its infectious period). In the presence of light pollution, a pathogen may become established more easily (e.g., at a low vector population size) and persist in the population via changes in host traits that contribute to infectivity, mortality, rates of recovery or other factors.

Many factors surrounding vector activity, abundance, and behavior affect R_0 parameters that increase and/or decrease outbreak potential. Culex nigripalpus, a known vector of WNV, is attracted to blue wavelengths emitted by LEDs (Ali et al. 1989). The presence of light at night, especially that of cool white LEDs, may increase the aggregation of mosquitoes near light sources and vector density (V). The maintenance of Culex mosquito abundance later in the fall could also prolong WNV transmission season (figure 1.2). Crepuscular feeding vectors can increase biting rate (b), throughout the night as they may misperceive light pollution as dusk or dawn and extend activity later into the night (figure 1.3). However, increased host activity at dawn and dusk may enhance vector avoidance behavior which would decrease biting rate (b) (Ouyang et al. 2017). Although not much is known about vector survival in light polluted habitats, this may affect the ability of vectors to transmit WNV further (i.e., fractions of mosquitoes surviving latent period). Moreover, several studies have found that mosquitoes thrive on “urban heat islands” (Paz and Albersheim 2008), so other aspects of urban sites, where dLAN predominates, could amplify or complicate dLAN effects on disease dynamics.

Exposure to pathogens might also be affected by dLAN through extended contact rates between hosts and vectors (Wright and Gompper 2005; Tompkins et al. 2011; Brearley et al.

2013; Becker and Hall 2014). Resource clumping strongly correlates with host density and contact rates within and among species (Bradley and Altizer 2007a). Combined, these effects have been directly related to increased parasite prevalence in fragmented habitats (Wright and Gompper 2005). WNV antibodies are often more prevalent in suburban avian reservoir hosts, perhaps because individuals are exposed to more infected vectors in these light-polluted habitats (Gibbs et al. 2006b). Many vertebrates prefer dark nights and actively avoid light at night (Dominoni et al. 2014; Lewanzik and Voigt 2014). In small habitat patches, it is possible that species are driven away from light sources at night along edges and increase contact rates and thus opportunity for transmission from vector to the host and from the host to the vector and/or nocturnal foraging vector density (V) further by congregating densely in darker niches.

Additionally, much evidence so far suggests that exposure to dLAN could increase host susceptibility by altering viral resistance mechanisms. Pathogens can be eliminated by the immediate innate immune responses, which are hindered by dLAN in laboratory rodents (Bedrosian et al. 2011; Fonken et al. 2013) and later during infection by adaptive immune responses (Cissé et al. 2017). For example, aspects of humoral immunity such as TNF-α and IL-6 play a large role in stimulating early immune responses but induce a great amount of immunopathology if not well regulated (Biron 1998; Fonken et al. 2013). The ability to eliminate pathogen burden and attenuate damage induced by the pathogen will determine whether and for how long hosts are infectious to vectors (IPH), however more work needs to be done to understand how the infectious period of vectors to hosts (IPV) may change with respect to light pollution (box 1.1).

As susceptibility is in part determined by within-individual characteristics, we assume that highly susceptible individuals are also more likely to become infected upon exposure.

European blackbird (*Turdus merula*) populations in urban habitats have greater longevity (Evans et al. 2009). Avian urban-exploiter species often have greater over-winter survival rates as well, due to the availability of resources throughout the season (Luniak 2004). Whether longevity decreases outbreak potential in hosts previously exposed (i.e., non-competent hosts [N]) to an immunizing pathogen such as WNV is complex, in part due to the lack of information on whether long living individuals are susceptible or resistant.

Several studies, including those on Lyme disease and WNV, found that stressors such as habitat loss or resource depletion will result in the reduction of community diversity, leaving primarily competent host species, enhancing transmission (Allan et al. 2003; Bradley and Altizer 2007a; Bradley et al. 2008). Although also an effect of urbanization, avian diversity is significantly lower in light-polluted regions than in those that are not (Blair 1996; Marzluff 2015). The most prominent bird species that remain (i.e., House sparrows, American robins, etc.) are those that serve as reservoir hosts of WNV (Kilpatrick et al. 2006). Competent host abundance (H) increases R_0 and transmission potential. Indeed, it's been suggested that the emergence of WNV in light polluted regions may be in part due to the disproportional abundance competent (H) hosts (Ortega-Álvarez and MacGregor-Fors 2009; Centers for Disease Control and Prevention 2017).

Conclusions:

As emphasized above, there are many ways in which light pollution can influence within and among individual disease dynamics. Although vector-transmitted diseases are the focus of this manuscript, contact, airborne, and sexually transmitted parasites are potentially influenced as well. Host competence to Mycoplasma gallisepticum, a contact-transmitted mycobacterium that causes conjunctivitis, is greatest among house finches who congregate around feeders in

developed habitats (Adelman et al. 2015). Host competence may increase further in the presence of light pollution, increasing contact rates and possibly transmission. Sexually transmitted pathogens may have an increased transmission rate among conspecifics that extend their reproductive behaviors for longer periods during the day and over more months throughout the year in the presence of light pollution (Dominoni et al. 2014). It is important to emphasize that light exposure will unlikely have the same consequences for all disease systems.

Recommendations can be provided to city planners and vector control agencies to prevent these potential outbreaks of infectious disease. Alternative lighting sources such as motion sensitive or timed lighting may limit sleep disturbance and suppression of melatonin, as well as activity levels of both hosts and vectors throughout the night. Switching from cool white to warm white LEDs will not eliminate the detriments to humans and wildlife but may limit the extent to which individuals suffer from blue light signals present throughout the night. Predictive disease modeling that integrates the effects of light pollution on model parameters may allow researchers to identify communities most susceptible to disease emergence and redirect vector control efforts to maximize outbreak prevention and minimize economic burden.

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Author contributions:

M. E. Kernbach contributed to conceptualization and writing; R. J. Hall contributed to conceptualization, formal analysis, visualization, and writing; N. Burkett-Cadena contributed to conceptualization, visualization, and revision; T. R. Unnasch contributed to conceptualization

and supervision; L. B. Martin contributed to conceptualization, funding acquisition, supervision, and writing.

There is no ethical statement for this study.

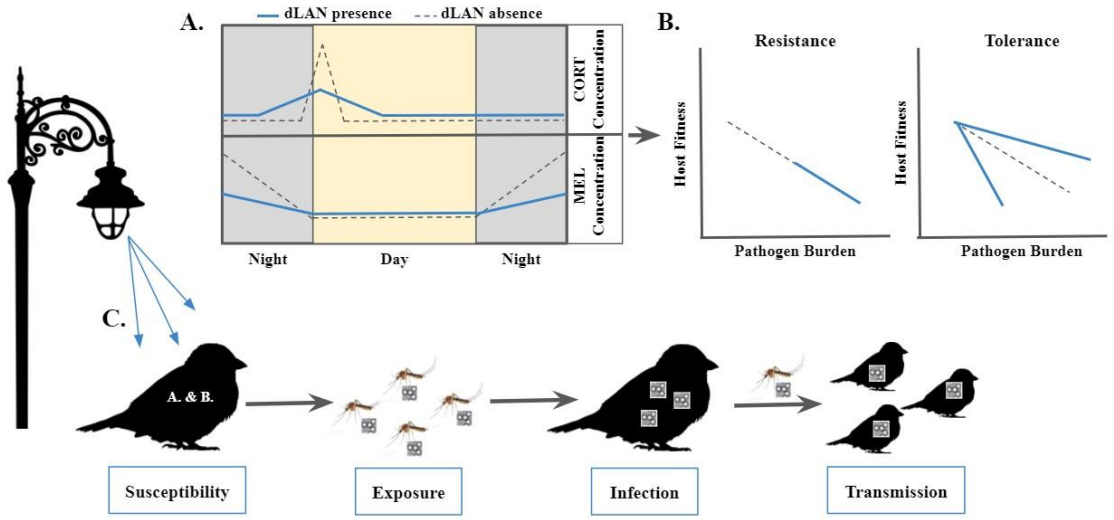

Figure 1.1:

The mechanisms by which dLAN can influence disease dynamics at the (A and B) within-individual and (C) among-individual levels. Hormonal dysregulation is depicted in (A) where the solid line represents individuals exposed to dLAN and the dashed line represents individuals who are not. In (A and B), individuals exposed to dLAN may increase corticosterone earlier in the morning due to the misperception of time of day but reach a lower peak concentration. In (C), individuals exposed to dLAN will attenuate melatonin secretion at night but may still produce low levels if dLAN is perceived as sunset. Hormonal alteration can in turn influence pathogen resistance and infection tolerance in (B). The image entitled "Resistance" shows that individuals exposed to dLAN may have a higher pathogen burden than those who are not exposed to dLAN. The graph entitled "Tolerance" shows that dLAN exposed individuals may have higher tolerance (upper solid line) and maintain fitness throughout the course of infection. Conversely, individuals exposed to dLAN may have lower tolerance (lower solid line) if they are unable to mount specific immunity and incur damage from inflammatory responses and viral replication. (C) dLAN altered circadian hormone fluctuations, resistance, and tolerance contribute to an individual's susceptibility to acquiring infection.

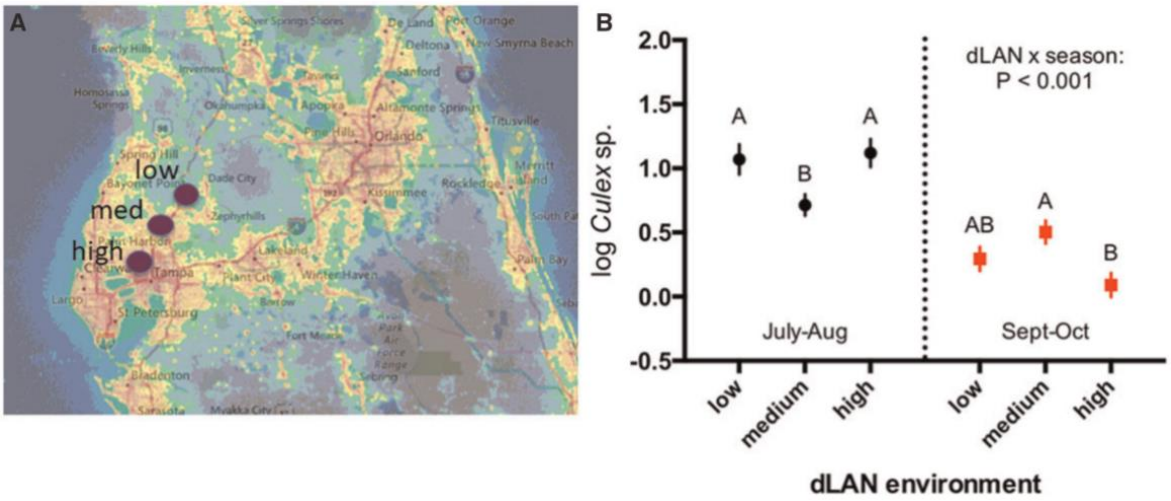

Figure 1.2:

(A) Light pollution across the Tampa Bay region; lighter shading denotes the highest light pollution. (B) Culex sp. numbers over time and by light-pollution environment (dLAN). All light-polluted locations were similar in habitat characteristics, rainfall, and temperature; however, we do acknowledge that there is likely some slight variation between sites. These sites were chosen because they were an intermediate between suburban and natural patches of habitat. Reservoirs, vectors, and humans actively come into contact at these sites, making them representative of potential origins of spillover events. Bars are means ± 1 SE. Letters denote group membership by post hoc comparisons. Dash line separates seasons, as a generalized linear model (with a negative binomial distribution for count data) revealed a significant season x site effect ($P < 0.001$). These data outline the significant interaction between light pollution sites and mosquito vector abundance across the transmission season. Culex sp. mosquitoes remain abundant later into the transmission season, which may indicate the exploitation of intermediate levels of light pollution.

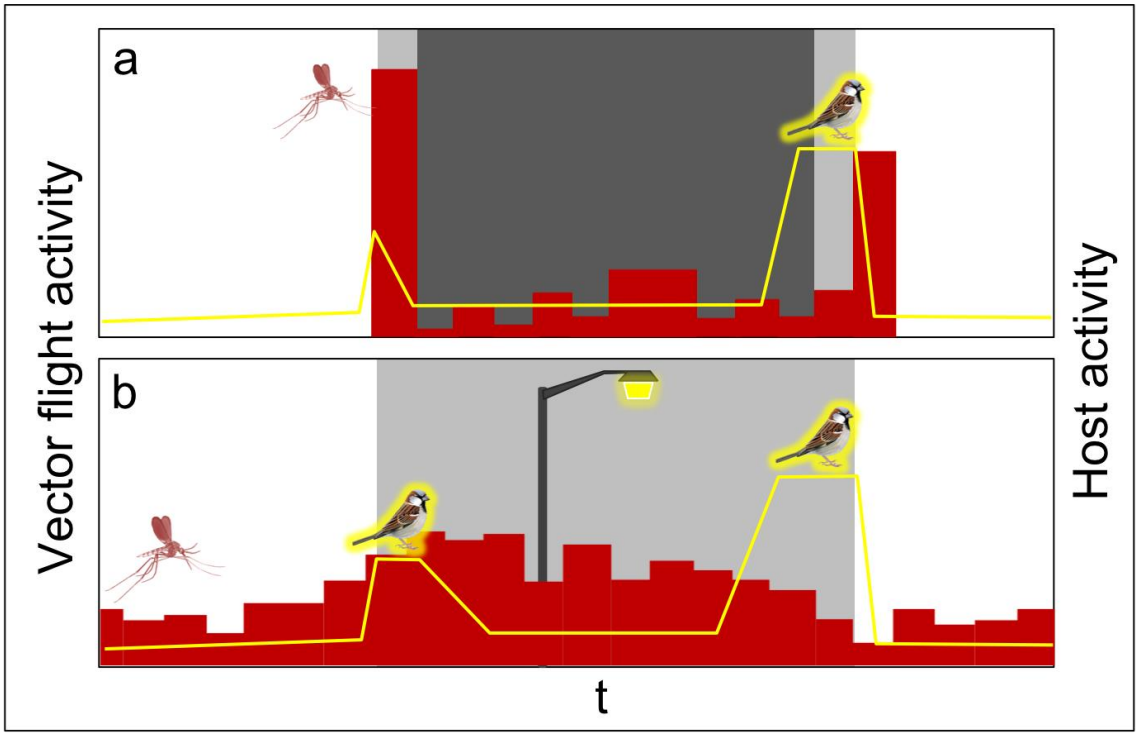

Figure 1.3:

Potential differences in mosquito–host interaction under natural (A) and light-polluted (B) conditions. Mosquitoes are most active during dawn and dusk as shown by the bars in panel (A). The yellow lines represent house sparrow activity level at dusk and dawn. Time (t) is defined along the x-axis, with the shaded background regions signifying nighttime hours. The bottom panel (B) shows an increase in house sparrow activity at dusk later into the night and earlier in the morning, and a greater overlap with mosquito activity that dissipates from peaks at dawn and dusk to fairly consistent feeding patterns throughout the entirety of night.

Box 1.1:

The expression for the pathogen basic reproductive number R_0 is based on the model derived by Wonham et al. (2004), with a slight modification that mosquitoes distribute their bites evenly among non-competent and WNV-competent hosts. The non-competent hosts could include non-competent, diluting species, and/or non-competent individuals of the focal species (i.e. those that are immune following prior exposure).

R_0 for a vectored parasite can be written as:

$$R_0 = \sqrt{b^2 p_{VH} p_{HV} \frac{V}{N+H} \frac{H}{N+H} f_L IP_H IP_V}$$

In which b represents biting rate, p_{VH}, p_{HV} represent probability of vector to host and host to vector transmission respectively, IP_H, IP_V represent infectious period of host and vector respectively, f_L is the fraction of mosquitoes surviving WNV latent period, V represents vector density, N is non-competent host density, and H represents WNV-competent host density. Here, we replace the vector to host ratio (V/H) from Wonham et al. (2004) with the ratio of vectors to all bitten species (non-competent and competent, $V/N+H$) and the probability that the bitten host is WNV-competent ($H/N+H$). R_0 allows us to integrate both host and vector characteristics with population and community dynamics to determine how the potential for transmission may change across contexts (i.e. light pollution exposure). The weight each parameter has on outbreak potential is also considered here, which allows us to identify and empirically model the impact each factor will have on disease dynamics in a particular system.

CHAPTER I:

LIGHT POLLUTION INCREASES WEST NILE VIRUS COMPETENCE OF A UBIQUITOUS PASSERINE RESERVOIR SPECIES

Note to Reader:

This is a pre-copyedited, author-produced version of an article accepted for publication in Proceedings of the Royal Society B following peer review. The version of record: Kernbach ME, Newhouse DJ, Miller JM, Hall RJ, Gibbons J, Oberstaller J, Selechnik D, Jiang RHY, Unnasch TR, Balakrishnan CN, Martin LB. 2019. Light pollution increases West Nile virus competence of a ubiquitous passerine reservoir species. Proc R Soc B Biol Sci 286:20191051.

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Abstract:

Among the many anthropogenic changes that impact humans and wildlife, one of the most pervasive but least understood is light pollution. Although detrimental physiological and behavioral effects resulting from exposure to light at night are widely appreciated, the impacts of light pollution on infectious disease risk have not been studied. Here, we demonstrate that artificial light at night (ALAN) extends the infectious-to-vector period of the house sparrow (*Passer domesticus*), an urban-dwelling avian reservoir host of West Nile virus (WNV). Sparrows exposed to ALAN maintained transmissible viral titers for two days longer than controls but did not experience greater WNV-induced mortality during this window. Transcriptionally, ALAN altered the expression of gene regulatory networks including key hubs (OASL, PLBD1, TRAP1) and effector genes known to affect WNV dissemination (SOCS). Despite mounting anti-viral immune responses earlier, transcriptomic signatures indicated that ALAN-exposed individuals likely experienced pathogen induced damage and immunopathology, potentially due to evasion of immune effectors. A simple mathematical modelling exercise indicated that ALAN-induced increases of host infectious-to-vector period could increase WNV outbreak potential by ~41%. ALAN likely affects other host and vector traits relevant to

transmission, and additional research is needed to advise management of zoonotic diseases in light polluted areas.

Introduction:

Among the many anthropogenic changes that impact humans and wildlife, one of the most pervasive but least understood is light pollution (Leu, M., Hanser, S., & Knick 2008). Artificial light at night (ALAN) is a common form of light pollution worldwide, in both urban centers and non-urban areas including farms, airports, warehouses, and even natural areas such as greenspaces near roadways (IDA 2009; Falchi et al. 2016). Early research on human health found that individuals working throughout the night routinely suffer higher rates of Type II diabetes, heart conditions and other non-infectious maladies compared to day-working staff (Navara and Nelson 2007). In domesticated rodents, exposure to short-wavelength light at night, similar to that of cool-white LEDs, has been linked to metabolic dysregulation, immunosuppression, and the development of some cancers (Navara and Nelson 2007). Levels of blue light (420-480nm) as low as 0.2 lux can suppress melatonin secretion in humans (Thapan et al. 2001; Pauley 2004), and in wildlife, comparable forms of ALAN alter many behavioral, life history, and physiological traits (Witherington and Martin 2000; Dominoni 2015).

Despite the diverse and strong effects of ALAN, no study has yet investigated whether and to what degree it might affect infectious disease risk, which is surprising given that many hosts and vectors use light cues to coordinate daily and seasonal rhythms (Hastings et al. 1985; Schibler 2005). Light is among the most reliable environmental cues, and light regimes induce temporal fluctuations in immune defenses and other factors that influence risk of infection (Bedrosian et al. 2011). Our goal here was to discern whether ALAN could alter zoonotic disease

risk for humans and wildlife by changing the ability of a reservoir host to amplify virus for subsequent transmission, and if so, to implicate some molecular mechanisms that might be responsible for these changes. Differences in transmission ability between individual hosts, which we term host competence (Paull et al. 2012; Barron et al. 2015; Gervasi et al. 2015), is partly mediated by endocrine-sensitive physiological processes (Bedrosian et al. 2011; Martin et al. 2016b). For example, melatonin and glucocorticoids both affect host behaviors driving exposure risk as well as immune defenses underlying resistance to infection and transmissibility (Sapolsky et al. 2000; Ouyang, De Jong, et al. 2015).

We investigated ALAN effects on WNV infections in house sparrows (*Passer domesticus*) because this species is among the most common infection reservoir in light-polluted areas and a close commensal of humans (Chamberlain et al. 2007). House sparrows are also among the more competent hosts for WNV (Nicholas Komar 2003), which we chose as our pathogen for two reasons: (i) more than 46,000 cases of WNV-induced human disease have been reported across the US since its introduction to New York in 1999 (Centers for Disease Control and Prevention 2017), and (ii) following its emergence in the US, WNV decimated avian populations, particularly corvids and other passerine species that commonly occupy light-polluted habitats (Marra et al. 2004).

Methods:

Capture and housing

We captured house sparrows using mist nets at two sites in the Tampa Bay area with comparable levels of light pollution. All birds were captured between the hours of 5:30 and 9:30 AM. Birds were then transported to the University of South Florida vivarium where they were housed individually in 13”x15”x18” cages for the next 7-25 days in visual and audial proximity

to each other. In captivity, birds were housed under ALAN/treatment (12h light: 12h ~8 lux artificial light; N= 23) or natural light/control conditions (12h light: 12h dark, N=22). Food (mixed seeds) and water were provided *ad libitum* throughout the study. Following this initial period, all birds were transported to the USF Biosafety level-3 (BSL-3) suite where they were kept individually in similar cages but inside bioBUBBLE containment systems (bioBUBBLE Inc, Fort Collins CO). Light conditions during this period were identical to conditions described above.

Dexamethasone suppression test

To examine HPA function, we performed the dexamethasone (DEX) suppression test twice: once at capture and once after 7-25 days in captivity. Blood samples for the DEX suppression test required: (1) a baseline corticosterone (CORT) sample obtained within 3 minutes of capture, (2) a post-stressor blood sample collected after 30 minutes of restraint in a cloth bag following initial capture, which was immediately followed by a DEX injection (s.q., 28ug dissolved in 50 uL peanut oil), and (3) a final sample collected 1h after injections. Blood samples were collected from the brachial vein using sterile 26-gauge needles and microcapillary tubes, and serum was frozen at -20°C until hormone assay.

WNV infection

Following transfer to the BSL-3 facility, we exposed all birds (N=45) to 10^1 (PFU) of WNV, NY99 strain via subcutaneous inoculation within the same time frame (ALAN exposure duration varied to determine if time of ALAN exposure affected infection outcomes but was not a significant term in models, so it is not further addressed). Following WNV exposure, all birds were maintained under the same light regimes while we sampled serum on days 2, 4, 6, and 10 to quantify WNV viremia in circulation (Gervasi et al. 2017). We also measured body mass (to 0.1g

prior to and on each blood sampling day) to assess effects on individual health and group WNV-induced mortality (mortality was closely monitored from the point of exposure through day 10, when the experiment was concluded). Serum and whole blood samples were frozen at -20°C until extraction and qPCR or sequencing methods protocols were performed.

Corticosterone assays

CORT concentrations were quantified in serum using an enzyme immunoassay (EIA) kit from Arbor Assays (Arbor Assays, Ann Arbor, MI, product # K014-H5; 13). Samples were run in duplicate and standardized across plates. Concentrations were derived from known values along the standard curve, and all values fell within the curve.

RNA extraction and RT-PCR for viremia

WNV RNA was extracted from 10uL of stored serum using the Qiagen QIAmp Viral Extraction Mini Kit (Qiagen Cat. No. 52906). Viremia was quantified using quantitative real-time polymerase chain reaction (qRT-PCR) using a one-step Taqman kit (iTaq Universal Probes One-Step Kit; Bio-Rad Cat. No. 1725141). Standards were extracted from known concentrations (via plaque-assay) of WNV stock and quantified using the same methods listed above. Forward and reverse primers and probe sequences are listed in supplementary text (Gervasi et al. 2017). All samples were run in duplicate with negative controls.

WNV and corticosterone statistical analyses

Linear mixed models were used in R studio and SPSS to analyze most data, after log10 transformation of WNV viremia which produced variable distributions amenable to model assumptions. Statistics in the supplementary text confirm that analyses conducted in both programs were consistent. We first modeled viremia as a dependent variable in which ALAN conditions, days post-exposure and their interactions were fit as fixed effects. Given the

repeated-measures nature of the study, we used individual bird as a random effect. We modeled WNV tolerance in a similar fashion except that in these models, body mass change (from pre-WNV values) was the dependent variable. To account for unequal variance among groups, we performed several iterations of this model that accounted for this and compared them using an ANOVA; because the models were not significantly different, we reported the conservative estimates provided by the linear mixed model allowing for unequal variance (supplementary text). We included all data over the course of the entire infection and used days post-exposure, ALAN, and WNV titer (as a continuous covariate) and all two- and three-way interactions as predictors (Adelman et al. 2015). As above for viremia, individual bird was included as a random effect and body mass prior to WNV exposure was included as a covariate to control for pre-existing differences in vigor among individuals (Adelman et al. 2015). CORT data were analyzed similarly to viremia with the following exceptions. First, we conducted an omnibus mixed model in which CORT was the dependent variable and time in captivity, ALAN and their interaction were fixed effects with individual bird as a random effect. In a second series of models, we analyzed each of four HPA traits separately: baseline CORT (first measurement), post-stressor CORT (second measurement), post-dexamethasone CORT (third measurement), and total CORT (area under the total concentration curve, AUC), as each variable serves a distinct physiological role across the time period which they were sampled and hence could affect WNV competence differently. In these simpler mixed models, time was binary (at capture versus after a period of captivity but prior to WNV exposure), but otherwise model composition was identical to the omnibus models. Finally, we used Cox regression to assess effects of ALAN on direct mortality risk to WNV. We set alpha to < 0.05 and used SPSS v24 and GraphPad Prism for analyses and figure production, respectively.

Outbreak potential modeling

Using a previously developed model (Wonham et al. 2004) the pathogen basic reproductive number can be written:

$$R_0 = \sqrt{\frac{a^2 bcIP}{m}\frac{k}{k+m}\frac{M}{B}}$$

where a is the bite rate, b and c are the respective probabilities that birds and mosquitoes are infected by a bite, IP is the bird host infectious period, m is the mosquito mortality rate, k is the WNV development rate in the mosquito and M/B is the ratio of adult mosquitoes to birds. Assuming that $ALAN$ only affects host competence traits measured in the experiment (i.e. infectious period) and does not affect vector traits, the proportionate change in the reproductive number due to ALAN is

$$r = \frac{R_0(ALAN) - R_0(control)}{R_0(control)} \times 100\% = \left(\sqrt{\frac{IP(ALAN)}{IP(control)}} - 1\right)$$

The infectious periods of ALAN and control birds were estimated as the total amount of time for which viremia exceeds the 10^5 transmission threshold, yielding respective values of approximately 4 and 2 days. This results in a change in outbreak potential, $r = 100 \times \left(\sqrt{4/2} - 1\right) \approx 41\%$.

To estimate the absolute outbreak potential in the presence and absence of ALAN ($R_0(ALAN), R_0(control)$ respectively), we estimated the remaining component parameters of the reproductive number from experiments and the literature; where ranges of parameters were reported, we took the approximate midpoint value (supplementary text). The parasite development rate and adult mosquito mortality were calculated as the inverses of the reported extrinsic incubation periods of WNV and *Culex quinquefasciatus*, a common and competent

WNV vector in Florida, lifespan, respectively (David et al. 2012). The resulting values for R_0 in the presence and absence ALAN were $R_0(ALAN) = 12.66$ and $R_0(control) = 8.95$.

Whole blood RNA extraction and sequencing

RNA was processed and sequenced following the protocols described by Louder et al. (Louder et al. 2018). Following sequencing, reads were adapter trimmed with Trim Galore v0.3.8 (Martin 2011). Trimmed reads were then aligned to the zebra finch (*Taeniopygia guttata*) v3.2.4 reference genome (Warren et al. 2010) with STAR v2.5.3 (Dobin et al. 2013) specifying '--sjdbOverhang 74'. We assigned Ensembl gene IDs and quantified reads with htseq-count v0.6.0 (Anders et al. 2015) specifying '-s reverse' to account for the strand specific library preparation. A total of 9,688 genes with an average count value > 5 across all 18 samples were used to generate a count matrix and retained for downstream analysis.

RNA sequencing – differential expression

DEseq2 v1.21.21 (Love et al. 2014) was used to read in the count matrix and perform normalization of counts to sequencing depth. Normalized counts of each sample were then rlog transformed and visualized via Principle Components Analysis (PCA) within the pcaExplorer R package (Figure A2.4; (Louder et al. 2018)). We generated the DEseq model '~ nested.ind + TreatDay', where 'nested.ind' accounts for repeated sampling of individuals and 'TreatDay' is a grouping variable of the interaction between treatment and day (i.e., 4 groups: 2dpe-Control, 2dpe-ALAN, 6dpe-Control, 6dpe-ALAN). We then extracted results from the model selecting pairwise contrasts between 2dpe-ALAN vs 2dpe-Control, 6dpe-ALAN vs 6dpe-Control, Control-6dpe vs 2dpe, and ALAN-6dpe vs 2dpe. In each case, we used the 'lfcShrink' function within DEseq2 to perform log2 fold change shrinkage to enhance visualization of individual gene expression plots. DEseq2 performs a Wald test followed by false discovery rate (FDR; (Martin

2011)) correction to determine differential expression (DE). We classified genes with an FDR <0.10 as DE, which is the standard for DEseq analyses and default determined by the package; values listed for the FDR <0.05 are in the supplementary text. As the interaction term between treatment and day on viremia was significant on day 6, we were primarily interested in the effects of light pollution on gene expression at this point. We further filtered the comparisons of 6dpe-ALAN vs 2dpe-ALAN and 6dpe-ALAN vs 6dpe-Control. For 6dpe-ALAN vs 2dpe-ALAN, we eliminated genes that were also DE in the 6dpe-Control vs 2dpe-Control. As the control birds were also infected with WNV, this isolates the genes responding to both WNV and ALAN treatment in the 6dpe-ALAN birds. For 6dpe-ALAN vs 6dpe-Control, we removed genes also DE in the 2dpe-ALAN vs 2dpe-Control comparison. This eliminates genes that did not change in relative expression level between sampling points. In each of these filtration steps, we only removed genes with the same regulation pattern (i.e., up or down), as we were interested in genes that show the opposite expression patterns between days and/or treatments.

For each of the four DEseq2 comparisons, we performed gene ontology (GO) analysis with the GOrilla webserver (Eden et al. 2009) after converting zebra finch Ensembl IDs to gene symbols in the Ensembl-BioMart web server (Kinsella et al. 2011; Zerbino et al. 2018). A total of 7,321 of 9,688 had associated gene symbols. We then sorted DEseq2 results by ascending FDR value and used the entire ranked list of 7,321 genes to perform GO analysis. A GO category was considered significantly enriched if the FDR value was < 0.05.

Lastly, we performed cell type enrichment analysis with the CTen tool (Shoemaker et al. 2012). CTen identifies cell types from heterogeneous tissue (e.g., whole blood) transcriptomic data. Here, we restricted our analysis to DE genes, separated into Up and Down regulated, in the d6 ALAN v Control and ALAN d6 v d2 contrasts. This approach helps distinguish whether gene

expression differences were due to changes in transcription or relative cell type abundance following infection and ALAN treatment. We followed the "Advanced Example" on the CTen webserver (http://www.influenza-x.org/~jshoemaker/cten/advanced_example.php) and a cell type was considered significantly enriched with an enrichment score of >2.

RNA sequencing – weighted gene correlation network analysis (WGCNA)

We used WGCNA v1.64-1 (Langfelder and Horvath 2008) to cluster genes with correlated gene expression into modules and then test these modules for associations with experimental groups. To generate the input for WGCNA, we first performed a variance-stabilized transformation of read counts on all 9,688 genes in DEseq2. We then removed 59 genes that had a median absolute deviation of zero, for a total input of 9,628 genes. We generated a signed network with the following parameters: soft threshold power (β) = 18, minimum module size = 30, and module dissimilarity threshold = 0.1. We then tested modules for associations with day, treatment, and individual treatment x day groups. For module-trait correlations of interest, we visualized module gene expression with heatmaps and performed a target vs background GO analysis in GOrilla testing module genes (target) against all genes (background) used in the analysis. Lastly, we visualized module hub genes with Visant (Langfelder and Horvath 2008). To do so, we restricted our visualization the top 300 genes ranked by intramodular connectivity from each module. Within these 300 genes, we calculated the topological overlap (i.e., strength of interaction) between each gene and ranked descending. We plotted the top 300 strongest interactions and identified the top 1-6 genes with the highest number of connections (degree distribution) to other genes and classified these as the module hubs.

Results:

We detected a significant effect of ALAN on the temporal course of WNV viremia in house sparrows (ALAN x day: $F_{4,124} = 2.9$, $P = 0.023$, 4 time points; Figure 2.1A [no main effect]). At 2-4 days post-exposure (dpe; all animals became infected), both ALAN-exposed and control birds had comparable viral titers. However, at 6 dpe, the interaction between ALAN treatment and dpe was significant ($t = 2.7$, $P = 0.009$). Post-hoc analyses (conducted using 'emmeans' in R studio) further confirmed the existence of a significant interaction (treatment x 6 dpe $t = -2.9$, $P = 0.005$ [supplementary text]). The conservative estimate for minimum circulating viral titer needed to transmit WNV to vectors is ~10^5 plaque-forming units (PFU; horizontal dashed line in Fig. 2.1A; (Turell et al. 2000)), suggesting the ALAN-exposed individuals remained infectious longer than controls. Specifically, eight ALAN-exposed sparrows possessed viral titers above the transmission threshold whereas no control birds were infectious at 6 dpe (Fig. 2.1C).

In previous studies, we found that CORT, an avian stress hormone, enhanced host-attractiveness to Culex mosquito vectors (Gervasi et al. 2016) and increased WNV viremia above the transmission-to-vector threshold (Turell et al. 2000). Given these results, we investigated whether any effects of ALAN on WNV competence could be explained by increases in CORT using the DEX suppression test (Liebl et al. 2013a). We found that CORT was unlikely to be involved in the observed ALAN effects in the present study, as there was little evidence that ALAN affected HPA function (treatment: $F_{1,40} = 2.8$, $P = 0.104$). To further probe whether ALAN effects on WNV competence were mediated by HPA dysregulation, we included CORT values in models to predict viremia and tolerance; however, no single measure (baseline,

stressor-induced or post-dexamethasone concentrations or the integral of CORT over the measurement period) was a significant predictor in any model.

Additionally, our study was designed to determine whether duration of ALAN exposure influenced corticosterone regulation or viremia. Hence, birds were exposed to ALAN in captivity for a range of 7-25 days. However, days in captivity had no effect on viremia in the mixed models ($P = 0.802$, supplementary text), thus we binned all birds under either ALAN or control groups and removed this term from further iterations of the models.

We next asked whether ALAN might modify the capacity of individuals to tolerate WNV, or ameliorate damage associated with infection (e.g., maintain body mass while infected sensu (Råberg et al. 2007)). A linear mixed model involving body mass as the dependent variable, treatment, day, and their interaction as fixed effects, and individual bird as a random effect was built using the 'nlme' software package in R. We found no effect of ALAN on WNV tolerance across the entire post WNV-exposure period (ALAN x integrated WNV titer: $F_{1,34} = 1.3$, $P = 0.257$). However, birds with the highest WNV titers overall lost more body mass than birds with lower cumulative titers (integral WNV titer: $F_{1, 34} = 6.6$, $P = 0.015$). Subsequently, we assessed directly whether body mass changed over time differently in ALAN-exposed and control birds after WNV infection. Birds in the control group gained mass post-infection, whereas body mass reached a nadir in ALAN-exposed birds 6 dpe (Fig. 2.1B); mass gain in virally-infected birds is counter-intuitive, but has precedent (Coon et al. 2011). Again, post-hoc analyses ('emmeans' in R studio) indicate that body mass differed at day 6 between controls and ALAN birds (treatment x 6 dpe $t = 2.8$, $P = 0.007$). On 10 dpe, body mass returns to comparable levels between groups (Fig. 2.1B). When we analyzed how WNV tolerance changed over the infectious period, we found that it varied with days post-exposure (dpe x ALAN x WNV titer: $F_{3,}$

$_{83}$ = 3.1, P = 0.030). This three-way interaction was driven by distinct WNV x ALAN effects on 6 dpe (β = 1.3 +/-0.60, t = 2.1, P = 0.041; Fig. 2.1C): at this time, only some ALAN-exposed birds (~50%) maintained WNV titers above the transmission threshold; no control birds were infectious on day 6. We found no effect of ALAN on survival of WNV infection post-exposure (χ21 = 0.26, P = 0.610; Fig. 2.1D); about 60% of birds in each group survived to 6 dpe. We confirmed that no collinearity existed among these three variables (ALAN, viremia, and body mass) using variance inflation factors and Eigenvalue condition indices (supplementary text).

To evaluate the epidemiological implications of the above effects, we compared the relative change in outbreak potential in the presence and absence of ALAN by evaluating the pathogen basic reproductive number, R_0, based on a simple single host, single vector model of WNV transmission (Wonham et al. 2004). We conservatively assumed that ALAN effects on house sparrows arise solely via extension of the infectious period; additional parameter values relating to demographic and transmission processes were estimated from the literature (supplementary text). Under these conditions, ALAN effects on host infectiousness increased R_0 from 8.95 to 12.66. In other words, assuming no prior exposure of hosts to WNV (i.e., no pre-existing immunity in the bird population) and no other effects of ALAN on house sparrow hosts or Culex vectors, ALAN would increase R_0 for WNV by 41%.

To implicate physiological mechanisms mediating ALAN effects on WNV competence, we conducted RNA-seq on whole blood samples at 2 and 6 dpe. Weighted gene co-expression network analysis (WGCNA; (Tag-El-Din-Hassan et al. 2012a)) identified 22 modules of co-regulated genes. One module (purple; Figure 2.2A-B) included genes associated with innate immunity and were relatively increased in abundance in 2 dpe ALAN individuals (r=-0.66, p=0.003; (Mashimo et al. 2002)). OASL, a gene linked to WNV resistance in both birds (Tag-El-

Din-Hassan et al. 2012b) and mammals (Mashimo et al. 2002; Perelygin et al. 2002), acted as hub (i.e., the most highly connected gene) within this module (Figure 2.2B). Suppressor of cytokine signaling 1 (SOCS1), responsible for suppressing IFN-gamma (anti-viral) activity, was also assigned to the purple module and transcript levels increased in day 2-ALAN individuals (Figure 2.3A), an outcome that may facilitate WNV dissemination through the host body (Guo et al. 2005; Mansfield et al. 2010; Ma and Suthar 2015). Conversely, transcript levels of SOCS3, which also suppresses cytokine signaling, were decreased in ALAN-exposed individuals at 6 dpe (Figure 2.3B). Two other modules revealed strong effects of ALAN treatment on the blood transcriptome, particularly at 6 dpe (turquoise module r = 0.84, p=1.000x10-5], figure 2.2C-D; tan module [r =0.84, p=1.000x10-5], 2.2E-F). In one module, both PLBD1 and ATP11B (Figure 2.2D) were hubs (Chovatiya and Medzhitov 2014; Guardado et al. 2016). PLBD1 is expressed during severe infection in malaria patients (Sobota et al. 2016a). Similarly, ATP11B is expressed in individuals experiencing innate immune hyperactivation (Hu 2013). In the other module, TRAP1, (i.e., Heat Shock Protein [HSP] 75), was a hub (Figure 2.2F); TRAP1 inhibits cellular apoptosis by reducing reactive oxygen species (Guardado et al. 2016; Sobota et al. 2016a). Altogether, these results demonstrate that ALAN alters various components of the immune system (Yoshimura et al. 2000; Newhouse et al. 2017).

Discussion:

In this study, we demonstrated that ALAN extended the infectious-to-vector window for a zoonotic pathogen in a wild reservoir species. Ecologically, this effect could enhance transmission risk, as suggested by changes in R_0 when only this parameter (duration of infection) was allowed to vary with light pollution. Although this approach is unarguably a great simplification of the true effects of ALAN in nature, this result should instigate additional

theoretical and empirical studies of ALAN and infectious disease. At the molecular level, transcriptomic data suggest that ALAN-exposed birds were less effective at tolerating infection on day 6 post-exposure, likely from a combination of pathogen induced damage or immunopathology, although neither of these were directly measured (Chovatiya and Medzhitov 2014; Guardado et al. 2016). The mechanism underlying body mass gain in control birds during WNV infection is not well-understood, but not unprecedented (Gervasi et al. 2017). Many of the birds exposed to ALAN with significant loss of body mass on day 6 died shortly thereafter; this may be why the group average on day 10 reflects a "catching-up" of body mass of individuals who survived the studied course of infection. Much is still unknown about body mass regulation during viral infections, so we emphasize the need to further investigate relationships between pathogen-induced or collateral damage and body mass in passerines. The higher abundance of gene transcripts of typical WNV anti-viral response genes earlier in ALAN-exposed than control birds also suggests that immune responses were generally dysregulated. These differences could have contributed to the loss of body mass in ALAN-exposed birds, as there are significant energetic costs involved in mounting immune responses, but direct investigations are necessary (Råberg et al. 2007; Bonneaud et al. 2012). Regardless of mechanism, ALAN did not cause greater WNV-induced mortality, a result that could enable infectious birds to transmit WNV to vectors for longer than in non-polluted areas.

Whereas antiviral immune defenses were bolstered earlier, ALAN birds remained infectious for longer than controls, which prompts questions regarding the mechanisms that allow high viral burden to persist. The dysregulation of the TRAP1 network indicates that inhibition of apoptosis may have been important (Guardado et al. 2016; Sobota et al. 2016a). Additionally, SOCS genes, which assist in negative feedback of immune mechanisms via the

JAK-STAT signaling pathway, might have attenuated cytokine secretion and thus enabled WNV to disseminate more easily. ALAN-exposed individuals upregulated SOCS1 on day 2 post-WNV exposure and downregulated SOCS3 on day 6 post-WNV exposure. Previous studies have found that upregulation of SOCS during WNV infection increases neuroinvasive capacity (Mansfield et al. 2010). SOCS has also been proposed as a mechanism by which flaviviruses, including WNV, actively evade host defenses (Mansfield et al. 2010). It is likely that high viral titers persisted as a result of a combination of these and other mechanisms (Martin et al. 2016a).

Prior studies on laboratory rodents found that individuals exposed to various forms of light at night had exaggerated immune responses, many with the capacity to induce collateral damage (Navara and Nelson 2007). Although the exact mechanisms by which ALAN altered immune defenses here is obscure, other hormones (i.e., melatonin) could play a role (Hastings et al. 1985). Our study ruled out corticosterone as a factor, despite other evidence in birds that ALAN alters the regulation of avian physiology via stress-response pathways (Ouyang, De Jong, et al. 2015; Ouyang et al. 2017). Because melatonin enhances viral resistance and attenuates cellular and tissue damage by acting as an antioxidant and free radical scavenger, ALAN-induced suppression may contribute to the increased viral titer observed in this study (Hastings et al. 1985; Valero et al. 2015). Alternatively, incoordination of biological rhythms may also have contributed to the effects we observed. Most organisms evolved to use photoperiod to synchronize endogenous circadian rhythms with the environment. Indeed, 10% of the mammalian genome shows intrinsic circadian oscillations, including immune parameters such as Toll-like receptor expression and neutrophil activity (Scheiermann et al. 2013). Shifting the time at which individuals are exposed to WNV (i.e., from crepuscular to nighttime periods) may also affect infection outcomes, as other studies have found oscillations in pathogen defenses that

impact the likelihood that viral dissemination occurs (Mideo et al. 2013; Hoyle et al. 2017). This issue is worthy of future study. Lymph nodes, which also influence viral dissemination, and the spleen, which is a key site of WNV replication, also display circadian patterns of gene expression (Keller et al. 2009). Peritoneal macrophages involved in inflammation upregulate the secretion of cytokines, including TNF-a and IL-6, at different points during the 24-hour period. ALAN cues that contradict zeitgeber time may mismatch circadian rhythms of hosts to their environments and hence induce upregulation of certain antiviral defenses at inappropriate times.

We must acknowledge that studies like ours, conducted in captivity, have some limits and should be cautiously extrapolated to the natural world. For example, gain of body mass during the course of infection may not occur in nature as resources typically are not as accessible (Liker et al. 2008). Furthermore, mortality could differ for ALAN-exposed birds if morbidity decreased survival probability via predation risk (Adelman et al. 2017). Ultimately, though, experimental WNV infections will never be realized in nature, so we advocate for additional work like ours, with study elements directed at emulating natural conditions (e.g., naturalistic food availability), which will be useful to the parameterization of epidemiological models (Griffith et al. 2017).

Our results also should motivate further investigation of mechanisms whereby ALAN affects epidemic risk. Indeed, light pollution might alter other drivers of R_0 such as vector and host diversity and the nature and timing of their interactions (i.e., over days and seasons; (Keller et al. 2009)). Most WNV vectors, for instance, take blood meals at dusk and dawn (Apperson et al. 2004a); with ALAN, the blood-meal feeding window might be extended, or vectors might arouse too early to find a blood meal (Kernbach et al. 2018a). Mosquito density also tends to be lower in urban than rural environments, however, urban heat islands make ideal breeding habitat for many species of vectors (Araujo et al. 2015; Ferraguti et al. 2016). More work must

determine which vector species thrive in light-polluted environments and how vector community composition affects local disease dynamics (Kernbach et al. 2018a). Incoordination of the immune system has also been noted in laboratory rodents and could result in increased susceptibility at time of exposure, thus increasing an important parameter in outbreak potential involving the likelihood that a host develops infection upon mosquito bite (i.e., exposure; (Wonham et al. 2004; Navara and Nelson 2007)). The pineal-derived hormone mentioned above, melatonin, also coordinates such circadian behaviors which could have complex effects on WNV dynamics, particularly as vectors also rely on melatonin for temporal coordination of behaviors (Gwinner and Benzinger 1978).

As we further explore ALAN effects on infectious disease risk, it will be important to study whether and how lighting spectra can be adjusted to mitigate risk. Motion-activated or directed light sources can be substituted for current illumination practices, and lighting overall could also be reduced when alterations would have the greatest positive impacts on wildlife (i.e., migrations, breeding seasons). The International Dark-Sky Association has led efforts to eliminate lighting in tall urban buildings during avian migrations to reduce extensive window strikes that occur during critical migratory periods (IDA 2009). An analogous example to curtail vectored-disease transmission in the southeastern US would be to reduce lighting of vulnerable areas during the height of arbovirus transmission season (e.g., late Fall; (Ezenwa et al. 2006a)). Additional mitigation opportunities likely reside in the advent of new technologies detectable by human, but less so wildlife, vision (e.g., high-wavelength (red) wavelengths versus the broad-spectrum options typically used; (Apperson et al. 2004a)).

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Author contributions:

M. E. Kernbach contributed to conceptualization, data curation, methodology, investigation, project administration, and writing; D. J. Newhouse contributed to data curation, methodology, formal analysis, and writing; J. M. Miller contributed to data curation, project administration, and investigation; R. J. Hall contributed to conceptualization, formal analysis, visualization, writing- original draft; J. Gibbons contributed to data curation and formal analysis; J. Oberstaller contributed to data curation and formal analysis; R. Jiang contributed to formal analysis; T. R. Unnasch contributed to conceptualization, funding acquisition, methodology, resources, and supervision; C. N. Balakrishnan contributed to data curation, methodology, formal analysis, writing, and supervision; L. B. Martin contributed to conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, supervision, and writing- original draft.

Ethical Statement:

All procedures and protocols were approved by and performed according to IACUC (#2716) and USF Biosafety (#1323).

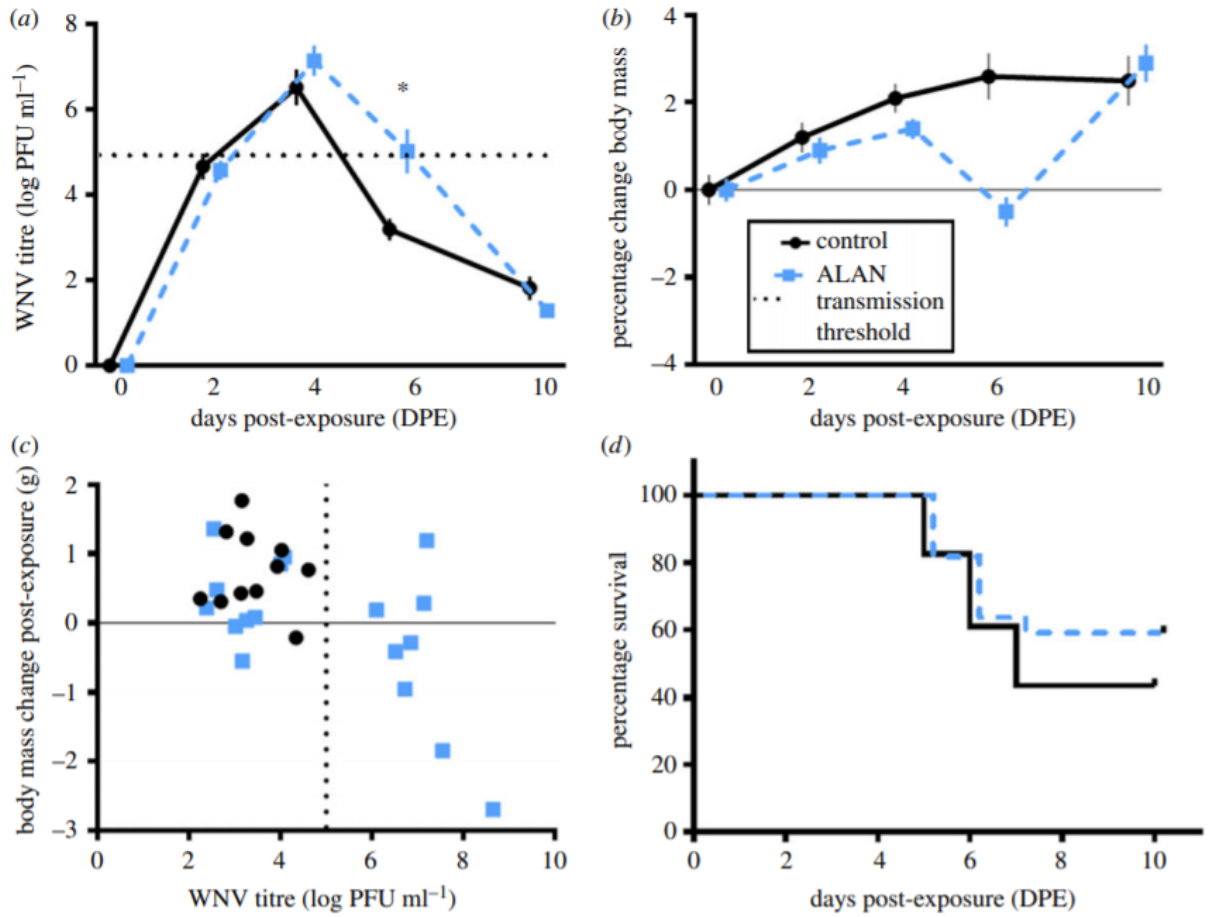

Figure 2.1:

West Nile virus infection viremia, body mass and WNV-induced mortality results. Effects of experimental West Nile virus exposure on house sparrows (Passer domesticus) exposed to artificial light at night (ALAN; 8 lx during night hours for two to three weeks prior to WNV exposure) versus controls (animals kept on 12 L : 12 D for duration of experiment). Blue points and dashed lines signify ALAN-exposed individuals, and black points and solid lines signify controls. (A) Individuals exposed to ALAN had significantly higher viral titers on d6 post-exposure, indicated by the asterisk. The horizontal dashed light represents the conservative transmission threshold or the minimum amount of virus in circulation required to transmit WNV to a vector (i.e. 105 PFU). (B) Effects of WNV and ALAN on change in group mean body mass

throughout the course of WNV infection. On d6, ALAN-exposed individuals lost appreciable mass whereas controls continued to gain body mass. (C) Relationship between WNV titer and body mass change on d6 post-WNV exposure. The vertical dashed line represents the WNV transmission threshold; individuals to the right of this dashed line are infectious to mosquitoes, and individuals to the left of this dashed line are not. Only ALAN-exposed individuals were infectious on d6. (d) No effect of ALAN on WNV-induced mortality.

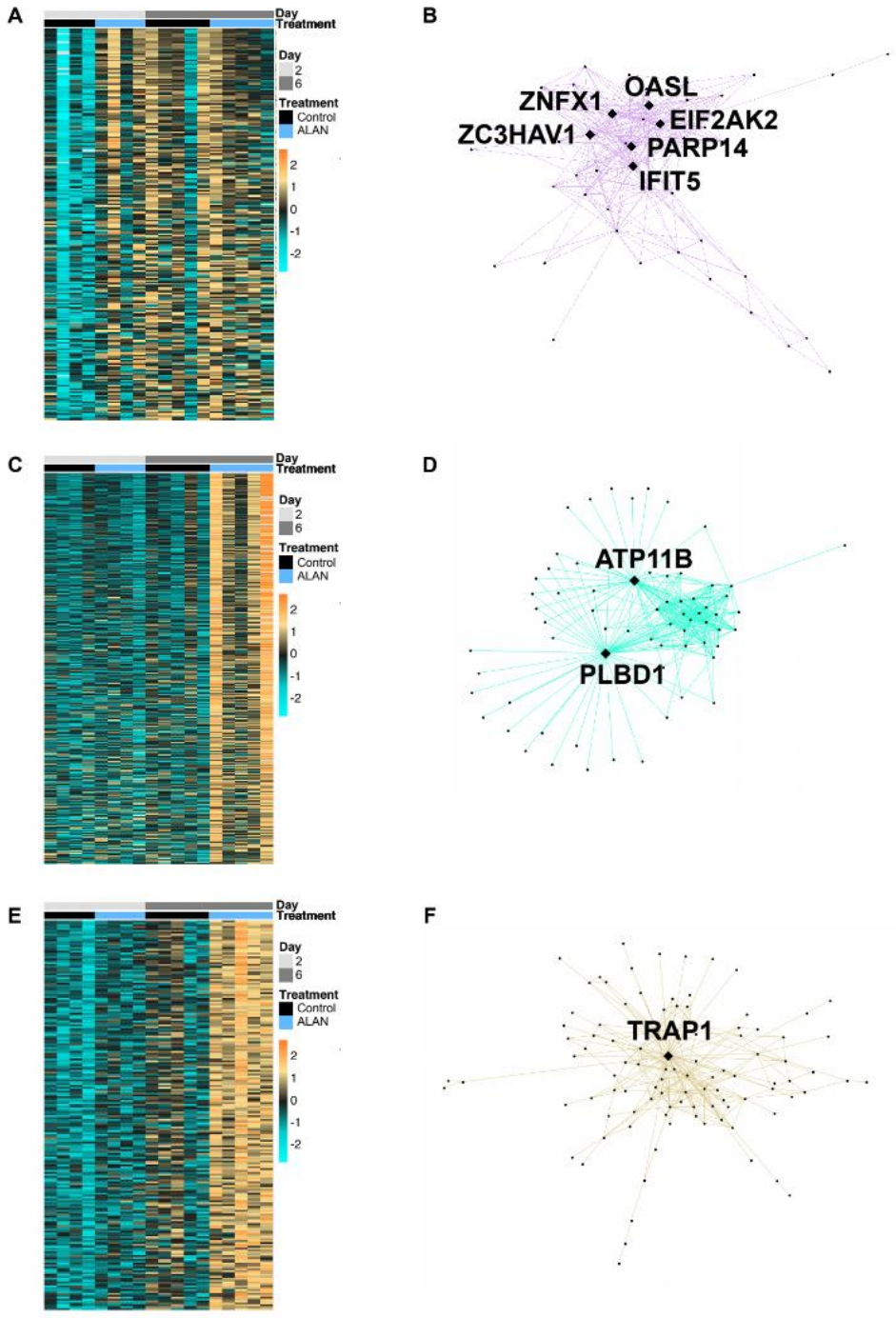

Figure 2.2:

WGCNA results for significantly enriched WNV immune defense modules. (A) Heatmap of eigengene expression for purple module (235 genes), showing downregulation in d2 control birds. Columns are organized by day and treatment; each row represents a module gene and row

colors correspond to relative expression levels, where orange represents upregulation and blue represents downregulation. (B) Visant network of the most interconnected genes in the purple module (greater than 28 connections). Each dot represents a gene and diamonds highlight hub genes. (C) Heatmap of eigengene expression for turquoise module (3274 genes), showing upregulation in d6 ALAN birds. Columns are organized by day and treatment, each row represents a module gene and row colors correspond to relative expression levels, where orange represents upregulation and blue represents downregulation. (D) Visant network of the most interconnected genes in the turquoise module (greater than 44 connections). Each dot represents a gene and diamonds highlight hub genes. (E) Heatmap of eigengene expression for tan module (206 genes), showing upregulation in d6 ALAN birds. Columns are organized by day and treatment, each row represents a module gene and row colors correspond to relative expression levels, where orange represents upregulation and blue represents downregulation. (F) Visant network of the most interconnected genes in the tan module (greater than 60 connections). Each dot represents a gene and diamonds highlight hub genes.

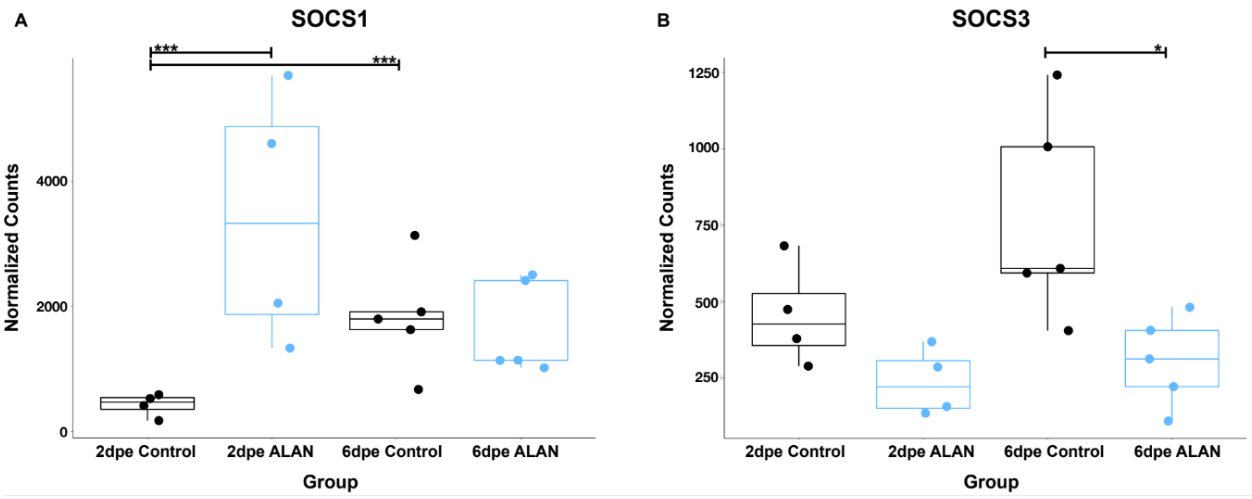

Figure 2.3:

Normalized counts for (A) SOCS1 and (B) SOCS3 across treatment groups. Each dot represents a sample. Black dots and boxplots correspond to control and blue dot and boxplots correspond to ALAN. Significance bars indicate ***$p < 0.001$ and *$p < 0.05$.

CHAPTER II:

BROAD-SPECTRUM LIGHT POLLUTION SUPPRESSES MELATONIN AND INCREASES WEST NILE VIRUS-INDUCED MORTALITY IN HOUSE SPARROWS (PASSER DOMESTICUS)

Note to Reader:

This is a pre-copyedited, author-produced version of an article accepted for publication in The Condor following peer review. The version of record: Kernbach ME, Cassone VM, Unnasch TR, Martin LB. 2020. Broad-spectrum light pollution suppresses melatonin and increases West Nile virus–induced mortality in House Sparrows (*Passer domesticus*). Condor.

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Abstract:

Artificial light at night (ALAN) has become a pervasive anthropogenic stressor for both humans and wildlife. Although many negative impacts of ALAN on human health have been identified, the consequences for infectious disease dynamics are largely unexplored. With the increase in popularity of energy efficient light-emitting diodes (LEDs), the effects of spectral composition of ALAN have also come into question. Previous studies showed that exposure to low levels of incandescent ALAN extended the infectious period of House Sparrows infected with West Nile virus (WNV) without affecting mortality rates, thus increasing the pathogen initial reproductive rate (R_0) by about 41%. Here, we asked whether exposure to broad-spectrum (3000K [Kelvin; unit of color temperature]) ALAN suppressed melatonin, a hormone implicated in ALAN-induced physiological consequences, in House sparrows (*Passer domesticus*). We then asked whether amber-hue bulbs (1800K) could ameliorate the effects of WNV on individual sparrows, and whether broad-spectrum or blue-rich bulbs (3000K and 5000K, respectively) could exacerbate them. We found that exposure to low intensity (~5 lux) broad-spectrum (3000K) ALAN significantly suppressed melatonin levels throughout the night. Second, we found that exposure to broad-spectrum and blue-rich (3000+5000K) lights did not affect WNV viremia but did increase WNV-induced mortality. Conversely, birds exposed to amber-hue (1800K) ALAN had lower viremia and mortality rates similar to controls (i.e., natural light conditions). This study demonstrates that ALAN affects melatonin regulation in birds, but this effect, as well as ALAN influences on infectious disease responses, can be ameliorated by particular lighting technologies.

Introduction:

Light pollution, especially artificial light at night (ALAN), is a widespread and influential anthropogenic stressor (Kyba, Kuester, et al. 2017). In addition to its prominence across busy highways and city centers, ALAN is found in rural greenspaces and along forest edges, many of which are occupied by wildlife (Longcore and Rich 2004). As the lightbulb was invented in the late 19th century, artificial nighttime lighting poses a relatively novel threat (Morison and Hughes 1991; Hedges and Kumar 2003). Alteration of light cycles, historically perhaps the most reliable environmental cue on Earth, can create mismatch and confusion in both circadian and circannual rhythms of the organisms exposed to such light pollution. Some of the best known examples of these effects include earlier reproduction in passerines exposed to light pollution (Miller 2006b; Dominoni et al. 2013a), as well as the multiple dimensions of immune dysregulation observed in laboratory rodents (Bedrosian et al. 2011; Fonken et al. 2013). Light at night has also induced broad physiological consequences in birds such as hormonal dysregulation, shifted circadian regulation, and modified behavior (Schoech et al. 2013; Dominoni 2015; Alaasam et al. 2018). Nevertheless, the full extent to which these organismal effects translate into ecological consequences remains elusive (Kernbach et al. 2018a).

Many effects of ALAN on animals are likely mediated by upstream circadian dysregulation of molecular timekeepers like melatonin. The brain's suprachiasmatic nuclei (SCN), known as the central pacemaker, is responsible for coordinating circadian rhythms across the body. The pineal gland coordinates with the SCN, and integrates light cues from melanopsin receptors to encode time of day (Bell-Pedersen et al. 2005b). Although the SCN and pineal gland are both important for maintaining daily rhythms, the pineal gland is responsible for the synthesis and secretion of the indoleamine hormone, melatonin (Hardeland et al. 2012).

Melatonin, in addition to synchronizing molecular clocks throughout the body, aids many other physiological processes including gonadal development, inflammation control and antioxidant activity, thermoregulation, and metabolism (Hing-Sing Yu 1992; Cassone et al. 2009). Melatonin secretion is suppressed during exposure to light (i.e., day) and typically peaks during the middle of the dark phase (Blask et al. 2002). Indeed, exposure to light at night suppresses melatonin secretion in many birds and mammals, including humans (Yamada et al. 1988; Redlin 2001). Given the many roles that pineal-derived melatonin plays in the body, it is unsurprising that its suppression has been linked to multiple physiological consequences (i.e., mental disorders and immune dysregulation) (Claustrat et al. 2005; Srinivasan et al. 2006; Castanon-Cervantes et al. 2010).

Some species affected by ALAN can serve as zoonotic reservoirs, i.e., hosts that naturally amplify pathogens that can spill into human populations. House sparrows, American robins (*Turdus migratorius*), Blue jays (*Cyanocitta cristata*), and Northern cardinals (*Cardinalis cardinalis)*, for instance, are all important reservoirs of West Nile virus (WNV) (Chace and Walsh 2006). They all also are quite common in ALAN-polluted habitats. Several bat species, too, are reservoirs of zoonoses, and these species alter their behavior when exposed to light at night (Calisher et al. 2006; Spoelstra et al. 2017). Peromyscus mice and related rodents, hosts to Borrellia burgdorferi (the causative agent of Lyme disease) and hantavirus among other infections, also alter their nighttime behaviors and social interactions in the presence of light pollution (Han et al. 2015; Gaitan and Millien 2016; Hoffmann et al. 2018, 2019).

In a recent study, we found that House sparrows exposed to a low intensity (5 lux) of broad-spectrum (3000K) light at night (ALAN) maintained higher WNV titers for longer than controls; mortality rates to WNV were unaffected by ALAN, however (Kernbach et al. 2019).

Using a traditional epidemiological framework (e.g., the comparison of the initial reproductive rate (R_0) changes in response to an environmental change), we found that these modest effects on individual birds meant that ALAN might increase WNV R_0 by as much as 41% (Wonham et al. 2004; Kernbach et al. 2019). Because ALAN has both organismal- and ecological-level effects, we felt it important to determine if changes in the practice of night-time lighting could moderate some of these effects (Khan and Abas 2011). First, we wanted to determine if melatonin was dysregulated by broad-spectrum (3000K) ALAN; if so, the blue-dominated spectra of these bulbs would be expected to blunt the natural circadian rhythms in melatonin. Melanopsin receptors are maximally sensitive to the blue-rich hues emitted by these LEDs (Cashmore et al. 1999; Mure et al. 2007; Pawson and Bader 2014). To save money, many cities have begun switching to 1800K, 3000K, and 5000K LED lighting, which is concerning because blue-rich hues are especially detrimental to both human and wildlife health (Falchi et al. 2011). Our second aim was to reveal whether blue-rich (5000K) LED lighting exacerbates the effects of ALAN on avian WNV responses and whether other lighting options that mostly emit in the amber-hue (1800K) part of the light spectrum could alleviate the impacts. These bulbs have had positive effects in some contexts (e.g., turtle nestling success), but their impacts in other ALAN contexts have been rarely investigated (Witherington and Bjorndal 1991; Ferenc and Leonard 2008; Gaston et al. 2012).

We focused on the House Sparrow, as ALAN affected competence of this species for WNV (Marra et al. 2004; Hanson et al. 2019). We studied WNV, a vector-transmitted arbovirus, because it continues to pose health threats to humans and wildlife across the United States (Centers for Disease Control and Prevention 2018). We predicted that exposure to broad-spectrum (3000K) ALAN would suppress melatonin secretion and hinder viral resistance. For blue-rich (5000K) ALAN, we expected even poorer resistance, allowing individuals to maintain

high viral titers (and hence remain infectious) for longer periods of time (Wyse and Hazlerigg 2009). We also predicted that individuals exposed to amber-hue (1800K) ALAN would manifest resistance comparable to controls (i.e., total darkness at night).

Methods:

Experimental procedures

Melatonin experiment: House Sparrows (N=48) were captured in the Tampa Bay area using mist nets during the months of January, February, and March 2018. All birds were captured between 5:30 and 9:30am. Following capture, birds were transported to the University of South Florida (USF) campus vivarium and housed individually in 13”x15”x18” cages in visual and auditory proximity to one another under assigned lighting conditions (N=24 control, N=24 ALAN) for 2 weeks. Food (mixed seeds) and water were provided ad libitum throughout the study. All birds were housed under 12 hours of light and 12 hours of darkness (12L:12D) such that light during the simulated day period was approximately 150 lux emitted at 3000K. At night, ALAN treatment birds were exposed to 5 lux of 3000K light [i.e., the intensity emitted by a typical street lamp at night (Dominoni et al. 2013a)], whereas control birds were housed in near darkness (~0 lux). Spectral composition of light is measured in units of Kelvin (K) and describes the light appearance in commercially sold lightbulbs. Although typically thought of as heat intensity, Kelvin in this instance describes the color temperature.

Blood samples were collected twice prior to treatment exposure (i.e., on two separate nights) at 8pm, 10pm, 12am, 2am, and 4am (Zeitgeber times 14, 16, 18, 20, 22). Individuals were only sampled once per night and at different timepoints between the two sampling nights to avoid anemia and any related adverse effects. Individuals were either exposed to ALAN or control conditions at night during the exposure duration of two weeks, and then sampled twice

again using identical methods described above, for a total of four sampling nights. None of the individuals from this melatonin suppression study were used in the subsequent WNV-infection experiment; following the conclusion of this study, all individuals were euthanized.

The serum was extracted and stored at -40°C until samples were processed. Melatonin was quantified using a commercially available enzyme-linked immunosorbent assay (ELISA; RE54021; Tecan Industries, Switzerland). Samples ranged in volume from 70-110ul and were diluted to 500ul in volume for the assay parameters. Then, samples were treated according to the published Tecan Industries protocol, and melatonin concentrations were calculated for each volume of serum and extrapolated to pg/ml serum. This approach was validated by dilution of a separate set of sparrow serum samples collected from previous experiments performed in the Cassone lab to determine parallelism with the standard curve.

Spectral composition experiment: House Sparrows (N=71; not including individuals from the melatonin suppression study) were captured in the Tampa Bay area using mist nets during October and November 2018. All husbandry conditions were identical to those described above with the exception of light at night treatments. At night, control birds were housed in complete darkness (0 lux; N=24) whereas all other groups were exposed to 5 lux [i.e., the intensity emitted by a street lamp at night (Dominoni et al. 2013a)] of one of three forms of light: amber-hue (1800K) LED light (SCS Exterior Wildlife & Habitat Lighting, catalogue no. GB030, certification no. 2018-057; N=12), broad-spectrum (3000K) LED light (N=11), or blue-rich (5000K) LED light (N=24). The amber-hue bulb was selected from the Florida Fish and Wildlife Commission (FWC) Certified Wildlife Lighting list which is required by state law to be used in coastal communities (Florida Fish and Wildlife Conservation Commission 2019). Sample sizes were unequal due to housing limitations and the original study design (see results). After the first

two weeks, birds were transported from the USF animal biosafety-level 2 (ABSL-2) facility where they were originally housed upon capture to the USF animal biosafety-level 3 (ABSL-3) facility to be housed under the same lighting conditions as used prior to transfer to the new facility. Birds were placed inside bioBUBBLE secondary containment systems (bioBUBBLE Inc, Fort Collins CO) within the ABSL-3 facility which enclosed bird cages to prevent aerosol circulation throughout the housing facility for the remainder of the study.

All birds were exposed to 10^1 plaque-forming units (PFUs) of New York 1999 strain (NY'99) WNV one day after transfer to BSL-3 (Kernbach et al. 2019). Following WNV exposure, blood samples were collected from birds on days 2, 4, 6, and 10, and serum was stored at -20°C until viral RNA extractions were performed. Body mass (to 0.1g) was measured at WNV exposure and all sampling timepoints thereafter, and mortality was monitored daily. All birds were euthanized by isoflurane overdose followed by rapid decapitation on day 10 post-exposure. This study was also originally designed to capture whether supplementing melatonin could promote viral resistance in ALAN-exposed birds. Therefore, approximately ½ of all birds were administered 200ug/mL crystalline melatonin dissolved in 0.5% EtOH in drinking water at night. As this treatment had no statistically significant effect on either treatment group (see supplementary text), this aspect of the experiment is not further addressed.

To quantify viremia, WNV RNA was first extracted from 10 uL of each frozen serum sample using the Qiagen QIAmp Viral Extraction Mini Kit (Qiagen Cat. No. 52906). WNV standards were also extracted from known concentrations stocks using the same methods. Following extractions, RNA was quantified using quantitative real-time polymerase chain reaction (qRT-PCR) using a one-step Taqman kit (iTaq Universal Probes One-Step Kit; Bio-Rad Cat. No. 1725141). All samples were run in duplicate with negative controls to detect potential

contamination. The stock titer was recently measured using plaque assays, and qPCR results closely mirrored plaque counts (Brien et al. 2013).

Data analysis

Melatonin experiment: Melatonin data were analyzed using a generalized linear model (GLM; Gamma distribution) with the R base and 'car' packages (Fox, John & Weisberg 2011). Melatonin concentration in pg ml-1 was the dependent variable and ALAN treatment and Zeitgeber time and their interaction were considered fixed effects. We also evaluated the generalized linear model with a type III ANOVA to determine main effects. To confirm there were no pre-existing differences among treatment groups, we built a GLM to compare melatonin concentrations between treatment groups before exposure (supplementary text). To determine whether ALAN exposure affected melatonin concentrations, we compared the group to its initial concentration (pre-exposure and post-exposure; supplementary material), as well as the concentrations between groups following 2 weeks in their respective treatments (ALAN and control).

Spectral composition experiment: Visual inspection of data indicated that 3000K and 5000K ALAN birds were quite similar in terms of viremia, body mass, and mortality rate. Statistical comparisons also indicated that these two groups were indistinguishable, so they were combined into a single "3000+5000K ALAN" group for all other analyses ([N=35]; see supplementary text). To evaluate effects of ALAN spectra (i.e., light type) on viremia, we modeled log10 transformed WNV titer as the dependent variable in a generalized linear mixed model (GLMM; distribution was non-normal) with light type (control, broad-spectrum/blue-rich [3000+5000K] ALAN, and amber-hue [1800K] ALAN), days-post exposure (2, 4, 6, and 10 dpe), and their interaction as fixed effects and bird id as a random effect in R studio using the 'lme4' package

(Bates et al. 2015). We then also asked whether changes in body mass (% since exposure) were affected by lighting treatment, viremia, their interaction, and vigor (i.e., body mass prior to WNV exposure), again using GLMM in the R studio 'lme4' package (Burgan et al. 2019).

To assess how birds tolerated infections (i.e., residual variation in performance at a given viremia), we calculated average viremia and average percent change in body mass for each individual across the course of its infection (Gervasi et al. 2017; Burgan et al. 2019). We plotted average viremia along the X-axis and average percent change body mass along the Y-axis and used a linear regression to determine whether there was a relationship between the two variables.

Finally, to discern how ALAN spectra and other factors affected mortality rate, we used the 'survival', 'survminer', and 'dplyr' packages for the Cox proportional hazards method in R Studio (Therneau and T. Lumley 2015; Wickham and Francois 2016; Kassambara 2018). We conducted two modeling exercises for WNV-dependent mortality. First, we checked whether ALAN spectra alone predicted mortality (with the body mass of an individual prior to infection as a covariate in the model). As body mass had no detectable effect it was excluded from further models (supplementary text). Our second Cox modelling effort incorporated average viremia and average percent change body mass from days 2 and 4 post-exposure as well as ALAN spectra and all two- and three-way interactions as potential predictors of mortality. We used only data from days 2 and 4 post-exposure here as no mortality occurred until after that period. Moreover, we were concerned that diminutions in health occurring late in the infection might confound our ability to detect drivers of mortality; in other words, we were interested to learn whether peak viremia or the nadir in body mass in response to infection predicted mortality. Other studies have taken a similar approach for similar reasons (Gervasi et al. 2017). To determine whether individuals died earlier or later than expected, we compared residual variation in days until death

based on average viremia (days 2 and 4) between treatment groups by conducting a one-way ANOVA followed by a Tukey pairwise comparison to more easily visualize the underlying differences in mortality.

Results:

Exposure to ALAN suppresses melatonin in House Sparrows

We found that exposure to 3000K ALAN significantly suppressed melatonin concentrations at night (ALAN*Exposure X^2 = 186.419, P = < 2e-16; Figure 3.1). Additionally, the melatonin concentrations within the ALAN-exposed groups were significantly suppressed from pre- to post-exposure timepoints (X^2 = 7.7698, P = 0.005). Finally, we confirmed that there were no pre-existing differences in melatonin secretion among the two groups (X^2 = 0.0225, P = 0.881).

Amber-hue ALAN enhances West Nile virus resistance

We detected a significant main effect of light type (X^2 = 6.9942, P = 0.030) on WNV viremia (Figure 3.2A). We found that the difference was driven by significantly lower viremia in the amber-hue ALAN treatment group (1800K, t = 2.530, P = 0.011; table 3.1), which persisted across the entire post-exposure period (1800Kxday linear, t = 2.724, P = 0.006; 1800Kxday quadratic, t = 1.999, P = 0.046). Viremia did not differ between broad-spectrum/blue-rich ALAN and control groups however (3000+5000K, t = 0.290, P = 0.772). Light type, day, and their interaction did not affect percent change in body mass since WNV exposure, but there was a significant interaction between 3000+5000K ALAN and day (3000K+5000Kxday linear, t = 2.071, P = 0.038), such that these birds lost more body mass than the other groups since WNV exposure (Figure 3.2B).

Broad-spectrum ALAN increases WNV-induced mortality

For tolerance, we found that there was no relationship between average viremia and average percent change body mass (R^2 = 0.015, P = 0.316). Therefore, we focused on percent change in body mass since WNV exposure, independent of viremia, as a proxy of health status during infection for further analyses. We found that light type alone affected survival rate post-WNV exposure, driven by significantly higher mortality in the broad-spectrum/blue-rich ALAN group (X^2 = 6.0217, P = 0.049; 3000+5000K, z = 1.893, P = 0.0583; Figure 3.2C). Additionally, both average viremia and average percent change in body mass affected mortality (Table 3.2); higher titer and greater mass loss exacerbated mortality risk. A type III ANOVA revealed that main effects of light type, average percent change body mass, average viremia, the interaction between average viremia and treatment, and the interaction between average percent change body mass and average viremia all had significant effects on mortality. In other words, broad-spectrum ALAN mortality rates to WNV remained significantly higher even after the addition of other parameters, meaning that light type alone explained differences in mortality rates (z = 2.205, P = 0.027). Additionally, average viremia from days 2 and 4 (z = 3.079, P = 0.002) and the interaction between average viremia and treatment (z = -2.189, P = 0.029) were significant predictors of mortality. When we calculated the residual variation in time (days) until death based on an individual's average viremia, we revealed a significant difference between light treatments (DF = 2, F-value = 3.561, P = 0.034): broad-spectrum/blue-rich ALAN exposed birds died on average approximately 1 day earlier than expected compared to controls (adjusted P = 0.035; Figure 3.3).

Discussion:

Light type affected how House Sparrows coped with WNV infections. Broad-spectrum (3000K) ALAN suppressed melatonin throughout the night after only two weeks of exposure. Alternatively, exposure to amber-hue ALAN (1800K) increased WNV resistance by maintaining lower WNV burdens for shorter periods of time and perhaps reduced competence to transmit to vectors (Sears et al. 2011; Burgan et al. 2018). Broad-spectrum/blue-rich ALAN (3000+5000K) exposure, however, did not reduce viral resistance, as seen previously, but it did increase WNV-induced mortality rates. Additionally, individuals exposed to the broad-spectrum/blue-rich ALAN died from WNV infection at lower viral burdens than control individuals. Altogether, our data indicate that the type of ALAN to which organisms are exposed likely affects melatonin secretion and could exacerbate or ameliorate how zoonotic diseases affect populations. Below we discuss both the organismal and ecological ramifications of our results as well as the mitigation opportunities they present in the interest of wildlife and human health.

Melatonin actions on antiviral immune defenses

Exposure to low intensities of 3000K ALAN is enough to significantly suppress melatonin concentrations at night. Melatonin, as emphasized above, plays many active roles in the body including coordinating and controlling immune defenses. Early research discovered a close relationship between the fluctuations of melatonin and differentiation of granulocytes (i.e., toxic-granulated white blood cells), suggesting that melatonin played a key role in synchronizing immunological processes throughout the body (Kuci et al. 1988). Since then, many other relationships between the rhythmicity and concentration of melatonin and immunity have been discovered, including effects important for antiviral defenses. For example, elevating melatonin concentrations in birds at night increases heterophil activity, which is important for recognizing

and engulfing apoptotic or virally infected cells (Rodríguez et al. 1999; Kogut et al. 2005; Uematsu and Akira 2006). Although phagocytes (i.e., white blood cells that engulf and destroy virally-infected cells) can generate a disproportionate amount of free radicals, melatonin also protects against potential oxidative damage by acting as an antioxidant itself (Reiter 1996; Babior 2000; Galano et al. 2011). In other words, the collateral damage that phagocytic antiviral responses generate via radical oxygen species can typically be attenuated by melatonin directly. Furthermore, melatonin induces an anti-apoptotic regulation of T cells during differentiation, which undoubtedly affects the identification and elimination of virally infected cells by CD4+ and CD8+ lymphocytes (Sainz et al. 1995). It is therefore reasonable to expect that melatonin suppression via ALAN might mediate how sparrows control WNV infection. Some studies revealed that melatonin suppression via light at night exposure can propagate a Th1-biased humoral response, which is typically inflammatory in nature, and when uncontrolled, can cause immunopathology (Carrillo-Vico et al. 2013). Indeed, in human neuroinvasive WNV cases, polarized T-cell responses are associated with increased pathogenesis (James et al. 2016). Blocking melatonin actions in the pineal gland via propranolol (a melatonin receptor antagonist) suppressed multiple aspects of cellular and humoral immunity (Claustrat et al. 2005).

ALAN exposure, viral resistance, and its relationship to melatonin

In a previous study, we found multiple indications that antiviral immune defenses and damage attenuation were affected in WNV-infected House Sparrows exposed to low-intensity 3000K ALAN (Kernbach et al. 2019). A weighted gene correlation network analysis (WGCNA) identified multiple networks of closely co-regulated genes that were differentially expressed between ALAN and control individuals. One network containing many aspects of the anti-WNV immune pathway, including the hub (i.e., most highly connected) gene, OASL, was upregulated

sooner during infection in ALAN-exposed than controls (Mashimo et al. 2002; Tag-El-Din-Hassan et al. 2012b; Kernbach et al. 2019). Although OASL stimulates antiviral responses, its advanced upregulation suggests that WNV infection disseminated more quickly in individuals that were exposed to ALAN (Choi et al. 2015). Dissemination of virus can be accelerated with the presence of reactive oxygen species; these reactive oxygen species are often neutralized by melatonin, which may be one mechanism whereby suppression of melatonin expedited viremia (Bonilla et al. 2004b). Furthermore, a different network containing the hub gene, TRAP1 (heat shock protein [HSP] 75), was upregulated later during infection, when viremia in ALAN-exposed individuals was very high (Kernbach et al. 2019). TRAP1 is often upregulated in response to excessive oxidative stress, which might become common in the absence of melatonin (Bonilla et al. 2004b; Hua et al. 2007). It has been suggested that TRAP1 and other heat shock proteins also aid the cellular entry of flaviviruses (i.e., a class of positive-sense and single-stranded RNA viruses) (Rastogi et al. 2016). In this light, TRAP1 might be upregulated to compensate for an absence of melatonin, and in the process, allow for enhanced viral replication by aiding cellular entry or inhibiting apoptosis of infected cells (Hua et al. 2007).

ALAN exposure, immunopathology, mortality, and a potential role for melatonin

Furthermore, the WGCNA analysis from our previous study revealed that individuals exposed to ALAN were also experiencing severe immunopathology. Another network upregulated by ALAN-exposed individuals contained two hub genes, ATP11B and PLBD1 (Kernbach et al. 2019). ATP11B is associated with sepsis, an extreme response of the immune system that can cause life-threatening organ damage (Hu 2013). Similarly, PLBD1 is expressed during severe malaria infection and exposure to oxidative or thermal stressors (Chovatiya and Medzhitov 2014; Sobota et al. 2016b). A handful of studies have explored the relationship

between melatonin and sepsis, including the administration of melatonin as a potential therapeutic (Şener et al. 2005; Galley et al. 2014). The antioxidant and anti-inflammatory properties of melatonin contribute to the control of collateral damage during an immune response. As above, melatonin suppression might have led to the septic symptoms we observed in ALAN-exposed individuals. In support, the administration of melatonin to WNV-infected mice reduced the risk of encephalitis and resulting mortality (Ben-Nathan et al. 1995). Additionally, mice infected with Venezuelan Equine Encephalitis Virus (VEEV) experienced similar protective benefits from melatonin supplementation with effects ranging from enhanced viral resistance (signified by lower viremia burdens) to decreased risk of mortality (Bonilla et al. 1997). Here, we observed that House Sparrows exposed to broad-spectrum ALAN experienced higher WNV-induced mortality rates and succumbed to infection at lower viral burdens than their control counterparts. Although we cannot directly attribute these effects to melatonin suppression, we encourage follow-up work to determine whether melatonin suppression is a mechanism by which light pollution induces increased mortality risk.

Melatonin Independent Effects of ALAN Exposure on Immune Defenses

In this study, we observed that exposure to amber-hue ALAN slightly, yet significantly, reduced WNV burden. Although we make a case that melatonin suppression partly mediates the effects of light at night exposure on antiviral immunity, we do not anticipate that circulating melatonin concentrations differed between control and amber-hue ALAN exposed individuals. Exposure to ALAN increases nighttime activity levels in captive birds and foraging opportunity in wild shorebirds (Dwyer et al. 2013; Alaasam et al. 2018). Perhaps amber-hue nocturnal illumination provided the opportunity for individuals to forage at night to offset the costs associated with melatonin suppression. Resource availability and quality are linked to immune

function; therefore the link between food intake, metabolism, and immunity should be investigated in individuals exposed to ALAN (Lochmiller et al. 1993; Siva-Jothy and Thompson 2002).

Alternatively, enhanced immune defenses may be related to an evolutionary association between nighttime illumination and risk of injury in prey species. Risk of predation for several organisms is highest during brightly lit nights including especially the full moon phase (Daly et al. 1992; Prugh and Golden 2014). Some nocturnally active rodents avoid illuminated areas including moonlight, where predation risk is high (Lima 1998; Upham and Hafner 2013). Perhaps exposure to light at night initiates anti-predator responses, which mobilize and activate of aspects of the immune system in anticipation of injury (Martin 2009). Indeed, predation risk is associated with exaggerated immune responses in insect species (Duong and Mccauley 2016). The association between nocturnal illumination and predation risk may contribute to the enhanced immune responses and lower WNV burdens we observed in here for amber-hue ALAN exposed birds.

Seasonality in ALAN effects on WNV resistance and mortality

When comparing the present study to our previous findings, we discovered a large seasonal difference in WNV responses. When individuals were exposed to broad-spectrum ALAN during spring months, individuals maintain significantly higher WNV burdens for longer without incurring increased mortality rates (Kernbach et al. 2019). However, in this study focusing on birds caught in fall, individuals exposed to broad-spectrum ALAN showed no significant differences in WNV viremia but did incur a higher WNV-induced mortality rate. There are several different phenomena to which may explain these seasonal effects.

Organisms naturally possess seasonal patterns in the properties of their immune system (Nelson and Demas 1996). These fluctuations may correlate with the risk of exposure during different times of year, such as influenza during winter or arboviruses during late summer (Dowell 2001; Blackmore et al. 2003). Additionally, immunity is often traded-off for other important fitness investments during energetically-demanding life history stages such as reproduction, migration, and molt (Sheldon and Verhulst 1996; Martin et al. 2008). Other stressful or unpredictable conditions such as resource shortage, habitat loss, or extreme weather events may force individuals to reroute energy intended for immune defenses towards other processes required for immediate survival (Lochmiller and Deerenberg 2000; Dobson 2009). A further investigation of the effects of ALAN on host competence over the annual cycle would both enhance our understanding of how light pollution alters infectious disease dynamics and create an opportunity to predict when and where exposure risk is highest (Wonham et al. 2004; Rushing et al. 2017). Although we are unsure what contexts drive seasonal patterns in WNV competence, the effects of ALAN across seasons likely affects within-individual dynamics and ecological level outcomes.

Alternative Lighting Options

Here, we found that exposure to amber-hue LED lightbulbs alleviated most, if not all, negative effects we observed of ALAN exposure on WNV infection results in House Sparrows. This suggests that amber-hue light types may be a viable alternative to other LED lighting methods. Indeed, energy-efficient LED lightbulbs in this spectra are commercially available in multiple models (Florida Fish and Wildlife Conservation Commission 2019). Because these bulbs are already widely used along shorelines, advocating for their use within neighborhoods, cities, roadways, and other developed areas with nighttime illumination should not be met by

harsh criticism (Ferenc and Leonard 2008). Although less feasible, lights-out programs advocated by organizations such as the International Dark-Sky Association (IDA) would likely also be beneficial to wild organisms. In both this and our previous studies, we found that control birds who are housed in near-complete darkness don't incur as much WNV-induced mortality or damage (Kernbach et al. 2019). Other studies have recognized the benefit of lights-out programs such as decreasing building collision rates in nocturnally migrating birds (Winger et al. 2019).

Other studies have suggested the benefits of alternative lighting (such as the technology we used in this study) for other wildlife taxa as well. The use of amber-hue filtered light at night is less impactful on several species of non-passerine wildlife such as Green turtles, Loggerhead turtles, and Newell's shearwater (Ferenc and Leonard 2008; Longcore et al. 2018). Furthermore, installation of amber-hue lighting should also have benefits for other organisms including humans. Indeed, exposure to blue-rich light at night can negatively affect sleep quality, thermoregulation, and resting heart rate in humans, all of which can be alleviated by shifting the spectral composition of nighttime lighting (Cajochen et al. 2005; Chellappa et al. 2013). Therefore, we advocate switching to amber-hue LED lightbulbs as this alternative still offers energy-efficient benefits while eliminating many health risks associate with blue-rich light exposure.

Conclusions:

Our experiment confirmed that melatonin was significantly suppressed by low intensity broad-spectrum (3000K) in House Sparrows. These data provide a plausible link between light at night exposure and antiviral immune dysregulation. However, more work needs to be done to directly link melatonin suppression to the aspects of anti-WNV immunity detailed above. We tested whether altering the spectral composition of ALAN would affect WNV resistance and

mortality; broad-spectrum ALAN enhanced WNV-induced mortality and amber-hue ALAN slightly enhanced viral resistance while maintaining low rates of mortality relative to controls. These results suggest that the substitution of blue-rich wavelengths with amber-hue light at night might be a viable alternative to limiting negative consequences. We stress that ALAN has the potential to impact other parameters that influence WNV transmission and outbreak potential such as vector biting rate, WNV extrinsic incubation period, and other parameters, all of which need to be considered when describing infectious disease dynamics (Kernbach et al. 2018a). Furthermore, it is important to consider other parameters that are known to impact infectious disease outbreak (e.g., area of impervious surfaces, human population density, climate) to determine where to focus intervention efforts. Although our findings here suggest viable lighting alternatives exist, it is still important to consider how spectral composition of ALAN affects other factors important to WNV transmission before recommending blanket lightbulb switches across the nation.

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Author contributions:

M. E. Kernbach contributed to conceptualization, data curation, methodology, investigation, project administration, and writing; V. M. Cassone contributed to data curation and analysis; T. R. Unnasch contributed to conceptualization, funding acquisition, methodology, resources, and supervision; L. B. Martin contributed to conceptualization, data curation, formal

analysis, funding acquisition, investigation, methodology, project administration, resources, supervision, and writing- original draft.

Ethical Statement:

All experimental work was pre-approved by and performed according to IACUC #2716 and IBC #1323.

Table 3.1:

Summary statistics of the generalized linear mixed model to determine effects of day treatment, and their interaction on WNV viremia accounting for random effects of bird ID. A type III ANOVA revealed the main effects of both day and treatment had an effect on WNV viremia. Upon further investigation of the GLMM output, we determined that these main effects are driven by significantly lower viremia in the amber-hue (1800K) ALAN exposed group and the interaction between day (linear (lin) and quadratic (quad)) and the amber-hue group. Significant terms are bolded.

ANOVA (Type III)				
Parameter	**X^2 Value**	**Degrees of Freedom**	**P Value**	
Day	76.3587	3	< 2e-16	
Treatment	**6.9942**	**2**	**0.03029**	
Day x Treatment	8.7008	6	0.19111	
GLMM Fixed Effects				
Parameter	**Estimate**	**Standard Error**	**T Value**	**P Value**
Day (linear)	0.101127	0.012972	7.796	6.41e-15
Day (quadratic)	0.073140	0.010849	6.742	1.57e-11
Day (cubic)	0.014650	0.008698	1.684	0.09212
1800K Treatment	**0.031555**	**0.012474**	**2.530**	**0.01142**
3000/5000K Treatment	0.002687	0.009257	0.290	0.77165
Day (lin) x 1800K	**0.067238**	**0.024680**	**2.724**	**0.00644**
Day (quad) x 1800K	**0.041429**	**0.020729**	**1.999**	**0.04565**
Day (cub) x 1800K	0.015780	0.016147	0.977	0.32842
Day (lin) x 3000/5000K	0.006164	0.018069	0.341	0.73300
Day (quad) x 3000/5000K	0.006026	0.015218	0.396	0.69214
Day (cub) x 3000/5000K	-0.003912	0.011954	-0.327	0.74351

Table 3.2:

Summary statistics of the Cox proportional-hazards analysis to determine effects of treatment, average percent change body mass, average viremia, and their interaction on mortality. A type III ANOVA revealed that all bolded parameters are significant predictors of mortality including treatment, average percent change in body mass since exposure (days 2 and 4), average viremia (days 2 and 4), the interaction between treatment and average viremia, and the interaction between average change on body mass and average viremia. The model outputs illustrated which parameters are driving the significant main effects observed, including significantly higher mortality in the broad-spectrum (3000+5000K) ALAN group and average viremia as a strong indicator of future mortality. Interestingly, the interaction between the broad-spectrum ALAN group and average viremia is significant. Significant terms are bolded.

ANOVA (Type III)				
Parameter	**X^2 Value**	**Degrees of Freedom**	**P Value**	
Treatment	**7.3586**	**2**	**0.02524**	
Avg % Δ Mass	**4.0220**	**1**	**0.04491**	
Avg Viremia	**15.2721**	**1**	**9.308e-05**	
Treat x % Δ Mass	3.9696	2	0.13741	
Treat x Avg Viremia	**6.5208**	**2**	**0.03837**	
% Δ Mass x Avg Viremia	**4.3120**	**1**	**0.03785**	
Treat x % Δ Mass x Viremia	4.1748	2	0.12401	
Cox Prop-Hazards Model				
Parameter	**Coefficient**	**Standard Error**	**Z Score**	**P Value**
1800K Treatment (2)	1.557e+01	9.729e+00	1.601	0.10943
3000+5000K Treatment (3)	**1.950e+01**	**8.843e+00**	**2.205**	**0.02743**
Avg % Δ Mass	-5.311e+00	2.932e+00	-1.811	0.07014
Avg Viremia	**3.577e+00**	**1.162e+00**	**3.079**	**0.00208**
Treat (2) x Avg % Δ Mass	2.685e+00	5.821e+00	0.461	0.64465
Treat (3) x Avg % Δ Mass	5.158e+00	2.946e+00	1.751	0.07993
Treat (2) x Avg Viremia	-2.028e+00	1.313e+00	-1.544	0.12260
Treat (3) x Avg Viremia	**-2.561e+00**	**1.170e+00**	**-2.189**	**0.02863**
% Δ Mass x Avg Viremia	7.055e-01	3.776e-01	1.868	0.06172
Treat (2) x Avg % Δ Mass x Avg Viremia	-3.491e-01	8.921e-01	-0.391	0.69558
Treat (3) x Avg % Δ Mass x Avg Viremia	-6.864e-01	3.796e-01	-1.809	0.07052

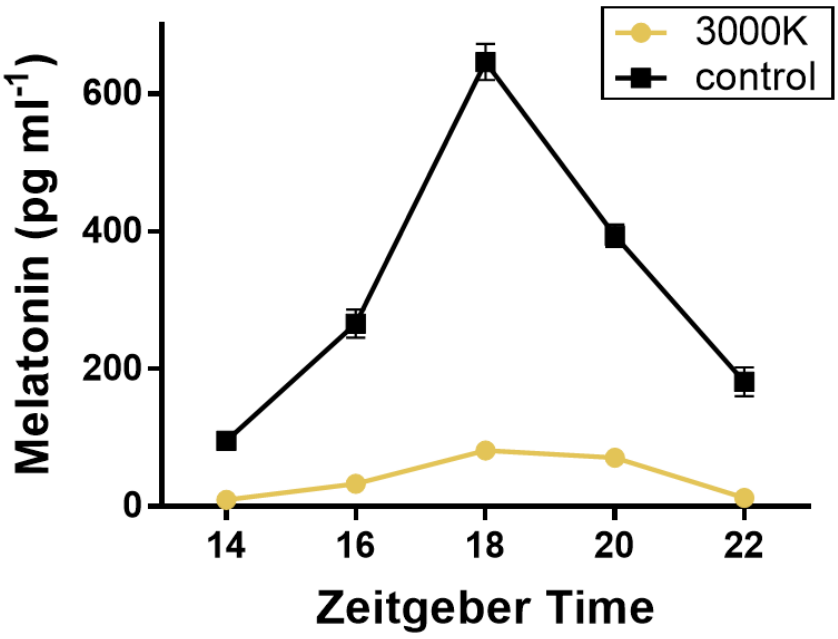

Figure 3.1:

Melatonin concentration in pg ml-1 measured during the dark phase at Zeitgeber times 14 (8pm), 16 (10pm), 18 (12am), 20 (2am), and 22 (4am) in both control (~0 lux, darkness; N=24) and 3000K ALAN exposed (N=24) birds post-exposure/captivity.

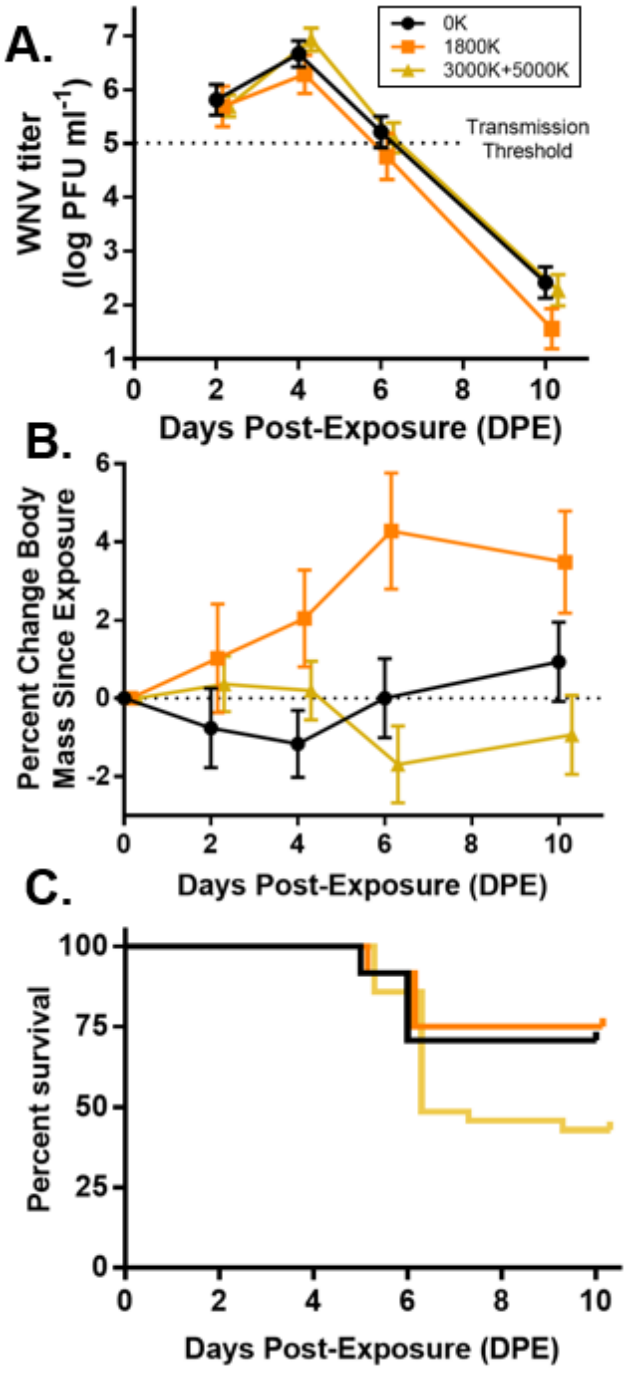

Figure 3.2:

The effects of spectral composition of ALAN on (**A**) WNV viremia (log10 PFU ml^{-1}), (**B**) percent change body mass since exposure (g), and (**C**) percent survival. Birds exposed to amber-hue (1800K) ALAN (N=12) had significantly lower viremia throughout the course of

infection and significant interaction across days post-exposure when day is described as both a linear and quadratic function (panel A). There are no main effects of treatment or day on percent change body mass, however, there is a significant interaction between broad-spectrum and blue-rich (3000K+5000K) ALAN (N=35) and day when day is modeled as a linear function (panel B). 3000+5000K ALAN exposed birds incurred a significantly mortality rate than control (N=24) and amber-hue exposed birds, which is partly driven by average viremia on days 2 and 4 post-exposure (panel C).

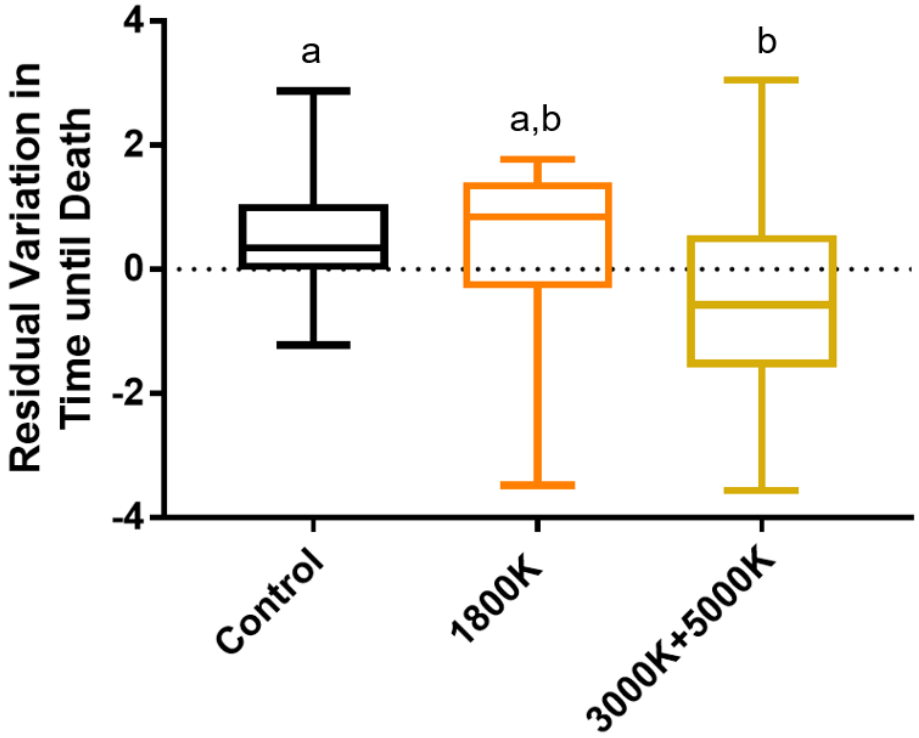

Figure 3.3:

Residual variation of the means of time (days) until death as a function of average viremia during days 2 and 4 post-exposure. A one-way ANOVA analysis followed by a Tukey pairwise comparison showed that there was a significant difference between broad-spectrum (3000+5000K) ALAN (N=35) and control (N=24) birds (P = 0.034). The significant difference exists between control (a) and broad-spectrum (b) treatments; amber-hue treatment (a,b; N=12) did not differ from either control (a) or broad-spectrum treatments (b).

CHAPTER III:

LIGHT POLLUTION AFFECTS WEST NILE VIRUS EXPOSURE RISK ACROSS FLORIDA

Note to Reader:

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Abstract:

Emerging infectious diseases (EIDs) present global health threats, and their emergences are often linked to anthropogenic change. Artificial light at night (ALAN) is one form of anthropogenic change that spans beyond urban boundaries and may be relevant to EIDs through its influence on behavior and physiology of hosts and/or vectors. Although West Nile virus (WNV) emergence has been described as peri-urban, we hypothesized that exposure risk could also be influenced by ALAN in particular, which is testable by comparing the effects of ALAN on prevalence while controlling for other aspects of urbanization. By modeling WNV exposure among sentinel chickens in Florida, we found strong support for a nonlinear relationship between ALAN and WNV exposure risk in chickens with peak WNV risk occurring at low ALAN levels. Although our goal was not to discern how ALAN affected WNV relative to other factors, effects of ALAN on WNV exposure were stronger than other known drivers of risk (i.e., impervious surface, human population density). Ambient temperature in the month prior to sampling, but no other considered variables, strongly influenced WNV risk. These results indicate that ALAN may contribute to spatiotemporal changes in WNV risk, justifying future investigations of ALAN on other vector-borne parasites.

Introduction:

Emerging infectious diseases (EIDs) are among the greatest threats to public health today (Binder et al. 1999; Morens et al. 2004). Most EIDs are zoonotic in origin (70%), in that causative agents spill over to human populations from other species (Woolhouse and Gowtage-Sequeria 2005; Jones et al. 2008). Anthropogenic effects on wildlife, such as habitat fragmentation, sensory pollutants, and toxin exposure, can become detrimental to humans in places where humans and wildlife come into contact (Estrada-Peña et al. 2014; Murray et al. 2019; Dominoni et al. 2020). The recent surge in many EIDs can be attributed to various forms of global change, including climate change and the structure and biological composition of landscapes (Jones et al. 2008; Lewis and Maslin 2015; McMahon et al. 2018). One recent example of an anthropogenically-driven zoonotic EID is West Nile virus (WNV), which was introduced to the United States in 1999 (Kilpatrick and Randolph 2012). WNV decimated susceptible bird populations, especially corvid species, within the first several years of its arrival, and its propensity to be transmitted by many vector species also made it a source of substantial human and livestock (i.e., horse) disease (Marfin et al. 2001; Turell et al. 2001; Hayes et al. 2005; Marm Kilpatrick and Wheeler 2019). Now, 20 years since its introduction, WNV continues to cause harm to diverse animal populations, particularly in or near highly human-modified habitats (Liu et al. 2011; Johnson et al. 2012).

WNV is recognized as a peri-urban arbovirus, as human incidence and songbird seroprevalence are much higher in or near urban habitats (Bradley et al. 2008; Kilpatrick 2011). Historically, higher incidence of WNV in or near cities was linked to aspects of environments that influence mosquito success such as local climate and availability of breeding sites (Bradley et al. 2008; Loss et al. 2009). The Florida Department of Health surveillance system has closely

monitored arbovirus transmission in mosquito breeding hotspots, such as peri-urban drainage systems, since the late 20th century (Day and Lewis 1991; Day et al. 2015). WNV dynamics resemble other zoonoses in that urban and agricultural predominance affect emergence and transmission (Gómez et al. 2008). For instance, Lyme disease (caused by *Borrelia burgdorferi*) and flavivirus infections including yellow fever, dengue, and chikungunya, emerge where competent host and vector communities occur in close proximity to humans (LoGiudice et al. 2003; Dhondt et al. 2005; Weaver 2013). Some anthropogenic stressors have been found to affect zoonotic risk, but many conspicuous and common ones have never been considered, including light pollution. One form of light pollution, artificial light at night (ALAN), now covers 18.7% of the continental U.S. and affects 99% of the human population with increases anticipated in the future [e.g., 2.2% increase globally per year from 2012 to 2016, (Kyba, Kuester, et al. 2017)]. Further, small areas of urban development can emit light into distant suburban and rural landscapes, suggesting that light pollution effects, and skyglow in particular, may be widespread (*Population, Housing Units, Area, and Density: 2010 - United States -- States; and Puerto Rico* 2010; Horton et al. 2019).

Light pollution affects multiple host traits with consequences for disease transmission. For instance, in night shift workers, ALAN can affect non-communicable disease risk (e.g., cancer, diabetes, etc.) (Navara and Nelson 2007), likely because vertebrate immune systems are 'fundamentally circadian in nature' (Navara and Nelson 2007; Cermakian et al. 2013; Becker and Ketterson 2020). House sparrows, a common passerine reservoir of WNV, experimentally infected with WNV and exposed to modest ALAN (i.e., 5 lux; a full moon on a clear night is 0.3 lux) maintained transmissible WNV titers for 2 days longer than controls but did not experience higher mortality (Kyba, Mohar, et al. 2017; Kernbach et al. 2019). Epidemiologically, this

extension of the infectious-to-vector period were estimated to increase outbreak potential by 41% (Kernbach et al. 2019). Host population characteristics also have the capacity to alter disease transmission. For example, avian species diversity is known to affect WNV transmission, but there are no studies to our knowledge that have quantified direct effects of ALAN on avian community composition (Dominoni et al. 2014; Evans et al. 2015).

ALAN also probably alters a multitude of vector traits that affect arbovirus transmission. Arthropod vectors of WNV, mainly *Culex spp*., are renowned for their flight-to-light behaviors; for those vectors that survive desiccation or depredation, flight-to-light behavior might concentrate infection risk where light pollution is common (Barghini and de Medeiros 2010; Donners et al. 2018). WNV can be transmitted by as many as 45 vector species, many of which bite wildlife (including songbirds) and humans, and are abundant across Florida (Sardelis et al. 2001; Marra et al. 2004; Hamer et al. 2008). WNV vectors are dense in urban areas, as surface imperviousness is one of the strongest predictors of *Culex spp.* distribution (Gangoso et al. 2020). Interestingly, *Culex spp.* are more abundant in areas of moderate ALAN than low or high ALAN during late summer months during peak transmission (Kernbach et al. 2018b).

Given the current pervasiveness of ALAN, we asked whether light pollution can affect infectious disease dynamics in a part of the US where arboviruses are common and influential, both economically and socially (Kernbach et al. 2018b). Specifically, we investigated whether ALAN affected risk of WNV exposure across several counties of Florida where emergence and spillover has occurred in the recent past ("Mosquito-Borne Disease Surveillance" 2020). We chose to focus on WNV because it is the most broadly distributed arbovirus and most common causative agent of viral encephalitis worldwide (Chancey et al. 2015; Davies and Smyth 2018). Using data from the Florida Department of Health (FDOH) sentinel chicken WNV surveillance

program (Day and Lewis 1991), we tested whether WNV exposure, as estimated by the number of sentinel chickens undergoing antibody seroconversion, would be related to ALAN exposure. We used mixed-effect models with and without spatial correlation structure to assess the effects of ALAN (in radiance, millicandela/m^2) on WNV exposure for four recent years across 5 counties based on 6468 samples from individual chickens from 1126 surveillance events across 105 unique geographical coordinates, including many peri-urban regions (Figure 4.1). These models also accounted for previously documented and other hypothesized predictors of WNV risk (i.e., variation in temperature, precipitation, soil moisture, and several aspects of urbanization).

We hypothesized that WNV exposure risk would have a nonlinear relationship with ALAN, being greatest in areas of moderate ALAN, and lowest in non- and intensely light polluted areas. We made this specific and yet complex prediction because we expected vector density and/or host competence to be highest in areas with intermediate light pollution (Kernbach et al. 2019). As above, house sparrows were more infectious under these ALAN conditions than natural light-dark conditions. Also, we found previously that some *Culex* mosquitoes were most abundant in areas of moderate ALAN during WNV transmission season, indicating there might be combined effects of flight-to-light behavior from dark areas, but increased vector predation in intensely illuminated areas (Eisenbeis and Hassel 2011; Krebs et al. 2014; Lewanzik and Voigt 2014; Kernbach et al. 2018b). Active avoidance of ALAN by passerines, likely in part due to increased predation risk or negative physiological effects, could also decrease host density in brightly illuminated places, subsequently reducing opportunities for transmission (Daly et al. 1992; Dominoni et al. 2014; Lewanzik et al. 2014; Fallows et al. 2016; Kernbach et al. 2020).

Methods:

Sentinel Data

Sentinel chicken data were shared by the Florida Department of Health offices in Leon, Manatee, Nassau, Sarasota, St. Johns, Volusia, and Walton counties for the years 2015-2018. The data were provided as monthly total number of sentinel chickens per site that tested positive for WNV antibodies. WNV case counts result from weekly sampling of either 6 (89.5% of observations) or 4 (10.5% observations) sentinel chickens located at each site ("Mosquito-Borne Disease Surveillance" 2020). Once a chicken tested positive for WNV antibodies, it was removed from a coop and replaced with a WNV-naïve chicken, ensuring that all positives are new exposures. Coops housing sentinel chickens were occasionally moved small distances (typically < 0.001 degrees or less than approximately 100m, but as much as 11km) resulting in small differences in coop locations across years. As such, we used the unique coordinates from each sentinel chicken sampling location to determine the anthropogenic components of night sky brightness in radiance (ALAN), weather variables (soil moisture, temperature, precipitation), and urbanization variables (human population density, anthropogenic impervious surface extent, human footprint index, see below) at the time and place of sampling of each chicken coop.

Environmental Data

Because multiple dimensions of the environment, including ALAN, vary along an urbanization gradient, understanding which aspects are responsible for elevated WNV risk is challenging. As such, we assembled geospatial data reflective of several dimensions of urbanization: percent anthropogenic impervious surface from the 2011 National Landcover Database [30m resolution; (Homer et al. 2015)], human population density from the 2010 US Census [1km resolution; (US Census Bureau 2010)], human footprint index data reflective of

conditions in 2009 [1km resolution; (Venter et al. 2016)], plus ALAN estimates from the world atlas of artificial night sky brightness [30-arc seconds, i.e., approx. 1 km resolution; (Falchi et al. 2016)], which is an improved measure of NASA's VIIRS data using zenith sky brightness confirmed with handheld sky quality monitors (Zhong et al. 2020). This resource provides the anthropogenic component of night sky brightness in radiance (microcandela/m^2, henceforth denoted as μcd/m^2) and is regarded as the most relevant available option at the spatial scale of our study, particularly as zenith sky brightness is more highly correlated with ground-level ALAN exposure than VIIRs satellite data (Simons et al. 2020). We temporally-harmonized high-resolution monthly cumulative precipitation and monthly mean temperature data (0.8km resolution) from the PRISM database (Schneider et al. 2013) and monthly mean soil moisture estimates (3km resolution) generated from NASA's Sentinel 1/SMAP platform (Das et al. 2017) to match the month of sentinel chicken sampling. Additionally, to account for potential lags in conditions that could favor vector abundance, we also collected data for these variables from the month prior to sampling. Because >94% of the positive detections of WNV occurred after May of each year, which is similar to other studies (Apperson et al. 2004b; Bolling et al. 2009), we restricted our analyses to June-December. Additionally, because mean soil moisture estimates for the month of surveillance and the prior month were not available for all records, we restricted analyses to those records with complete environmental data, resulting in a final dataset of 6,468 samples from individual chickens from 1,126 surveillance events spanning 80 sites and 105 unique spatial locations.

Data analysis

We modeled incidence of WNV seroprevalence using mixed-effect models with and without a spatially-explicit exponential correlation structure with negative binomial error and

implemented in the fitme function of the R package *spaMM* 2.7.5 (Rousset and Ferdy 2014) and the glmm.nb function of the R package *lme4* 1.1-21 (Bates et al. 2015). We used a negative binomial error rather than Poisson error in our models as many preliminary Poisson models did not converge. Models that did converge received less support from the data than identical models with negative binomial error (i.e., $\Delta AIC \geq 30$). Because of the high number of zeros in the response variable, we also checked models for zero-inflation using the testZeroInflation function in the R package *DHARMa* 0.2.4 (Hartig 2020) but found no evidence that a zero inflated term was necessary.

Given the hierarchical and repeated sampling regime of sentinel chicken surveillance programs, we included nested random effects of sampling month within site within county. For fixed effects, we built a model that included ALAN, the three variables reflective of urbanization (i.e., impervious surface extent, population density, human footprint) and monthly mean soil moisture, precipitation total, and mean temperature, plus year to account for interannual variation. All continuous variables were centered and scaled to facilitate direct comparisons of effects and, based on our hypothesis that WNV seroprevalence would peak at intermediate ALAN exposure levels, we also modeled the effect of ALAN as a second-order polynomial. Preliminary data exploration revealed a nonlinear relationship between WNV seroprevalence and monthly mean temperature, which was also best explained by a second-order polynomial.

From the fully parameterized model described above, we explored whether substituting monthly soil moisture, precipitation total, and mean temperature values with the corresponding values from the previous month improved model performance using Akaike information criterion (AIC) values. We retained soil moisture and precipitation totals from the months in which chickens were sampled. However, subsequent analyses included monthly mean

temperature of the prior month because this time-lagged form of the temperature variable received stronger support than monthly mean temperature of the month of sampling (ΔAIC > 90). We used AIC scores to gauge whether polynomial terms for ALAN and mean temperature of the previous month improved model performance over linear effects of each. We also used AIC scores to arbitrate between spatial and non-spatial models, to evaluate the support for using an offset to account for the number of chickens sampled per site per month, and to test whether there was any evidence for polynomial effects of predictor variables other than ALAN that reflected urbanization. We considered models with ΔAIC scores $\leq$ 2.0 as equally competitive and determined that a parameter had a strong effect on WNV seroprevalence patterns when its 95% confidence interval (95% CI) did not overlap zero. Finally, because different approaches to model selection can result in different results (Posada and Buckley 2004), we evaluated relative model support using two additional information criteria in the *spaMM* package: the conditional AIC (cAIC) (Vaida and Blanchard 2005), which is conditional on the realized values of the random effects, and the dispersion AIC (dAIC), which focuses on dispersion parameters (Ha et al. 2007).

Finally, we conducted two forms of model diagnostics. First, we checked for potential multicollinearity and redundancy among predictor variables with variance inflation factor (VIF) and considered VIF > 10 as potentially problematic (F. Dormann et al. 2007). Mean temperature of the previous month, and its quadratic term, were the only parameters with suspect VIF scores. However, centering this variable at its mean resulted in VIF < 2.0 for each variable. Second, we assessed model performance with qqplots and residual vs. expected value plots using the simulateResiduals function in the R package *DHARMa* 0.2.4 (Hartig 2020).

Results and Discussion:

ALAN was a strong but nonlinear predictor of WNV exposure risk in Florida (Figure 4.2A). Models with and without spatial correlation structure (or an offset to account for variation in the number of chicken samples per site; see supplementary text) were equally competitive (Table 4.1) and included qualitatively similar parameter estimates for ALAN. Alternative model selection criteria also confirmed model rankings via AIC and relative competitiveness. Specifically, we found that WNV risk rises rapidly from dark conditions and peaks at low anthropogenic radiance intensities (i.e., <1), but then declines with higher ALAN exposure (Figure 4.3A), which was slightly lower than expected but overall consistent with our hypothesis. Besides the consistent influence of ALAN, all models provided strong evidence for a lag effect of temperature whereby high temperature (>25 °C) during the previous month strongly and positively influenced WNV seroprevalence (Figure 4.2B, Figure 4.3C). In contrast, increases in monthly cumulative precipitation were negatively related to WNV seroprevalence (Figure 4.3D), which is also consistent with prior work (Paull et al. 2017). In contrast with previous reports (Bradley et al. 2008; Kilpatrick 2011), we found no influence of two metrics of urbanization, human footprint index or percent anthropogenic impervious surface, on WNV seroprevalence in chickens. We found mixed support for an influence of human population density on WNV seroprevalence where two of the four models suggested there was a positive relationship (Figure 4.3B; supplementary text). Additionally, there was also some evidence for inter-annual variation in WNV prevalence; nonetheless, we found consistent polynomial ALAN effects on WNV risk across several years (Figure 4.3A).

To ensure that the relationship between WNV seroprevalence and ALAN did not reflect nonlinear effects of other variables characteristic of urbanization, we also tested for polynomial

effects of human population density, human footprint index, and percent anthropogenic impervious surface on WNV exposure risk. No polynomial terms for these variables were supported, and the model with only linear terms for all predictors (including weather variables and ALAN) received the least support (Table 4.2). Alternative model selection criteria (cAIC and dAIC) led to the same conclusions. Finally, based on the polynomial effects of both temperature of the previous month and the natural log of ALAN, we conducted a post hoc analysis with a model containing the interaction between the two, but the interaction decreased model performance (ΔAIC = 7.564; Table 4.2).

The use of radiance in units of microcandelas per m^2 in this study is admittedly hard to translate into values of light pollution measurable at fine spatial scales. Radiance values in our dataset appear relatively low, but supplemental figures show most ALAN values range from 0-2.5, so the peak in WNV cases around <1 can be interpreted as low to moderate light pollution. Previous experimental studies measured ALAN in units of lux using handheld light meters in enclosed facilities, which served as the foundation for our hypotheses (Kernbach et al. 2020). Whereas others have emphasized that data from the New World Atlas of Artificial Night Sky Brightness that we chose to use here are highly related to light pollution levels at ground-level (Bustamante-Calabria et al. 2020; Zhong et al. 2020), a critical component to future work involving ALAN will be understanding how remotely-sensed values translate to other common metrics for quantifying light exposure, such as lux (Zamorano et al. 2017). Thus, although our models suggest that WNV risk increases from the darkest sites in our dataset to peak in areas exposed to some degree of light pollution, we are limited by the current availability of data, namely that our ALAN measure captures only the artificial component of night sky brightness. Notwithstanding the importance of developing methods to better describe light pollution at finer

spatial scales, below we discuss how light pollution might exacerbate risk of WNV and other EIDs, and why WNV risk exhibited a nonlinear relationship with ALAN.

Why does ALAN affect WNV exposure?

The relationship between ALAN and WNV exposure risk we described was not altogether surprising. As highlighted earlier, ALAN can extend the infectious period of one avian host; perhaps similar effects occur in other host species (Kernbach et al. 2018a). Other studies strongly suggest that ALAN could also increase the local density and feeding period of crepuscular vectors, which might significantly alter opportunities for transmission (Wonham et al. 2004). Such effects are particularly likely because many vectors exhibit flight-to-light behavior, which could further concentrate risk spatiotemporally (Barghini and de Medeiros 2010).

Nonlinearity of ALAN effects on WNV exposure risk

Our results suggest that although WNV exposure risk initially increased with ALAN, it subsequently declined towards the most intensely lit sites in our study. Previously, we found that hosts exposed to broad-spectrum ALAN incurred greater mortality risk to WNV, which may create a disease "sink" for some avian hosts in highly light polluted areas (Kernbach et al. 2020). As above, surveys of *Culex* mosquitoes in the Tampa Bay region also suggest that vectors are more abundant in moderately light-polluted areas than non- and highly light-polluted areas during the WNV transmission season (Kernbach et al. 2018a). Hosts and vectors are probably both at higher risk of predation under a full moon or street lights or other sources of ALAN, which may contribute to lower host and vector densities in intensely light-polluted areas (Lewanzik and Voigt 2014; Minnaar et al. 2015). Similarly, individual birds avoid light exposure at night, which would further decrease host density in intensely light polluted areas (Dominoni et

al. 2014). Many characteristics of highly light polluted areas (e.g., fragmented habitat) support fewer hosts and fewer vector breeding sources, and may be subject to extensive vector control efforts (Norris 2004; Wright and Gompper 2005; Luz et al. 2011). Altogether, recruitment of vectors to light polluted areas, combined with reduced viral resistance, predator avoidance behavior, and/or host or vector mortality effects could all contribute to the observed nonlinear relationship between ALAN intensity and WNV exposure risk. To better understand the mechanisms underlying the nonlinear relationship between WNV exposure and ALAN, we advocate for future research on the light intensity-dependent effects of ALAN on vector survival, vector bite rate, and host susceptibility (Wonham et al. 2004).

Weather, precipitation, and WNV

In addition to ALAN, average temperature of the prior month predicted WNV exposure in parts of Florida. It is unsurprising that this climate component was important in our models, as many aspects of WNV transmission are temperature-dependent (e.g., vector development, survival, and competence) (Paz 2015). Indeed, most vectors of WNV thrive when temperatures increase over summer months, as high temperatures accelerate vector growth rates and extrinsic incubation periods (i.e., the time required to develop transmissible virus in salivary glands) (Paz and Semenza 2013). Periods of heavy rainfall and the availability of water sources for breeding can also sustain large populations of *Culex nigripalpus*, a moderately competent WNV vector in south Florida (Shaman et al. 2003; Turell et al. 2005). Relatedly, irrigation in the Western United States has sustained populations of WNV vectors during dry periods, creating suburban hotspots (DeGroote and Sugumaran 2012). Although water sources are required for mosquito breeding, drought is a significant predictor of high WNV infection rates in *Culex pipiens* and *restuans* (Johnson and Sukhdeo 2013). Drought can indirectly increase vector abundance by decreasing

mosquito predator density during drought years, however, these effects are typically time-lagged (Chase and Knight 2003; Lebl et al. 2013). Alternatively, contradicting data indicate that higher WNV incidence may reflect changes in host competence rather than vector success (Paull et al. 2017). While drought could be considered in future studies, we argue that the inclusion of rainfall data here enhanced our ability to control for natural factors at a higher resolution when asking how other variables affect WNV exposure risk.

Urbanization and zoonotic exposure risk

Urbanization has long been viewed as a driver of WNV prevalence (Bradley et al. 2008), but here, multiple metrics of urbanization had no to little explanatory power for WNV risk. Other studies have concluded that urban land use and human population density were important predictors of inter-annual WNV prevalence over large geographic regions (Begon et al. 2002; Gibbs et al. 2006a; Gómez et al. 2008). These environmental features are hypothesized to drive WNV incidence due to decreased host diversity in urban areas, altered vector ecology (e.g., small water sources ideal for mosquito breeding), and/or increased host susceptibility to infection (Ezenwa et al. 2006b; Bradley and Altizer 2007b). However, besides mixed support for a positive influence of human population density, we found minimal effects of urbanization on risk. One potential reason we did not detect relationships with urbanization might be due to a lack of data from the most urbanized areas of Florida. However, our study did include locations where anthropogenic surface was 67% and the human footprint index was 46.28 on a scale of 0-50, so we were able to capture diverse intensities of human inhabitation (Venter et al. 2016). It is also possible that our urbanization metrics are distinct to the ones used in previous studies, as sentinel chicken sites typically are not located in city centers (supplementary text). Further

exploration of how ALAN interacts with other aspects of urbanization across the landscape to influence WNV risk will be valuable.

Conclusions and Implications

Our study suggests that light pollution might affect arbovirus infection risk in Florida and perhaps elsewhere. Investigating how host and vector responses to ALAN vary with intensity level of light will be required to fully understand patterns in WNV exposure risk. The consideration of wild bird or vector data in the future would provide a more comprehensive description of how light at night might be affecting WNV outbreaks, whether it be *Culex spp.* derived or reservoir driven, and provide information regarding effective intervention strategies. Although additional studies are needed to assess the commonness of relationships between ALAN and WNV risk in other areas, mitigation opportunities (e.g., alternative lighting technologies) exist that could ameliorate the negative consequences of ALAN on wildlife and humans (Navara and Nelson 2007). Furthermore, we emphasize the need to investigate effects of ALAN on other passerine reservoirs of WNV, as songbirds, rather than sentinel chickens, drive transmission (Komar et al. 2003). In particular, we should consider important peri-urban and rural reservoirs such as Northern Cardinals (*Cardinalis cardinalis*) and American Robins (*Turdus migratorius*) as they dominate avian communities in areas with low-intensity ALAN and are quite competent for WNV (Evans et al. 2015). Many cities and neighborhoods are switching to cool white light-emitting diodes (LEDs), which could be especially harmful given the sensitivity of wildlife and humans to blue wavelengths (Witherington and Bjorndal 1991; Ferenc and Leonard 2008; Dimovski and Robert 2018). Attenuating spectral composition while substituting high pressure sodium and halogen street lights would provide an energy efficient

alternative while potentially alleviating broad light pollution effects on other ecological systems (Gaston et al. 2012).

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Author Contributions:

M. E. Kernbach solicited data, assisted in data cleaning, and wrote manuscript; L. B. Martin solicited data, assisted in data cleaning, and revised manuscript; T. R. Unnasch solicited data and revised manuscript; R. J. Hall assisted in data anlyses and revised manuscript; R. H. Y. Jiang revised manuscript; C. D. Francis solicited data, assisted in data cleaning, performed analyses, and revised manuscript.

There is no ethical statement for this study.

Table 4.1:

Rankings among models with and without spatial correlation structure and offsets to account for variation in number of sentinel chickens surveyed. All models contained the following fixed effects: 2nd order polynomial terms for mean temperature of the previous month and natural log of ALAN, plus human footprint index, human population density, percent impervious surface, monthly total precipitation, monthly soil moisture and year.

Model	logLik	AIC	DAIC
Spatial	-727.492	1492.984	0.000
Non spatial	-729.800	1493.601	0.617
Non spatial with offset	-729.939	1493.878	0.895
Spatial with offset	-729.272	1496.543	3.560

Table 4.2:

Sensitivity analysis of polynomial effects on WNV exposure. Rankings among models where anthropogenic predictors and mean temperature of the previous month were modeled with linear or polynomial effects. All models contained the following fixed effects: mean temperature of the previous month (temp), natural log of ALAN, human footprint index (hum foot), human population density (pop den), percent impervious surface (imp sur), monthly total precipitation, monthly soil moisture, and year. For each model, all predictors remained in each iteration of the model and we denote which parameters were included as polynomial effects with "poly". "All linear" reflects a model with no polynomial terms. "poly ALAN * poly temp" reflects the *post hoc* analysis of adding an interaction to the top ranked model in Table 4.1. All models were spatially explicit and did not contain an offset (i.e., such as top ranked model from Table 4.1).

Model	logLik	AIC	ΔAIC	cAIC	dAIC
poly ALAN, poly temp	-727.492	1492.981	0.000	1460.880	1466.98
poly temp	-730.790	1497.580	4.596	1463.940	1473.58
poly pop den; poly temp	-730.753	1499.506	6.522	1465.320	1473.51
poly imp sur; poly temp	-730.754	1499.508	6.524	1465.544	1473.51
poly hum foot; poly temp	-730.781	1499.561	6.577	1465.450	1473.57
poly ALAN	-736.806	1509.611	16.627	1478.040	1485.61
all linear	-740.050	1514.100	21.116	1481.260	1492.11
poly ALAN * poly temp	-727.272	1500.545	7.564	1468.182	1466.54

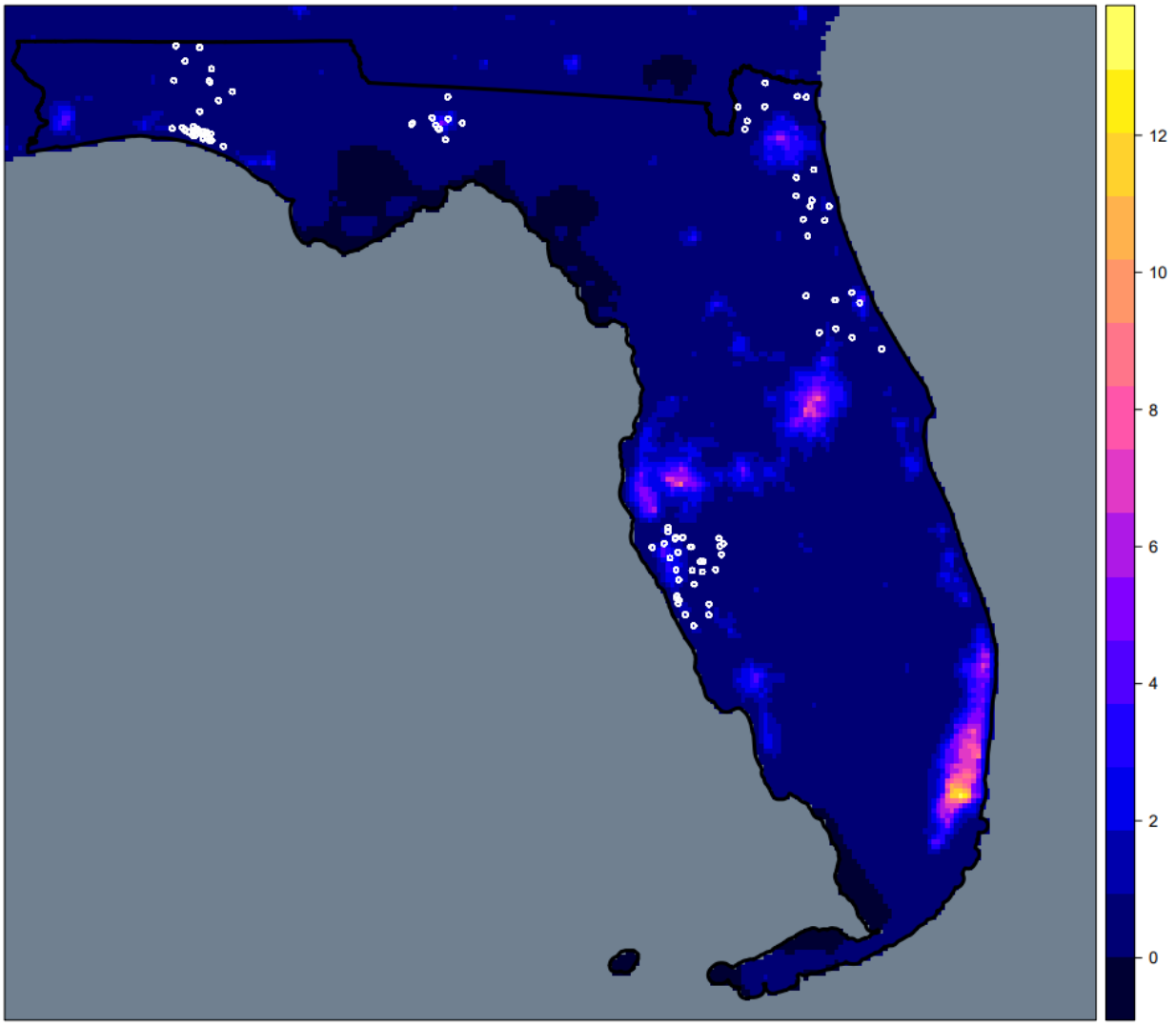

Figure 4.1:

Sentinel chicken sampling locations (white circles) throughout Florida overlaid on the artificial component of night sky brightness in radiance (μcd/m^2) estimates from Falchi et al. (Falchi et al. 2016).

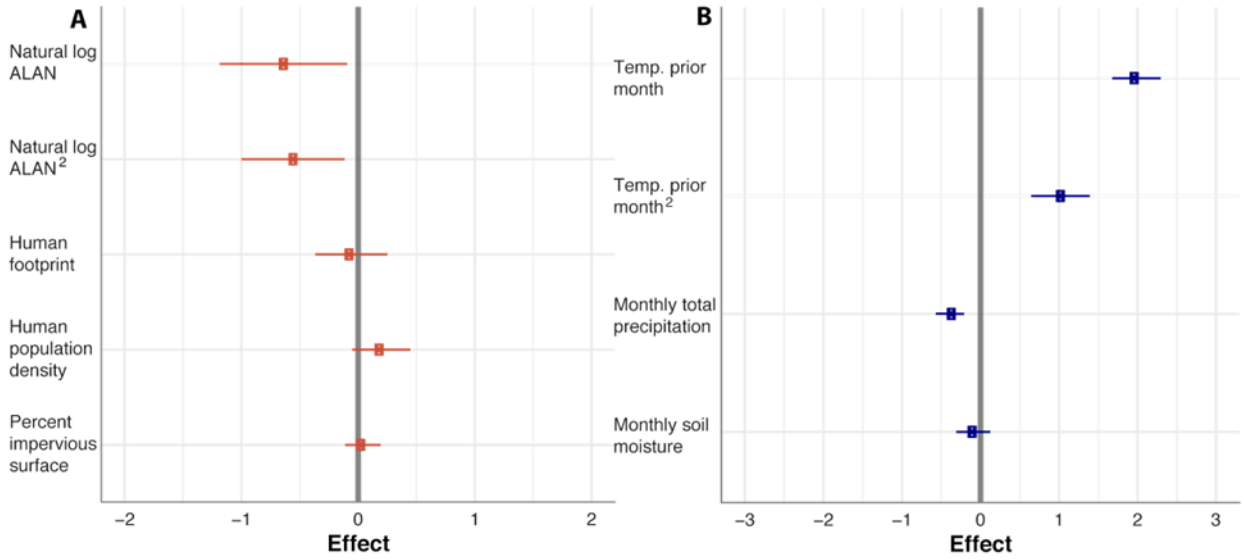

Figure 4.2:

Effect sizes of (A) ALAN and urbanization, and (B) temperature and weather variables on West Nile virus risk. Standardized effect sizes (square points) and 95% CI (lines) for (A) anthropogenic and (B) weather-related variables from top ranked model in Table 4.1. Natural log of ALAN and ALAN^2 in radiance (μcd/m^2) had the largest effects on WNV risk compared to urbanization parameters, whereas mean temperature and mean temperature^2 of the prior month had the largest effect sizes compared to precipitation and soil moisture variables. Variables are natural log transformed to account for the extreme values in the distribution of data.

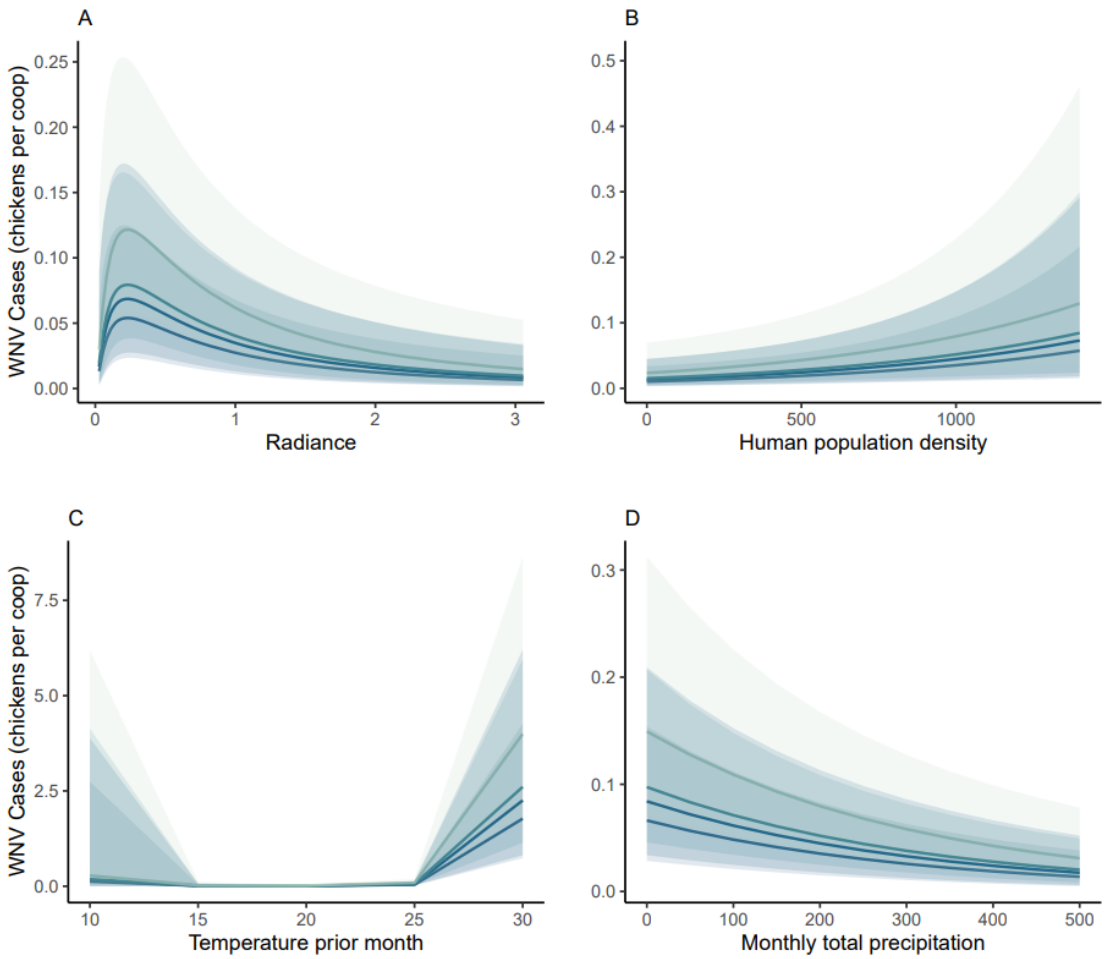

Figure 4.3:

Interannual effects of (A) ALAN, (B) human population density per km^2, (C) mean temperature of the previous month in degrees Celsius, and (D) monthly total precipitation (mm) on incidence of WNV seroprevalence. Marginal effects of each plotted by year with 95% CI band. Darkest color denotes earliest year (i.e., 2015) with lighter colors denoting subsequent years. Effects of ALAN (anthropogenic component of night sky brightness in radiance ($\mu cd/m^2$), human population density, temperature, and precipitation on WNV exposure were consistent across 5 years.

CONCLUSION

Through my dissertation research, I have started to uncover the effects of ALAN on WNV disease dynamics. Ultimately, light pollution was often overlooked when considering urban stressors, but has gained much attention over recent years. Here, I expanded upon the current knowledge about ALAN, immunity, and impacts on wildlife by considering the many points at which ALAN could affect vector-transmitted pathogens, and then asking whether ALAN affects the ability of house sparrows to cope with WNV, whether ALAN spectral composition affects coping ability, and whether these effects of ALAN on host competence observed in the lab translate ecologically (Kernbach et al. 2018a, 2019, 2020). Through these studies, I found that even modest levels of ALAN do affect the ability of individuals to resist and tolerate infection (Kernbach et al. 2019). Further, I found that these effects could potentially be resolved by altering spectral composition of ALAN (Kernbach et al. 2020). Lastly, I found that ALAN is one of the best predictors of WNV exposure risk in across Florida. Below, I consider the consequences of these studies and areas for future study.

The centerpiece of ALAN effects on WNV dynamics tends to hinge on circadian dysregulation and the mismatch of organisms with their environments. Previously, many studies have found that physiological mismatch with an organisms (Lof et al. 2012; Walker et al. 2019). Here, the mismatch of organisms with the correct time of day resulting from masking or incorrect light cues leads to false or dysregulated physiology. For example, fluctuation of immune cells throughout the day coordinated with relative risk of infection can become out of synch with consequences for host competence and spillover (Westermark et al. 2009; Lange et

al. 2010). Hormonal fluctuations throughout the day such as glucocorticoids with the onset of activity or melatonin with sleep/wake cycles are important to synchronizing organismal functions with environmental demands (Dickmeis 2009; Pevet and Challet 2011). When glucocorticoids and melatonin are dysregulated, the coordinated timing of activity and sleep/wake cycles will follow. Subsequently, the ability of a host or vector to combat infection pivots on environmental mismatch, ultimately generating consequences for disease dynamics.

Effects of ALAN on host immune responses to WNV were not altogether surprising but provide new insights into how environmental stressors may impact that ability of wildlife to battle infection. Many other stressors, including forest fragmentation, resource limitation, and habitat loss all affect host health, including immunity (Aguirre and Tabor 2008). Given that ALAN also affects wildlife health, it is important to consider whether urban expansion should occur in certain areas, and if existing effects of urban areas might be mitigated through the installation of new lighting. City planners and urban developers should collaborate with ecologists and wildlife physiologists in the future to determine whether certain aspects of development are acceptable in various regions (Murray et al. 2019). For example, building brightly illuminated agricultural facilities along the edge of forests that are home to species that harbor infectious diseases would likely generate many negative consequences for both wild organisms and the people that reside nearby if spillover occurs (Weaver 2013). Some of these species include bats or flying foxes, mice or related rodents, and corvids or other birds that harbor diseases that spill over to humans (Allan et al. 2003; Luniak 2004; Ferraguti et al. 2016). A proper assessment of species presence and disturbance level, including extent of light pollution, should be incorporated in further urbanization plans.

As mentioned previously, altering the spectral composition of light pollution is also an alternative that requires further consideration. As many organisms are most sensitive to short-wavelength light, reducing blue-hues in outdoor lighting technologies might prevent effects of ALAN on wildlife (Gaston et al. 2012). However, it is important to note that amber-hue ALAN is not without effect on sparrows, as I found here (Kernbach et al. 2020). While amber-hue ALAN enhanced viral resistance, which is beneficial to both bird and community, it is still unknown whether this also alters behavior or other physiological functions that might affect host competence in other ways (Wonham et al. 2004). Additionally, it is important to consider that not all organisms in the environment are uniform in visual spectral sensitivity (Osorio and Vorobyev 2008). Insect species respond differently to various spectral compositions of ALAN, which in turn affects their behavior and predation risk (Eisenbeis and Hassel 2011; Pawson and Bader 2014). While I emphasize that more work on this front must be completed to determine whether amber-hue lights at night are "safer", I must also stress that the current switch to more energy-efficient lighting provides a unique opportunity to alter spectral composition at little to no additional cost.

Given that ALAN affects WNV exposure risk across Florida, further arbovirus control efforts should take ALAN intensity into account. Focusing vector control efforts in areas of low to moderate ALAN intensities may prevent community transmission in these areas, thus preventing spillover ("Mosquito-Borne Disease Surveillance" 2020). Additionally, more surveillance in these areas should provide insights on why low to moderate ALAN increases exposure risk. Perhaps fine-scale lights in these areas draw in mosquitoes closer to suburban communities, thus exposing more sentinel chickens and people to WNV (Frank 1988; Eisenbeis and Hassel 2011). Alternatively, bright light at night may generate similar effects, but at certain

intensities renders mosquitoes vulnerable to predation, thus decreasing exposure risk where mosquitoes have low odds of survival (Spoelstra et al. 2017). Therefore, mosquito control efforts should also adjust their focus based on light pollution intensity in the future.

Ultimately, much work is to be done to fully understand how ALAN affects arbovirus dynamics. For example, it is unknown whether ALAN affects host-vector interactions, which is considered and heavily weighted in the WNV disease model (Woolhouse et al. 1997; Wonham et al. 2004). Biting rate could be affected by ALAN in two ways: hosts may be better able to reduce biting rate with increased visibility in light polluted areas and therefore kill more mosquitoes that are attempting to take a bloodmeal (Prugh and Golden 2014). On the other hand, metabolic dysregulation generated by light at night exposure in hosts may increase carbon dioxide emissions at night, known to attract mosquitoes (Allan et al. 2006; Dominoni et al. 2016). Along the same lines, the probability that a host or a vector is infected by a bite is considered in the WNV outbreak potential model, both of which could be affected by ALAN if hosts and vectors both experience dysregulated immune defenses early after exposure (Wonham et al. 2004; Bedrosian et al. 2011). Lastly, infection-induced and background mortality have an effect on whether WNV transmission is sustained in a population (Wonham et al. 2004). While these metrics seem trivial to measure, this phenomenon is not easily observed in nature. Even though infection-induced mortality can be observed in the laboratory, it is hard to determine whether individuals experience increased risk of mortality under natural conditions, with limited food availability and limited protection from predators. Additionally, observing natural background mortality rates of birds and mosquitoes in areas with different light pollution intensities is a large and laborious study, this metric is more attainable than the former. Therefore, I advocate that more metrics that contribute to transmission potential are considered in the future.

Consequences of light pollution on spillover to humans should also be considered in the future. Above, I considered the ways in which ALAN exposure likely affects other R_0 terms, with consequences for transmission among birds and mosquitoes (Wonham et al. 2004). This is however applicable to transmission to humans in the vicinity as well. While I found through my research that light pollution affects wildlife host infection responses, it is likely that similar intensities of ALAN exposure generate physiological consequences for humans, thus suppressing their antiviral responses as well (Kernbach et al. 2019). Additionally, two common WNV vector species (*Culex pipiens* and *Culex quinquefasciatus*) reside within close proximity of humans, which could increase further with human-affiliated light pollution and vector flight-to-light behavior (Eisenbeis and Hassel 2011; Farajollahi et al. 2011). Lastly, although humans are dead-end hosts of WNV, no research has yet asked whether light at night affects the ability of humans to limit viral replication, thus raising into question whether humans are actually dead end hosts of WNV under certain contexts (Higgs et al. 2005).

Importantly, there are many implications of my research for public health and human disease. Research first investigating the negative effects of ALAN occurred in the biomedical field, but to date no research on the effects of ALAN on communicable disease in humans has been conducted (Navara and Nelson 2007). However, there are likely effects of ALAN on communicable disease in humans that have yet to be uncovered. First, there's much evidence that exposure to short-wavelength light at night suppresses melatonin secretion (Brainard et al. 1988b). Additionally, humans are known to experience chronological disruption when exposed to light at night, dysregulating their body's natural rhythms (Reiter et al. 2011). Further, work in night shift workers found that individuals exhibit increased incidence of several types of cancer and non-communicable disease (Haim and Zubida 2015; Zubidat and Haim 2017). Various

technologies have taken these effects into consideration by reducing the amount of short-wavelength light emitted by technology at night (Nagare et al. 2019). While no work exploring how these effects relate to communicable disease in humans, light at night clearly affects immunity and daily rhythms enough to cause significant dysregulation of other diseases.

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APPENDIX A:

SUPPLEMENTARY MATERIAL FOR CHAPTER I

Materials and Methods:

Experimental procedures

House sparrows were captured using mist nets at two sites in the Tampa Bay area with comparable levels of light pollution as determined by satellite imaging and handheld light meters ("NOAA/NGDC - Earth Observation Group" 2019). All birds were captured between the hours of 5:30 and 9:30 AM. Males and females were evenly distributed throughout treatments to account for differences among sexes. Additionally, all birds captured were adults (i.e., between 1-3 years of age). To assess how light pollution affects the hypothalamic-pituitary-adrenal HPA axis, we performed dexamethasone (DEX) suppression tests using methods described below on each bird immediately after capture in the field and after ALAN/control lighting exposure. Birds were then transported to the University of South Florida vivarium where they were housed individually in 13"x15"x18" cages for the next 7-25 days in visual and audial proximity to each other. Control birds were exposed to ~0 lux at night and kept on 12h light:12h dark cycle consistent with late spring in Florida for the project duration. All ALAN birds were exposed to ~8 lux of incandescent white light during what was the dark period for control birds (12h light:12h dim light). Food (mixed seeds) and water were provided *ad libitum* throughout the study and IACUC (#2716) and USF Biosafety (#1323) approved the studies prior to the work.

Two days prior to WNV exposure, all birds were administered second DEX suppression tests according to the below bleeding timeline and procedures. The next day, all birds were transported to the USF Biosafety level-3 (BSL-3) suite where they were kept singly in similar cages but inside bioBUBBLE containment systems (bioBUBBLE Inc, Fort Collins CO) to prevent WNV escape into rooms. Light conditions during this period were identical to conditions described above. One day after acclimation to the BSL-3 facility, all birds were inoculated with 10^1 plaque-forming units (PFUs) each of New York 1999 strain at ~5 minute intervals between 7 and 9 am to account for any effect of inoculation time on competence (NY 1999; Gervasi, Burgan, Hofmeister, Unnasch, & Martin, 2017). Due to space constraints in the BSL-3 facility, this study was conducted in two cohorts, but all birds from both cohorts were inoculated using a common WNV stock.

Although we did not assess prior exposure to or current infection with WNV in these birds, unpublished research by our lab found that individuals are unable to be infected with WNV twice. We used infection as a proxy for whether individuals had any prior exposure to WNV. As all individuals became infected once they were exposed, we concluded that none of the individuals had any prior exposure to WNV.

Birds were sampled on days 2, 4, 6, and 10 at the same time of day following WNV inoculation. ~70 uL of blood was extracted using procedures described below. Bird mass was also measured prior to WNV inoculation, and during sampling periods on days 2, 4, 6, and 10 using methods described below. Mortality was monitored twice daily during infection period, and birds were euthanized when expressing sickness behaviors, which are typically only expressed when death appears imminent in the near future. All birds were euthanized on d10 following inoculation using deep isoflurane anesthesia and rapid decapitation.

Sample collection

Blood samples for the DEX suppression test required a baseline CORT sample, which was obtained within 3 minutes of capture, a post-stressor blood sample which was collected after 30 minutes of restraint in a cloth bag following initial capture, which was immediately followed by a DEX injection (s.q., 28 ug dissolved in 50 uL peanut oil), and final samples were collected 1h after injections. Blood samples were collected from the brachial vein using sterile 26-gauge needles and microcapillary tubes, and serum was frozen at -20C until hormone assay.

Blood samples for viremia were collected using sterile 26-gauge needles and microcapillary tubes rinsed with sodium citrate to prevent clotting of blood. Serum was extracted from the blood samples and frozen at -20C until viral RNA extraction and qPCR.

Body mass

Body mass measurements were recorded using a Pesola spring scale. Mass was recorded to the 0.01 gram on the day of inoculation, and days 2, 4, 6, and 10 following WNV exposure.

RNA extraction and qPCR for WNV titer

WNV RNA was extracted from 10 uL of stored serum using the Qiagen QIAmp Viral Extraction Mini Kit (Qiagen Cat. No. 52906). Viremia was quantified using quantitative real-time polymerase chain reaction (qRT-PCR) using a one-step Taqman kit (iTaq Universal Probes One-Step Kit; Bio-Rad Cat. No. 1725141). Standards were extracted from known concentrations (via plaque-assay) of WNV stock and quantified using the same methods listed above. Forward and reverse primers and probe sequences are listed below (Gervasi et al. 2017). All samples were run in duplicate with negative controls.

Forward Primer: 5’ CAGACCACGCTACGGCG 3’

Reverse Primer: 5’ CTAGGGCCGCGTGGG 3’

Probe: 5’ [6~FAM] CTGCGGAGAGTGCAGTCTGCGAT [BHQ1a~6FAM]

Corticosterone Assays

Corticosterone concentrations were quantified in serum using an enzyme immunoassay (EIA) kit from Arbor Assays (Arbor Assays, Ann Arbor, MI, product # K014-H5; Gervasi et al., 2017). Samples were run in duplicate and standardized across plates. Concentrations were derived from known values along the standard curve, and all values fell within the curve.

Supplementary text:

Days in captivity results

Our study was designed to capture the effects of duration of exposure to ALAN on corticosterone and viremia by housing birds under their designated conditions for a range of 7-25 days. We added “days in captivity” as a fixed effect in the mixed model analysis using the nlme package in R studio and found that days in captivity had no significant effect on the models (P=0.8024). A second set of mixed models intended to determine the effect of days in captivity on CORT area under the curve (AUC) was unable to be run using the ‘nlme’ package or the ‘lme4’ package in R studio. After a series of diagnostic tests, we discovered the reason the mixed model was unable to be run was that the random effects explained almost all of the variance (i.e., there was no difference in the CORT AUC between treatment groups).

Corticosterone results

First, we queried effects of ALAN on baseline (i.e., prior to a stressor), post-restraint (i.e., after a 30-minute psychological stressor), and post-dexamethasone (i.e., a synthetic

glucocorticoid that induces down-regulation of endogenous corticosterone release; Liebl, Shimizu, & Martin, 2013) concentrations, but there was little evidence that ALAN affected HPA function when all aspects were analyzed in a single model (treatment: $F_{1,40} = 2.8$, $P = 0.14$). HPA function changed over the course of the study (time: $F_{5,197} = 38.2$, $P < 0.001$; time x treatment: $F_{5,197} = 2.4$, $P = 0.04$), but most of this variation was due to captivity, which we have observed previously to affect HPA function in house sparrows (**Figs. S1 & S2**; Martin, Kidd, Liebl, & Coon, 2011). The only statistically significant effect of ALAN on HPA function was on baseline CORT (time x treatment: $F_{1,75} = 4.6$, $P = 0.03$); baseline CORT was lower just prior to WNV exposure in ALAN compared to control birds (**Fig. S3**).

Cell type enrichment results

Following a principal components analysis visualized in **Fig S4**, significant cell type enrichments are presented in **Fig S5.** Down regulated genes for both contrasts were strongly enriched for CD71+ Early Erythroid cells, an early precursor of red blood cells (RBCs). This down regulation occurs in ALAN birds at d6 relative to d2 and at d6 relative to Control. Additionally, up regulated genes in both comparisons are enriched for a wide variety of cell types, including many immune functioning cells (**Fig S6**). Thus, this represents a decrease in RBCs and increase in circulating lymphocytes. The down regulation of hemoglobin (Supplemental DEseq2 results) and up regulation of immune related genes in ALAN birds could result from a shift in cell type abundance. Nearly 3000 genes across several networks were differentially expressed and likely impacted the outcome of WNV infection in ALAN exposed individuals.

Body mass analysis

We have analyzed body mass throughout the course of infection using two models. The first model was a linear mixed model conducted in the nlme r software package where equal variances were assumed between groups. The dependent variable was body mass, the fixed effects were treatment, day, and their interaction, and the random effect was bird ID. There was a significant effect of treatment (P=0.0023), day (day4 P=0.0277; day6 P=0.0104) and their interaction (treatment*day6 P=0.0215) on body mass. A second mixed model using the same terms but allowed for variances to differ between groups was built; again, treatment (P=0.0021), day (day4 P=0.0253, day6 P=0.0147) and their interaction (treatment*day6 P=0.0461) had a significant effect on body mass. We performed an ANOVA to compare the two models, but there was no significant effect of allowing for variance to differ on the linear mixed model (P=0.1392). We therefore chose to report the statistics from the linear mixed model that allowed for differing variances as a conservative estimate of the observed effects. See tables for details of models.

Collinearity Diagnostics

We performed collinearity diagnostics in R studio using the 'olsrr' package between treatment, day, and viremia (Hebbali 2018). We used variance inflation factors (VIF) to detect any variance that may have been inflated by a collinear relationship between variables. VIF values above 4 demand further diagnostics, where values above 10 are strong signals of collinearity; there were two values that were between 4 and 10, so we conducted a follow-up Eigenvalue condition index diagnostic test. No two values had large variances denoted by Eigenvalue condition indices greater than 30, so we further concluded that there was no collinearity between variables in this model. See tables for detailed output information.

Table A2.1:

Terms used in the linear mixed model to determine effects of days in captivity on viremia. These two models were conducted with the 'nlme' package in R studio; the first model included days in captivity as a fixed effect and the second model removed this term. The ANOVA comparison revealed that these models did not significantly differ and that days in captivity (i.e. duration of ALAN exposure) did not influence viremia.

R package	Dependent Variable	Fixed Effects	Random Effects	AIC	BIC	LogLikelihood	P value
nlme	viremia	dayscaptivity+treatment*day	id	568.105	637.3499	-262.0525	0.8024
nlme	viremia	treatment*day	id	555.459	596.3767	-264.7296	

Table A2.2:

Parameters used for the survival analysis. This table shows the number of birds alive at each day throughout the course of infection; notice that mortality only occurs between days 4 and 8 post exposure.

Day	Treatment	Number Alive
0	Control	22
0	ALAN	23
1	Control	22
1	ALAN	23
2	Control	22
2	ALAN	23
3	Control	22
3	ALAN	23
4	Control	22
4	ALAN	23
5	Control	18
5	ALAN	19
6	Control	14
6	ALAN	14
7	Control	13
7	ALAN	10
8	Control	13
8	ALAN	10
9	Control	13
9	ALAN	10
10	Control	13
10	ALAN	10

Table A2.3:

Terms used in the linear mixed models to determine effects of ALAN, day post-exposure, and their interactions on body mass throughout the course of infection. These two models were conducted with the 'nlme' package in R studio; the first model assumed equal variance among groups and the second model allowed for unequal variance. The ANOVA comparison determined these models did not significantly differ, therefore, the statistics for the more robust model allowing for unequal variance were reported.

R package	Variance	Dependent Variable	Fixed Effects	Random effects	AIC	BIC	LogLikelihood	P Value
nlme	equal	mass	treatment*day	id	437.24	478.23	-205.62	
nlme	unequal	mass	treatment*day	id	441.69	511.06	-198.85	0.139

Table A2.4:

Output from type III test of fixed effects in SPSS to analyze relationship between fixed effects (day, treatment, day*treatment) and dependent variable (viremia), accounting for random effects (id).

Software	Dependent Variable	Fixed Effects	Random Effects	AIC	numerator df	denominator df	F value	P value
SPSS	viremia	day	id	540.466	4	123.594	270.47	0
SPSS	viremia	treatment	id	540.466	1	39.337	0.655	0.423
SPSS	viremia	treatment*day	id	540.466	4	123.594	2.945	0.023

Table A2.5:

Output from type III test of fixed effects in R studio to analyze relationship between day, treatment, and their interaction on viremia; output is nearly identical between SPSS and R studio, so we were confident that reporting statistics from both software programs would not impact the output.

Type III Analysis of Variance Table with Satterthwaite's method

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)	
treatment	0.67	0.670	1	39.336	0.6552	0.42315	
day	476.99	119.248	4	123.702	116.6442	< 2e-16	***
treatment:day	12.00	3.001	4	123.676	2.9351	0.02334	*

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Table A2.6:

Full statistics from the linear mixed model in SPSS.

Estimates of Fixed Effects[a]

Parameter	Estimate	Std. Error	df	t	Sig.	Interval Lower Bound	Interval Upper Bound
Intercept	1.960458	0.368851	159.805	5.315	0.000	1.232006	2.688911
[treatment =.00]	0.321883	0.525684	157.318	0.612	0.541	-0.716426	1.360191
[treatment =1.00]	0[b]	0					
[time=.00]	-1.960458	0.396255	127.842	-4.947	0.000	-2.744526	-1.176391
[time=2.00]	2.577890	0.400585	128.896	6.435	0.000	1.785318	3.370463
[time=4.00]	5.178810	0.396255	127.842	13.069	0.000	4.394742	5.962877
[time=6.00]	3.203637	0.414423	126.126	7.730	0.000	2.383514	4.023759
[time=10.00]	0[b]	0					
[treatment =.00] * [time=.00]	-0.321883	0.568058	127.781	-0.567	0.572	-1.445902	0.802137
[treatment =.00] * [time=2.00]	-0.147725	0.571087	128.301	-0.259	0.796	-1.277693	0.982243
[treatment =.00] * [time=4.00]	-0.593375	0.570009	127.290	-1.041	0.300	-1.721295	0.534546
[treatment =.00] * [time=6.00]	-1.693626	0.606943	122.947	-2.790	0.006	-2.895037	-0.492214

a. Dependent Variable: PFU.

b. This parameter is set to zero because it is redundant.

Table A2.7:

Linear mixed model outputs from ‘nlme’ package in R studio; these values account for repeated measures in the output.

Fixed effects: titer ~ treatment * day					
	Value	Std.Error	DF	t-value	p-value
(Intercept)	0.000000	0.2871384	122	0.000000	1.0000
treatment	0.000000	0.3880181	40	0.000000	1.0000
day2	4.711579	0.3276110	122	14.381628	0.0000
day4	6.868464	0.3332894	122	20.608111	0.0000
day6	3.796707	0.3931153	122	9.657999	0.0000
day10	2.284077	0.4066811	122	5.616384	0.0000
treatment:day2	-0.173625	0.4453719	122	-0.389842	0.6973
treatment:day4	0.270231	0.4469282	122	0.604642	0.5465
treatment:day6	1.369194	0.5118253	122	2.675119	0.0085
treatment:day10	-0.319135	0.5675739	122	-0.562279	0.5750

Table A2.8:

Parameters used to estimate West Nile virus basic reproductive number. The table provides a definition of each parameter, values used in models, how such values were obtained or estimated (e.g., midpoint and reported range in parentheses), and relevant citations.

Parameter	Definition	Value	Source	Citation
a	Bite rate	0.479/day	Based on 47.9% of *C. quinquefasciatus* females feeding each night	(Uttah et al. 2013)
b	Prob (vector infected by bite)	0.51 (range: 0.17-0.85)	Average proportion of vectors infected when host viremia is in infectious range ($>10^5$)	(Sardelis et al. 2001)
c	Prob (host infected by bite)	1	Derived from experiment	N/A
IP (control)	Infectious period (control birds)	2 days	Derived from experiment (days viremia > 10^5)	(Turell et al. 2000)
IP (ALAN)	Infectious period (ALAN birds)	4 days	Derived from experiment (days viremia > 10^5)	(Turell et al. 2000)
m	Mosquito mortality rate	1/(25.6 days) (range: 2.93-48.2)	Derived from average adult lifespan of *C. quinquefasciatus*	(David et al. 2012)
k	WNV development rate	1/13 days	Derived from extrinsic incubation period	(Richards et al. 2010)
M/B	Vector:host ratio	20.16	Derived from vector:host data	(Sallam et al. 2017)

Table A2.9:

Variance inflation factors (VIF).

	Variables	Tolerance	VIF
	<chr>	<dbl>	<dbl>
1	treatment	0.989	1.01
2	day2	0.247	4.05
3	day4	0.137	7.32
4	day6	0.322	3.10
5	day10	0.677	1.48
6	titer	0.185	5.42

Table A2.10:

Eigenvalue condition indices used to determine whether collinearity exists; there are no values above 30, therefore, collinearity does not exist between two variables.

	Eigenvalue	Condition Index	intercept	treatment	day2		day4	day6	day10	tite
1	3.30363942	1.000000	1.505112e-02	2.680385e-02	0.004605917	1	0.0036952618	0.0046253228	0.003877121	5.514081e-0
2	1.08014630	1.748860	3.565186e-03	9.220352e-03	0.001881470	2	0.0343264661	0.0121026917	0.269171776	3.627459e-0
3	1.00121935	1.816485	1.063383e-04	5.160158e-04	0.017081323	3	0.0035019340	0.1957529311	0.088235993	1.855106e-0
4	1.00000000	1.817592	0.000000e+00	2.018500e-33	0.112735252	4	0.0186520409	0.0002191967	0.131713136	2.016315e-3
5	0.43460146	2.757089	5.823986e-05	7.079713e-01	0.021598093	5	0.0024236320	0.0399779437	0.095115061	1.803158e-0
6	0.14680278	4.743831	8.943558e-01	2.427874e-01	0.027944388	6	0.0007816947	0.0259071622	0.174303243	6.754369e-0
7	0.03359069	9.917150	8.686336e-02	1.270103e-02	0.814153558	7	0.9366189705	0.7214147517	0.237583671	9.052830e-0

Table A2.11:

Number of differentially expressed genes classified by DEseq2 at FDR <0.05 and <0.10. In the text, we opted to report the 0.10 values as this is the standard for DEseq2 analysis and the default in the 'DEseq2' R package used to analyze the data (Love et al. 2014).

	FDR<0.05	FDR<0.10
Day 2 ALAN vs Control	101	162
Day 6 ALAN vs Control	1989	2775
Control Day 6 vs Day 2	674	997
ALAN Day 6 vs Day 2	2170	2794

Table A2.12:

Measurements of ALAN at capture sites using VIIRS satellite radiance data from lightpollutionmap.info and handheld lux meters. Handheld measures reported as a range because of the variation in light pollution at a local scale.

Location	Satellite Radiance	Handheld Lux
Lutz	9.53	3.8-4.2
St. Pete Beach	8.35	3.9-4.2

Table A2.13:

Post-hoc analyses using ‘emmeans’ in R studio for linear mixed models accounting for repeated measures.

Dependent Variable	Day	Contrast	Estimate	SE	DF	T ratio	P value
Viremia	6	ALAN-control	-1.371	0.465	40	-2.948	0.0053
ΔBody mass	6	ALAN-control	3.111	1.097	40	2.836	0.0071

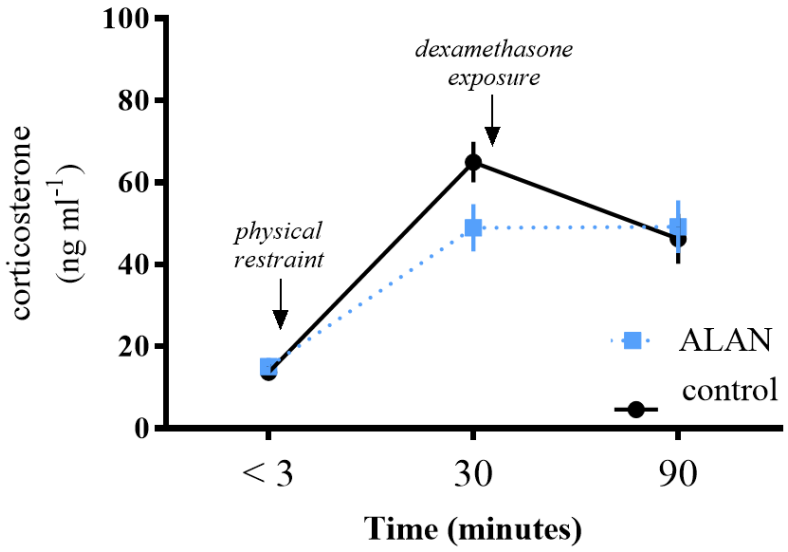

Figure A2.1:

Corticosterone levels at capture in the wild: baseline (<3 min of hitting a mist net), 30 (after 30 min restraint in a cloth bag), and 90 (after DEX-induced negative feedback in ALAN (blue) and control (black) individuals. This regulatory profile represents the ability of birds to mount a corticosterone response to a stressor and respond to agonism of glucocorticoid receptors in the brain with attenuation of corticosterone release from the adrenals. Note that all of these values were collected before any individuals were exposed to ALAN.

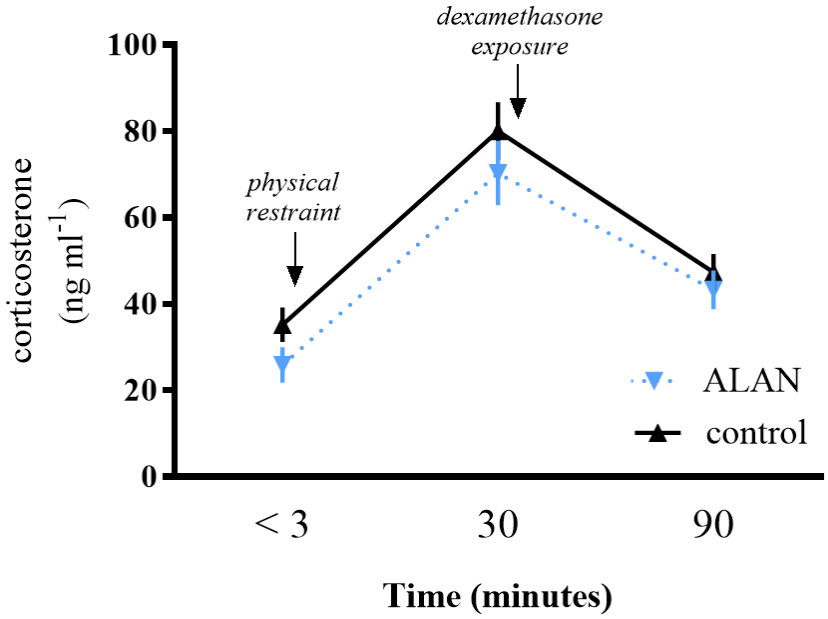

Figure A2.2:

Corticosterone regulatory profiles after ALAN exposure; ALAN-exposed individuals are depicted as blue symbols and control individuals are black. As above in Fig. A2.1, < 3 depicts baseline measures, 30 minutes depicts post-stressor measures, and 90 minutes depicts post-DEX negative feedback measures.

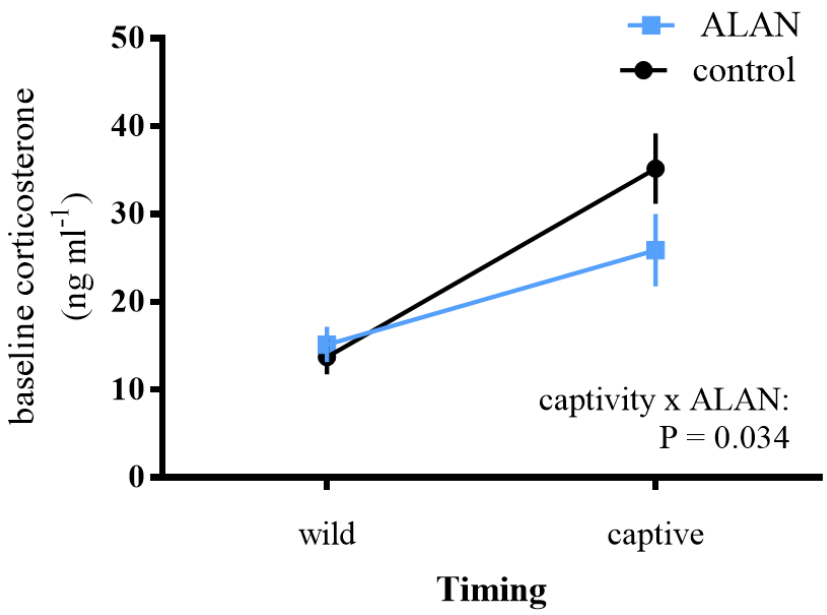

Figure A2.3:

Baseline corticosterone levels of birds at capture (wild) and after a period of time in captivity (captive). As above, ALAN exposed birds are depicted in blue and control birds in black. Both groups increased baseline corticosterone after time in captivity, a typical response for this species (Martin et al. 2011), but this increase in baseline corticosterone was more modest in ALAN-exposed birds.

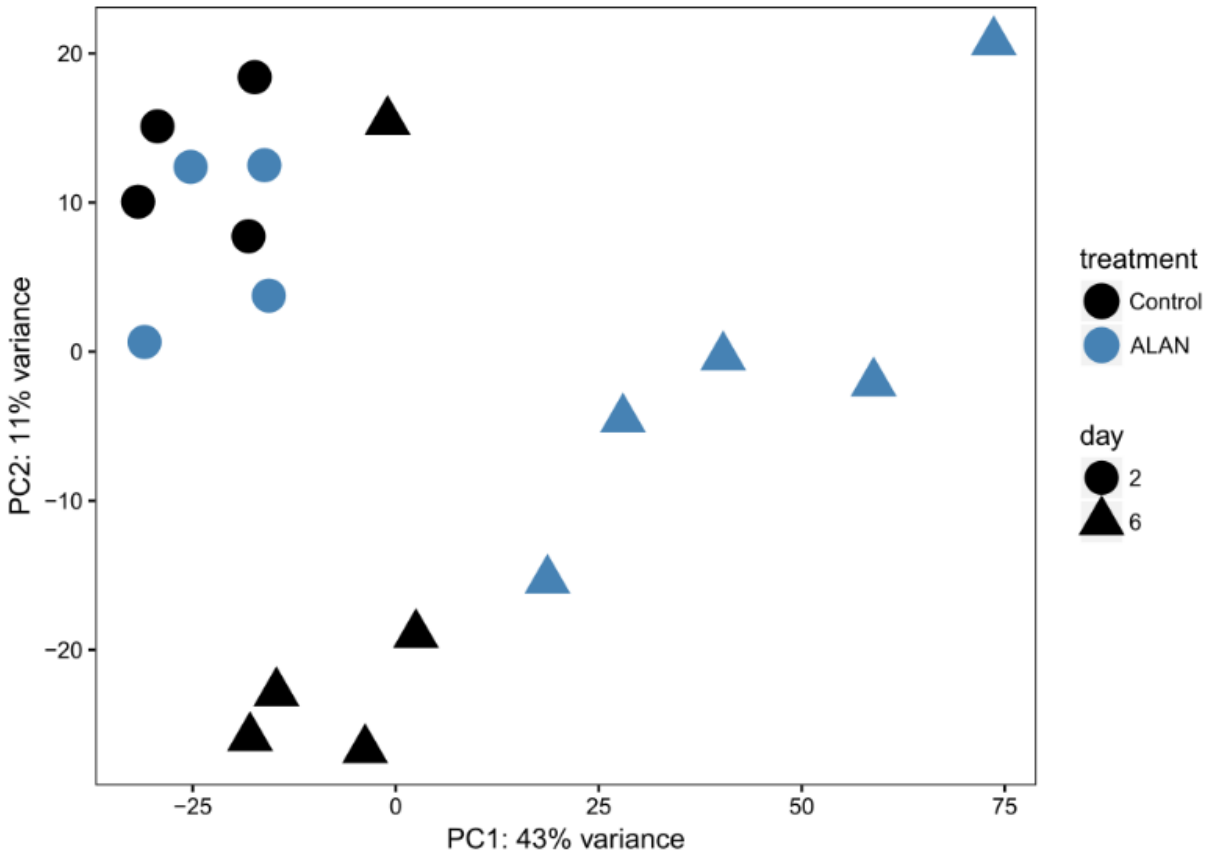

Figure A2.4:

PCA of all 18 RNAseq libraries used in the study.

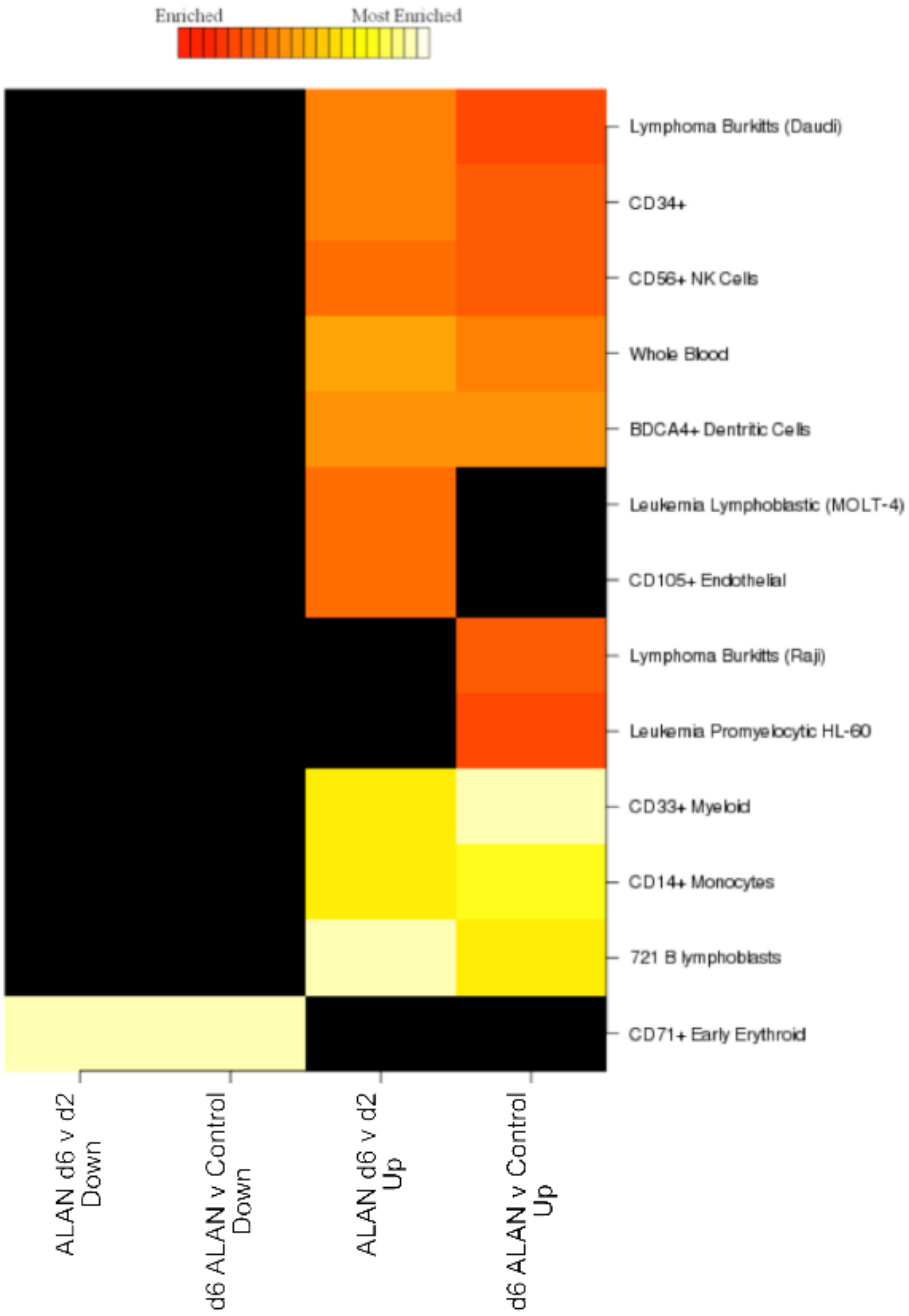

Figure A2.5:

Cell type enrichment analysis based on up and down regulated genes in the ‘d6 ALAN v Control’ and ‘ALAN d6 v d2’ DEseq2 results. Only significant enrichments are shown, with lighter colors indicating a stronger enrichment.

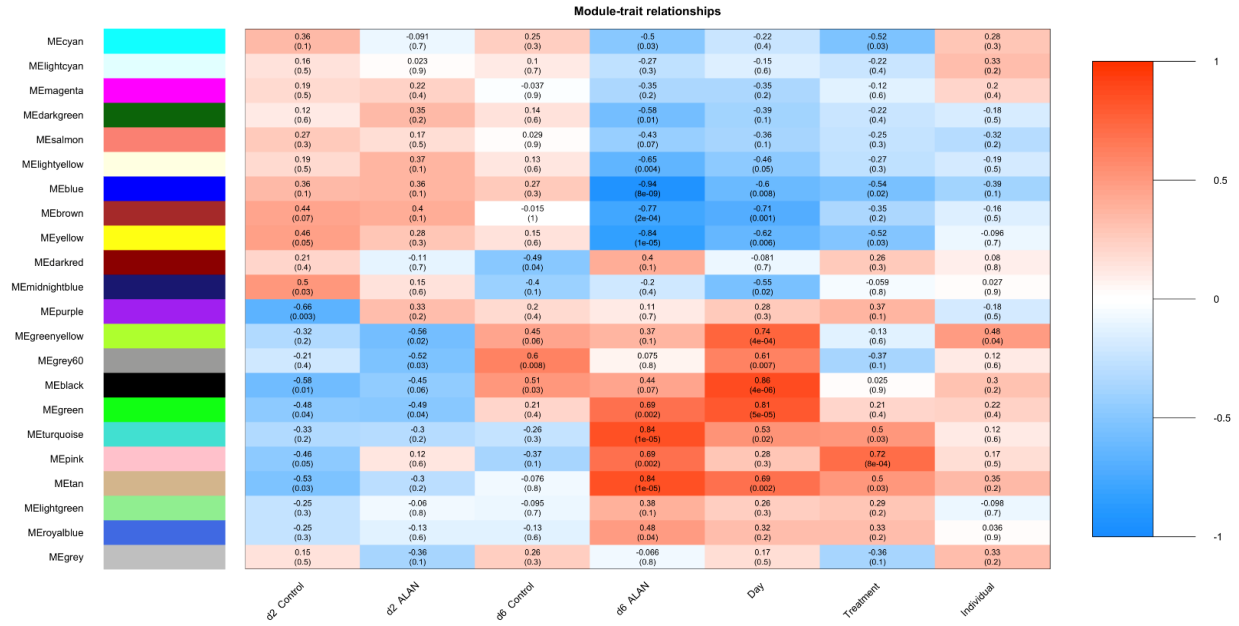

Figure A2.6:

WGCNA module trait correlations. Each box contains the correlation value, ranging from -1 to 1, and corresponding p value. The heatmap color shading corresponds to the correlation value, with red colors representing positive correlations and blue colors representing negative correlations.

APPENDIX B:

SUPPLEMENTARY MATERIAL FOR CHAPTER II

Experimental procedures:

House Sparrows (*Passer domesticus*; N=71) were captured in the Tampa Bay area using mist nets during the months of October and November 2018. All birds were captured between the hours of 5:30 and 9:30 AM. Following capture, birds were transported to the USF campus vivarium and housed individually in 13”x15”x18” cages in visual and auditory proximity to one another under assigned conditions for 2 weeks. Food (mixed seeds) and water were provided *ad libitum* throughout the study. All birds were housed under a 12L:12D cycle; control birds were housed in complete darkness (0 lux; N=24) at night, wildlife-safe ALAN exposed birds were housed under 5 lux of 1800K LED light at night (N=12), broad-spectrum ALAN exposed birds were housed under 5 lux of 3000K LED light at night (N=11), and cool-white ALAN exposed birds were housed under 5 lux of 5000K LED light at night (N=24). After the first two weeks, birds were transported to the USF ABSL-3 facility and housed under identical lighting conditions inside bioBUBBLE containment systems (bioBUBBLE Inc, Fort Collins CO).

All birds were exposed to 10^1 PFUs per individual of NY’99 WNV one day after transfer to BSL-3. Half of the birds in the control group (N=12) and the 5000K ALAN group (N=12) were administered 200ug/mL crystalline melatonin dissolved in 0.5% EtOH in drinking water at night, a method used in similar experiments to elevate melatonin levels in pinealectomized House Sparrows, in attempt to restore circadian rhythms and alleviate melatonin suppression

caused by ALAN exposure. Following WNV exposure, birds were sampled on days 2, 4, 6, and 10, and serum was stored at -20°C until viral RNA extractions were performed. Body mass (to 0.1g) was measured at exposure and all sampling timepoints to quantify individual and group health; mortality was closely monitored daily. All birds were euthanized on day 10 post-exposure at the conclusion of the study.

To determine viremia, WNV RNA was first extracted from 10 uL of stored serum using the Qiagen QIAmp Viral Extraction Mini Kit (Qiagen Cat. No. 52906). Standards were also extracted from known concentrations using the same methods. Following extractions, RNA was quantified using quantitative real-time polymerase chain reaction (qRT-PCR) using a one-step Taqman kit (iTaq Universal Probes One-Step Kit; Bio-Rad Cat. No. 1725141). All samples were run in duplicate with negative controls to detect potential contamination.

Data analysis:

All data analyses were performed in R studio (R Core Team 2013). Our first goal was to determine whether melatonin administration in the control or 5000K ALAN exposed birds affected viremia, mortality, and tolerance. We first used a generalized linear mixed model in R studio package ‘lme4’ (non-normal distribution indicated by Shapiro test) with WNV titer as the dependent variable, day, treatment (only including control and 5000K), melatonin administered or sham, and their interactions (two and three-way) as fixed effects; bird id was a random effect. Next, we asked whether melatonin influenced mortality rates or tolerance during the infectious-to-vector period. Indeed, melatonin, treatment, and their interaction had no significant effect on mortality or tolerance. Detailed statistical outputs are depicted below.

To confirm that the 3000K and 5000K treatments did not differ, we constructed a linear mixed model in R studio package ‘nlme’ (normal distribution without heteroskedasticity

indicated by Shapiro and Bartlett tests) with WNV titer as the dependent variable, day, treatment (only including 3000K and 5000K), and their interaction as fixed effects, and bird id as a random effect. Day was the only significant term in the model; the statistics are portrayed in the tables below. To further confirm that there was no statistical difference between the 3000K and 5000K treatment groups, we built a Cox proportional hazards analyses to determine effects of treatment, viremia, and vigor on mortality. We found that average viremia was the only significant predictor of mortality. Below are the specific statistics for this analysis. Finally, we confirmed that there was no difference between 3000K and 5000K groups regarding tolerance. To calculate tolerance, we first calculated the average change in body mass since exposure and the average viremia from days 2, 4, 6, and 10 post-exposure to summarize an individual's health across the course of infection. The population average was then calculated and each individual's residual from their predicted value served as their tolerance parameter (how much more body mass or less body mass did the individual maintain per their average viremia compared to all other individuals). We used a generalized linear model (non-normal distribution indicated by Shapiro test) with treatment, viremia, treatment*viremia, and vigor as fixed effects. Vigor was the only significant predictor of tolerance in this model, so we concluded 3000K and 5000K bird tolerance did not differ. Model statistics are detailed below.

When assessing residual variation in health, we first calculated average percent change body mass since exposure across the course of infection and total average viremia in each individual. Using a linear regression, we could then calculate each residual value from their predicted average percent change body mass as a function of their average WNV viremia based on the population average. This residual value thus served as an individual's measure of health; basically, how much more or less an individual maintained their body mass than was predicted

based on their average viral burden. We used a generalized linear model (GLM) in R studio's 'lme4' package where residual variance was the dependent variable and treatment, viremia, their interaction, and vigor were fixed effects.

Finally, the survival analyses were performed in R studio using 'survival', 'survminer', and 'dplyr' packages for the cox proportional hazards method. Time to death and censorship (died or survived until the conclusion of experiment) were incorporated as the dependent variables. The initial analysis was performed using just treatment as a predictor of mortality. Subsequently, once initial differences were detected, two different analyses were performed to avoid over-complicating models. The first iteration incorporated treatment, residual variation of health, their interaction, and vigor as fixed effects and the second iteration incorporated treatment, average viremia, their interaction, and vigor as fixed effects. This was done to determine if differences in mortality among treatment groups were driven by interactions between viremia and health status.

Table A3.1:

Melatonin Administration Viremia Distribution. Shapiro-Wilk test for normal distribution of data. Significant p-value indicated that WNV titer between MEL admin and sham individuals was not normally distributed.

Shapiro-Wilk Normality Test	W value	P value
Viremia	0.95821	0.0006078

Table A3.2:

Melatonin Administration Viremia Type III ANOVA. Summary of main effects of type III ANOVA of the GLMM model below where WNV viremia (titer) is the dependent variable, melatonin (MEL), day, and their interaction are fixed effects, and bird id is a random effect. Indeed, melatonin and its interaction with day do not affect viremia.

Type III ANOVA	Chi Sq value	Degrees of Freedom	P value
MEL	0.0529	1	0.8181707
Day	17.5512	3	0.0005443
MEL:day	0.6182	3	0.8922593

Table A3.3:

Melatonin Administration Alone on Viremia GLMM Output. Statistical output for a GLMM in R studio 'lme4' package. Treatment term as a fixed effect was dropped from the model as the model would not converge at this level of complexity and our main question was regarding the role of melatonin in viral resistance. This confirms that melatonin had no effect on WNV titer.

GLMM fit by 'ML', Gamma Distribution	Estimate	Std error	T value	P value
MEL	-0.0024998	0.0108736	-0.230	0.81817
Day(L)	0.1303988	0.0332830	3.918	8.93e-05***
Day(Q)	0.0860951	0.0283174	3.040	0.00236**
Day(C)	0.0148198	0.0232082	0.639	0.52311
MEL:day(L)	-0.0157864	0.0208790	-0.756	0.44960
MEL:day(Q)	-0.0059293	0.0176832	-0.335	0.73740
MEL(C)	0.0001207	0.0140931	0.009	0.99317

Table A3.4:

Melatonin Administration*Treatment on Viremia GLMM Output**.** Statistical output of cox proportional hazards analysis to determine whether melatonin administration influenced mortality rates. Indeed, the only marginally significant predictor of mortality was vigor (body condition in absence of infection).

CoxPH Survival Analysis	Coef	Exp(coef)	Se(coef)	Z score	P value
Treatment	-1.42e+01	6.84e-07	1.47e+01	-0.97	0.333
MEL	-1.20e+01	5.95e-06	8.63e+00	-1.39	0.163
Viremia	-2.90e+00	5.51e-02	2.23e+00	-1.30	0.193
Vigor	4.66e-01	1.59e+00	2.48e-01	1.88	0.061
Treatment:MEL	1.33e+01	6.20e+05	1.03e+01	1.29	0.196
Treatment:viremia	3.08e+00	2.17e+01	2.56e+00	1.21	0.228
MEL:viremia	2.09e+00	8.06e+00	1.49e+00	1.40	0.161
Treatment:MEL:viremia	-2.64e+00	7.11e-02	1.81e+00	-1.46	0.145

Table A3.5:

Residual Variation in Body Mass Distribution. Shapiro-Wilk test to determine normality. The p-value is significant, indicating that the data is not normally distributed.

Shapiro-Wilk Normality Test	W value	P value
Residual Variation in Body Mass	0.84972	2.137e-05

Table A3.6:

Melatonin Administration*Treatment*Viremia on Residual Variation in Body Mass GLM. Statistical output of a generalized linear model where we asked whether treatment, melatonin, viremia, or vigor predicted tolerance measurements. Indeed, no differences in tolerance existed between melatonin administered and sham individuals and the only significant predictor of tolerance was vigor.

GLM Residual Var Body Mass	Estimate	Std. Error	T value	P value
Treatment	-0.0002726	-0.1652612	-0.002	0.998692
MEL	-0.0093786	0.1023457	0.092	0.927456
Viremia	-0.0027277	0.0233191	-0.117	0.907483
Vigor	0.0112710	0.0031364	3.594	0.000903***
Treatment:MEL	-0.0353297	0.1171529	-0.302	0.764583
Treatment:Viremia	-0.0011187	0.0268656	-0.042	0.966997
MEL:Viremia	-0.0028335	0.0175894	-0.161	0.872851
Treatment:MEL:Viremia	0.0063113	0.0198555	0.318	0.752285

Table A3.7:

Viremia Distribution between 3000K and 5000K Treatments. Shapiro-Wilk test to determine normal distribution of data. Non-significant P-values indicate normal distribution.

Shapiro-Wilk Normality Test	W value	P value
Viremia	0.97697	0.1145

Table A3.8:

Bartlett Test of Homogeneity. Bartlett test to determine homogeneity of variances. Non-significant P-values indicate there is no heteroskedasticity or unequal variance among groups.

Bartlett Test of Homogeneity	K squared	Degrees of Freedom	P value
Viremia~treatment*day	11.175	7	0.1312

Table A3.9:

3000K and 5000K Type III ANOVA. Type III ANOVA test of main effects of the linear mixed model built in 'nlme'. Day is the only significant fixed effect in this model, so we concluded there are no difference between 3000K and 5000K treatments on viremia.

Type III ANOVA	Chi Sq	Degrees of Freedom	P value
Treatment	4.8539	3	0.1828

Table A3.10:

3000K and 5000K Effects on Mortality in a Cox Proportional Hazards Model. Statistical output for the cox proportional hazards model to determine effects of survival where treatment, viremia, their interaction, and vigor were integrated as fixed effects. The only significant predictor of mortality was viremia, so we concluded that treatments 3000K and 5000K again did not statistically differ in mortality rates.

Cox PH Model	Coef	Exp(coef)	SE(coef)	Z score	P value
Treatment	0.19382	1.21388	3.19598	0.061	0.95164
Viremia	1.09994	3.00397	0.35933	3.061	0.00221**
Vigor	-0.03809	0.96263	0.25329	-0.150	0.88047
Treatment:Viremia	0.03031	1.03077	0.43621	0.069	0.94460

Table A3.11:

3000K and 5000K Effects on Body Mass in a GLM. Statistical output of the generalized linear model used to determine whether 3000K or 5000K treatments differed in change in body mass. Vigor was the only significant predictor of tolerance, therefore, we concluded that the treatments did not differ regarding tolerance statistically.

GLM, Gamma Distribution	Estimate	Std. Error	T value	P value
Treatment	0.009165	0.041454	0.221	0.8266
Viremia	-0.001570	0.005246	-0.299	0.7668
Vigor	0.010455	0.004215	2.480	0.0192*
Treatment:Viremia	-0.001771	0.006367	-0.278	0.7829

Table A3.12:

Melatonin Pre-Exposure. Type III ANOVA output to determine melatonin pre-exposure concentrations between the control and treatment groups. No significant differences existed between groups.

Type III ANOVA	ChiSq	Degrees of Freedom	P value
Treatment	0.02255	1	0.8806
Time	0.69236	1	0.4054
Treatment:Time	0.17504	1	0.6757

Table A3.13:

Melatonin Pre-Exposure. Output of the generalized linear model where melatonin concentration was the dependent variable, and treatment, time, and their interaction were fixed effects pre-exposure. We confirmed that there were no significant pre-existing differences.

GLM Gamma Distribution	Estimate	Std Error	T value	P value
Treatment	4.131e-04	2.749e-03	0.150	0.8809
Time	-9.931e-05	1.193e-04	-0.832	0.4075
Treatment:Time	-6.109e-05	1.461e-04	-0.418	0.6768

Table A3.14:

Pre-/Post-ALAN Exposure Effects on Melatonin [Within-Group]. Main effects determined by Type III ANOVA for Pre-/Post-Exposure comparison of ALAN exposed birds GLM model with a gamma distribution. There is a significant main effect of exposure on melatonin concentration.

Type III ANOVA	ChiSq	Degrees of Freedom	P value
Pre-/Post-Exposure	7.7698	1	0.005313**
Time	2.8776	1	0.089817
Pre-/Post-Exposure:Time	1.9442	1	0.163214

Table A3.15:

Pre-/Post-ALAN Exposure Effects on Melatonin [Within-Group]. Output of the generalized linear model where melatonin concentration was the dependent variable, and pre-/post-exposure, time, and their interaction were fixed effects. ALAN exposure had a significant effect on melatonin concentration.

GLM Gamma Distribution	Estimate	Std Error	T value	P value
Pre-/Post-Exposure	4.155e-02	1.556e-02	2.670	0.00897**
Time	-1.604e-04	9.504e-05	-1.688	0.09485
Pre-/Post-Exposure	-1.135e-03	8.176e-04	-1.389	0.16827

Table A3.16:

Effects of ALAN Exposure on Melatonin Concentrations. Main effects determined by Type III ANOVA for ALAN-exposed vs control melatonin concentration GLM model with a gamma distribution. There is a significant main effect of the interaction between treatment and exposure on melatonin concentration, indicating that melatonin concentrations were only suppressed when individuals are exposed to ALAN.

Type III ANOVA	ChiSq	Degrees of Freedom	P value
Treatment	2.952	1	0.08575
Pre-/Post-Exposure	0.829	1	0.36262
Time	6.369	1	0.01161*
Treatment:Exposure	186.419	1	< 2e-16***

Table A3.17:

Effects of ALAN Exposure on Melatonin Concentrations. Output of the generalized linear model where melatonin concentration was the dependent variable, and treatment, time, and their interaction were fixed effects post-exposure. The interaction between treatment and exposure significantly affected melatonin concentrations, further confirming that only exposure to ALAN affected melatonin.

GLM Gamma Distribution	Estimate	Std. Error	T value	P value
Treatment	-7.254e-04	4.294e-04	-1.689	0.0929
Pre-/Post-Exposure	-4.104e-04	4.531e-04	-0.906	0.3663
Time	-1.555e-04	6.173e-05	-2.518	0.0127*
Treatment:Exposure	2.099e-02	2.245e-03	9.349	< 2e-16***

Table A3.18:

Main Effects of Treatment, Time, and Their Interaction on WNV Viremia. Main effects on viremia determined by Type III ANOVA for GLMM fit by 'ML' and gamma distribution. Treatment and day are both significant main effects on viremia (log10 WNV PFU).

Type III ANOVA	ChiSq	Degrees of Freedom	P value
Day	76.3587	3	2e-16***
Treatment	6.9942	2	0.03029*
Day:Treatment	8.7008	6	0.19111

Table A3.19:

Detailed Statistics of Treatment, Time, and Their Interaction on WNV Viremia GLMM Output. Main statistic output for the GLMM fit by 'ML' and gamma distribution to determine the effects of treatment, day, and their interaction on WNV viremia (log10 PFU). Day as a linear and quadratic function are both highly significant, which is unsurprising because it is just saying that viremia is different across time. The 1800K treatment and its interaction across time are both significant.

GLMM fit by 'ML', Gamma Distribution	Estimate	Std Error	T value	P Value
Day (linear)	0.101127	0.012972	7.796	6.41e-15***
Day (quadratic)	0.073140	0.010849	6.742	1.57e-11***
Day (cubic)	0.014650	0.008698	1.684	0.09212
1800K Treatment	0.031555	0.012474	2.530	0.01142*
3000+5000K Treatment	0.002687	0.009257	0.290	0.77165
Day(L):1800K	0.067238	0.024680	2.724	0.00644**
Day(Q):1800K	0.041429	0.020729	1.999	0.04565*
Day(C):1800K	0.015780	0.016147	0.977	0.32842
Day(L):3000+5000K	0.006164	0.018069	0.341	0.73300
Day(Q):3000+5000K	0.006026	0.015218	0.396	0.69214
Day(C):3000+5000K	-0.003912	0.011954	-0.327	0.74351

Table A3.20:

Detailed Statistics of Treatment, Time, and Their Interaction on Percent Change Body Mass GLMM Output. GLMM output for percent change body mass across the course of infection where treatment, day, and their interaction were fixed effects and bird ID was a random effect. There was a significant interaction between broad-spectrum (3000+5000K) and day as a quadratic function.

GLMM fit by 'ML', Gamma Distribution	Estimate	Std Error	T value	P Value
1800K (2)	-0.0117398	0.0099515	-1.180	0.2381
3000+5000K (3)	0.0006638	0.0074137	0.090	0.9287
Day (linear)	-0.0064685	0.0046877	-1.380	0.1676
Day (quadratic)	-0.0035572	0.0044511	-0.799	0.4242
Day (cubic)	0.0017896	0.0043874	0.408	0.6834
1800K:Day (L)	0.0026512	0.0073996	0.358	0.7201
3000+5000K:Day (L)	0.0130326	0.0062909	2.072	0.0383*
1800K:Day (Q)	0.0064073	0.0068622	0.934	0.3505
3000+5000K:Day (Q)	0.0019145	0.0060900	0.314	0.7532
1800K:Day (C)	-0.0031822	0.0066844	-0.476	0.6340
3000+5000K:Day (C)	-0.0044053	0.0060896	-0.723	0.4694

Table A3.21:

Effects of Treatment and Vigor on Mortality. Cox proportional hazards model in Rstudio using packages 'survival' and 'survminer'. Vigor has no effect on mortality, as demonstrated here, so it is not included in further iterations of the model.

Cox Proportional Hazards Model	X^2	DF	P Value
Treatment	5.7400	2	0.0567
Vigor	0.8902	1	0.3454

Table A3.22:

Effects of Treatment Alone on Mortality. Cox proportional hazards model after removing vigor from the model and only accounting for fixed effects of treatment on survival.

Cox Proportional Hazards Model	X^2	DF	P Value
Treatment	6.0217	2	0.0493*

Table A3.23:

Main Effects of Treatment, Average Percent Change Body Mass, and Average Viremia on Mortality. Type III ANOVA for main effects of the Cox proportional-hazards model in R studio with treatment, average % change body mass days 2 and 4 post-exposure), and average viremia (days 2 and 4 post-exposure) as fixed effects. We also asked about the interaction between these fixed effects.

Type III ANOVA	X^2	DF	P Value
Treatment	7.3586	2	0.02524*
Average % Δ Body Mass	4.0220	1	0.04491*
Average Viremia	15.2721	1	9.31e-05***
Treatment:Average % Δ Body Mass	3.9696	2	0.13741
Treatment:Average Viremia	6.5208	2	0.03837*
Average % Δ Body Mass:Average Viremia	4.3120	1	0.03785*
Treatment:Avg%ΔBodyMass:AvgViremia	4.1748	2	0.12401

Table A3.24:

Detailed Output of Treatment, Average Percent Change Body Mass, and Average Viremia on Mortality. Output from the Cox proportional-hazards analysis with treatment, average % change body mass days 2 and 4 post-exposure), and average viremia (days 2 and 4 post-exposure) as fixed effects. We also asked about the interaction between these fixed effects.

Cox Proportional-Hazards Model	Coef	Exp(coef)	SE(coef)	Z score	P value
1800K (2)	1.557e+01	5.802e+06	9.729e+00	1.601	0.10943
3000+5000K (3)	1.950e+01	2.953e+08	8.843e+00	2.205	0.02743*
Avg%ΔMass	-5.311e+00	4.939e-03	2.932e+00	-1.811	0.07014
AvgViremia	3.577e+00	3.578e+01	1.162e+00	3.079	0.00208*
(2):Avg%ΔMass	2.685e+00	1.466e+01	5.821e+00	0.461	0.64465
(3):Avg % Δ Mass	5.158e+00	1.738e+02	2.946e+00	1.751	0.07993
(2):Avg Viremia	-2.028e+00	1.316e-01	1.313e+00	-1.544	0.12260
(3):Avg Viremia	-2.561e+00	7.723e-02	1.170e+00	-2.189	0.02863*
%ΔMass:AvgViremia	7.055e-01	2.025e+00	3.776e-01	1.868	0.06172
(2):Avg%ΔMass:AvgViremia	-3.491e-01	7.053e-01	8.921e-01	-0.391	0.69558
(3):Avg%ΔMass:AvgViremia	-6.864e-01	5.034e-01	3.796e-01	-1.809	0.07052

Table A3.25:

Comparison of Days Until Death Between Treatments. One-way ANOVA analysis to determine whether the residual of the mean of days until death (i.e. mortality occurs earlier or later than predicted based on viremia) in treatments differ.

One-way ANOVA	DF	Sum Sq	Mean Sq	F value	P value
Treatment	2	13.51	6.754	3.561	0.0341*
Residuals	65	123.30	1.897		

Table A3.26:

Pairwise Comparison of Time Until Death Between Treatments. Tukey multiple comparison of means analysis used to determine between which treatment groups there were significant differences. Here, we see that the significant effect of treatment is driven by the difference between the control and broad-spectrum [3000+5000K] ALAN group. This indicates that individuals in the broad-spectrum ALAN group are dying earlier than anticipated based on the residual variation of the means of time until death based on average viremia days 2 and 4 post-exposure.

Tukey Comparison of Means	Diff	Lower	Upper	P value adjusted
1800K - control	-0.2397	-1.4253	0.9458	0.8787
3000+5000K - control	-0.9612	-1.8651	-0.0573	0.0346*
3000+5000K - 1800K	-0.7215	-1.8307	0.3878	0.2702

APPENDIX C:

SUPPLEMENTARY INFORMATION FOR CHAPTER III

Table A4.1:

Spatial iteration of the model predicting WNV exposure via fitme with no offsets.

Parameter	Estimate	Cond. SE	t-value	2.5% CI	97.5% CI
scale(temp)	**1.959**	**0.158**	**12.387**	**1.651**	**2.285**
scale(I(temp^2))	**1.020**	**0.189**	**5.400**	**0.612**	**1.380**
scale(log(ALAN))	**-0.665**	**0.284**	**-2.342**	**-1.264**	**-0.085**
scale(I(log(ALAN)^2))	**-0.624**	**0.238**	**-2.621**	**-1.120**	**-0.150**
scale(hum foot)	-0.059	0.155	-0.379	-0.369	0.259
scale(pop den)	0.214	0.123	1.738	-0.032	0.458
scale(imp sur)	0.014	0.080	0.179	-0.144	0.169
scale(precip)	**-0.401**	**0.084**	**-4.784**	**-0.569**	**-0.234**
scale(soil moisture)	-0.098	0.089	-1.106	-0.282	0.079
year2016	-0.034	0.458	-0.074	-0.924	0.881
year2017	0.280	0.393	0.713	-0.478	1.079
year2018	0.640	0.379	1.690	-0.092	1.421
nu	0.500				
rho	4.436				
AIC	1492.984				
logLik	-727.492				

Table A4.2:

Nonspatial iteration of the model predicting WNV exposure via fitme with offsets.

Parameter	Estimate	Cond. SE	t-value	2.5% CI	97.5% CI
scale(temp)	**1.991**	**0.161**	**12.363**	**1.680**	**2.323**
scale(I(temp^2))	**1.036**	**0.191**	**5.433**	**0.628**	**1.399**
scale(log(ALAN))	**-1.079**	**0.284**	**-3.796**	**-1.674**	**-0.491**
scale(I(log(ALAN)^2))	**-0.893**	**0.245**	**-3.643**	**-1.417**	**-0.419**
scale(hum foot)	0.082	0.158	0.517	-0.268	0.400
scale(pop den)	**0.301**	**0.135**	**2.236**	**0.037**	**0.573**
scale(imp sur)	-0.047	0.091	-0.516	-0.225	0.133
scale(precip)	**-0.402**	**0.085**	**-4.752**	**-0.572**	**-0.236**
scale(soil moisture)	-0.115	0.094	-1.229	-0.308	0.071
year2016	0.057	0.465	0.123	-0.839	0.978
year2017	0.343	0.408	0.841	-0.429	1.159
year2018	**0.763**	**0.393**	**1.942**	**0.016**	**1.559**
nu	NA				
rho	NA				
AIC	1493.878				
logLik	-729.939				

Table A4.3:

Nonspatial iteration of the model predicting WNV exposure via fitme without offsets.

Parameter	Estimate	Cond. SE	t-value	2.5% CI	97.5% CI
scale(temp)	**1.965**	**0.157**	**12.491**	**1.654**	**2.296**
scale(I(temp^2))	**1.015**	**0.186**	**5.458**	**0.606**	**1.378**
scale(log(ALAN))	**-0.860**	**0.260**	**-3.313**	**-1.432**	**-0.350**
scale(I(log(ALAN)^2))	**-0.728**	**0.209**	**-3.485**	**-1.189**	**-0.320**
scale(hum foot)	-0.061	0.151	-0.402	-0.366	0.258
scale(pop den)	**0.285**	**0.120**	**2.374**	**0.047**	**0.535**
scale(imp sur)	0.042	0.081	0.522	-0.122	0.201
scale(precip)	**-0.392**	**0.083**	**-4.692**	**-0.564**	**-0.224**
scale(soil moisture)	-0.123	0.088	-1.387	-0.315	0.054
year2016	-0.085	0.459	-0.184	-0.991	0.840
year2017	0.242	0.394	0.615	-0.524	1.060
year2018	0.636	0.380	1.675	-0.108	1.431
nu	NA				
rho	NA				
AIC	1493.601				
logLik	-729.800				

Table A4.4:

Spatial iteration of the model predicting WNV exposure via fitme with offsets.

Parameter	Estimate	Cond. SE	t-value	2.5% CI	97.5% CI
scale(temp)	**1.965**	**0.157**	**12.491**	**1.654**	**2.296**
scale(I(temp^2))	**1.015**	**0.186**	**5.458**	**0.606**	**1.378**
scale(log(ALAN))	**-0.860**	**0.260**	**-3.313**	**-1.432**	**-0.350**
scale(I(log(ALAN)^2))	**-0.728**	**0.209**	**-3.485**	**-1.189**	**-0.320**
scale(hum foot)	-0.061	0.151	-0.402	-0.366	0.258
scale(pop den)	**0.285**	**0.120**	**2.374**	**0.047**	**0.535**
scale(imp sur)	0.042	0.081	0.522	-0.122	0.201
scale(precip)	**-0.392**	**0.083**	**-4.692**	**-0.564**	**-0.224**
scale(soil moisture)	-0.123	0.088	-1.387	-0.315	0.054
year2016	-0.085	0.459	-0.184	-0.991	0.840
year2017	0.242	0.394	0.615	-0.524	1.060
year2018	0.636	0.380	1.675	-0.108	1.431
nu	NA				
rho	NA				
AIC	1493.601				
logLik	-729.800				

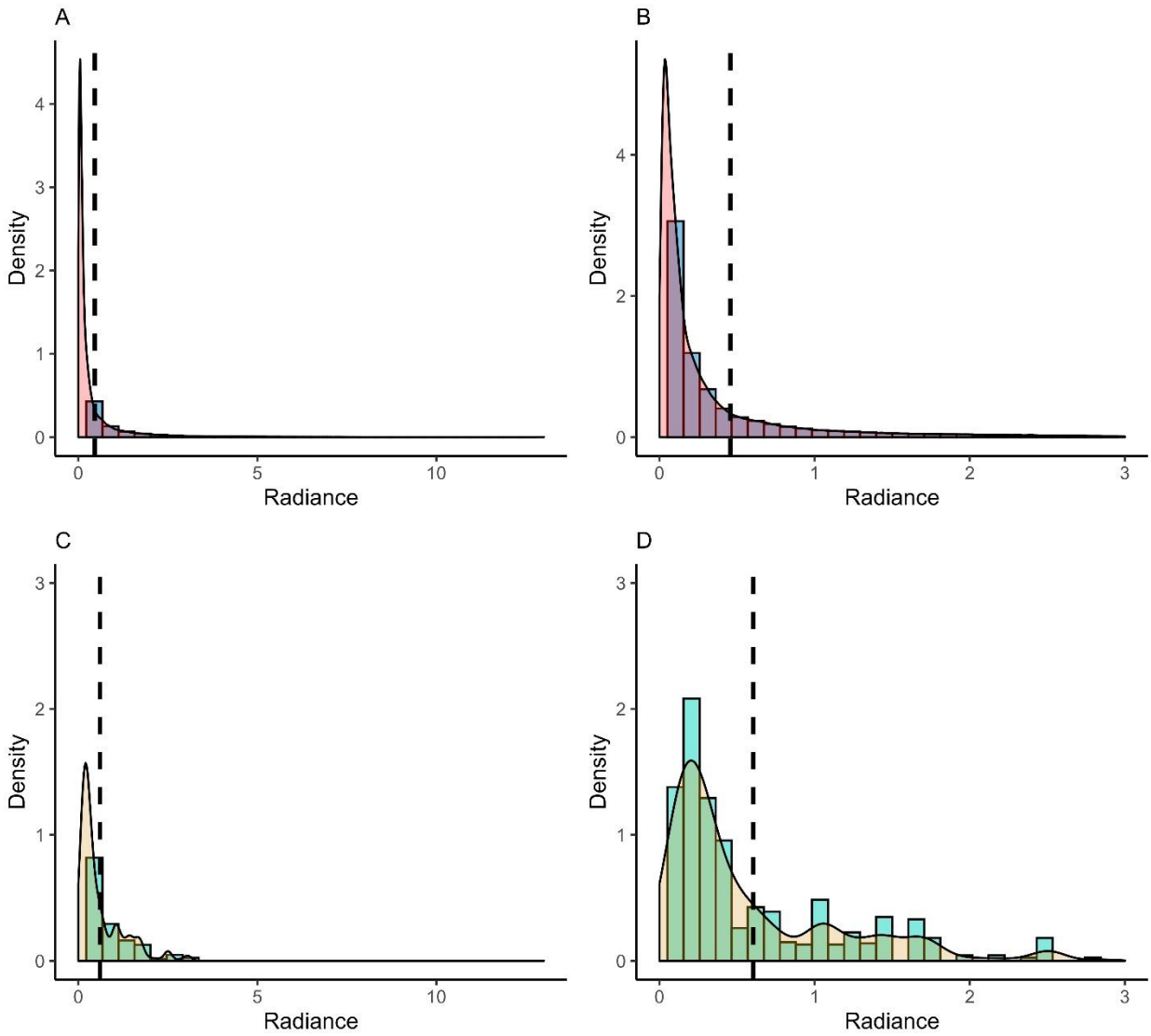

Figure A4.1:

Histograms and density distribution of artificial night lighting across Florida (A and B) and at our sentinel chicken sample locations (C and D). Radiance units reflect the artificial component of night sky brightness (μcd/m^2). X axis for A and C span the full range of values across Florida and our study sites, respectively whereas the x axis for B and C has been restricted to lower values to more clearly display the distribution of values. Vertical dashed line in all plots denotes mean values.

APPENDIX D:

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) AND INSTITUTIONAL BIOSAFETY COMMITTEE (IBC) APPROVAL

USF UNIVERSITY OF SOUTH FLORIDA

RESEARCH INTEGRITY AND COMPLIANCE
INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

MEMORANDUM

TO:	Lynn Martin,
FROM:	Farah Moulvi, MSPH, IACUC Coordinator Institutional Animal Care & Use Committee Research Integrity & Compliance
DATE:	9/26/2016
PROJECT TITLE:	Effects of Light Pollution on Immune Response to WNV
FUNDING SOURCE:	USF department, institute, center, etc.
IACUC PROTOCOL #:	R IS00002716
PROTOCOL STATUS:	**APPROVED**

The Institutional Animal Care and Use Committee (IACUC) reviewed your application requesting the use of animals in research for the above-entitled study. The IACUC **APPROVED** your request to use the following animals in your **protocol for a one-year period beginning 9/26/2016:**

Sparrow: house sparrow (adult/22-28g/F and M) 96

Please take note of the following:

• **IACUC approval is granted for a one-year period at the end of which, an annual renewal form must be submitted for years two (2) and three (3) of the protocol through the eIACUC system.** After three years all continuing studies must be completely re-described in a new electronic application and submitted to IACUC for review.

• **All modifications to the IACUC-Approved Protocol must be approved by the IACUC prior to initiating the modification.** Modifications can be submitted to the IACUC for review and approval as an Amendment or Procedural Change through the eIACUC system. These changes must be within the scope of the original research

hypothesis, involve the original species and justified in writing. Any change in the IACUC-approved protocol that does not meet the latter definition is considered a major protocol change and requires the submission of a new application.

• **All costs invoiced to a grant account must be allocable to the purpose of the grant.** Costs allocable to one protocol may not be shifted to another in order to meet deficiencies caused by overruns, or for other reasons convenience. Rotation of charges among protocols by month without establishing that the rotation schedule credibly reflects the relative benefit to each protocol is unacceptable.

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UNIVERSITY OF SOUTH FLORIDA
USF RESEARCH & INNOVATION

RESEARCH INTEGRITY & COMPLIANCE
INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

MEMORANDUM

TO:	Lynn Martin,
FROM:	Farah Moulvi, MSPH, IACUC Coordinator Institutional Animal Care & Use Committee Research Integrity & Compliance
DATE:	7/31/2019
PROJECT TITLE:	Light pollution and seasonality in passerine responses to West Nile virus
FUNDING SOURCE:	USF department, institute, center, etc.
IACUC PROTOCOL #:	R IS00006820
PROTOCOL STATUS:	**APPROVED**

The Institutional Animal Care and Use Committee (IACUC) reviewed your application requesting the use of animals in research for the above-entitled study. The IACUC **APPROVED** your request to use the following animals in your **protocol for a one-year period beginning 7/31/2019:**

Sparrow: house sparrow ((adult/22-28g/F and M))	108
Bird: Blue Jay ((adult/70-100g/ F and M))	30
Bird: Carolina Wren ((adult/ 18-22g/ F and M))	30
Bird: Great Crested Flycatcher ((adult/ 27-40g/ F and M))	30
Bird: Gray Catbird ((adult/ 23-57g/ F and M))	30
Bird: White-eyed Vireo ((adult/ 10-14g/ F and M))	30
Bird: Northern Mockingbird ((adult/ 45-58/ F and M))	30
Bird: Tufted Titmouse ((adult/ 18-26g/ F and M))	30
Bird: Carolina Chickadee ((adult/8-12g/F and M))	30
Bird: Northern Cardinal ((adult. 42-48g/ F and M))	30
Bird: Common Grackle ((adult/ 74-142g/ F and M))	30

Please take note of the following:

• IACUC approval is granted for a one-year period at the end of which, an annual renewal form must be

submitted for years two (2) and three (3) of the protocol through the eIACUC system. After three years all continuing studies must be completely re-described in a new electronic application and submitted to IACUC for review.

• **All modifications to the IACUC-Approved Protocol must be approved by the IACUC prior to initiating the modification.** Modifications can be submitted to the IACUC for review and approval as an Amendment or Procedural Change through the eIACUC system. These changes must be within the scope of the original research hypothesis, involve the original species and justified in writing. Any change in the IACUC-approved protocol that does not meet the latter definition is considered a major protocol change and requires the submission of a new application.

• **All costs invoiced to a grant account must be allocable to the purpose of the grant.** Costs allocable to one protocol may not be shifted to another in order to meet deficiencies caused by overruns, or for other reasons convenience. Rotation of charges among protocols by month without establishing that the rotation schedule credibly reflects the relative benefit to each protocol is unacceptable.

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UNIVERSITY OF SOUTH FLORIDA
USF RESEARCH & INNOVATION

From:	Institutional Biosafety Committee **Date:** September 12, 2019
To:	Lynn Martin
Re:	**PROTO2019-065** Light pollution effects on West Nile virus dynamics via heterogeneity in host competence
BSL:	**BSL-3 ABSL-3**
Status:	**Approved**

The above referenced protocol was reviewed by the Institutional Biosafety Committee (IBC). The IBC grants approval for this project.

Approval is for a one-year period beginning 9/12/2019.

- All modifications to the IBC approved protocol must be approved by the IBC prior to initiating the modification.
- All experimental procedures must be conducted at Biosafety Containment Level practices as described in your application. In accordance with the NIH/CDC publication Biosafety in Microbiological and Biomedical Laboratories 5th Edition.
- Report any significant problems, violations of the CDC/NIH Guidelines, or any significant research-related accidents and illnesses to Research Integrity and Compliance at 974-5091 or biosafety@usf.edu.

RESEARCH INTEGRITY & COMPLIANCE
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UNIVERSITY OF SOUTH FLORIDA

Lynn Martin, Ph.D.
Biology
SCA 110

8/15/2016

IBC Study number: **1323 Infectious Agent**

IBC Study Title: ***Stress hormone effects on host disease resistance, tolerance and transmission***

Dear Dr. Martin:

Your above entitled registration application was reviewed by the Institutional Biosafety Commitee (IBC) at its **07/19/2016** meeting. The IBC acknowledges you have fulfilled the IBC review requirements. The IBC hereby grants the approval for this project. In addition, please take note of the following:

- Approval is for a one-year period beginning: **8 /15/2016**
- The Principal Investigator shall not modify any research project approved by the IBC until that proposed modification has been registered with and approved by the IBC.
- Report any significant problems, violations of the CDC/NIH Guidelines, or any significant research-related accidents and illnesses to Research Integrity and Compliance at 974-0954.
- All operations must be conducted at Biosafety Containment Level Practices as described in your application in accordance with the NIH/CDC publication Biosafety in Microbiological and Biomedical Laboratories, 5th Edition.
- When using research animals with an IBC registered biological agent, initiation of the animal component of the study is contingent upon the completion of and approval by the Institutional Animal Care and Use Committee (IACUC) process.
- IBC registrations are approved for a one-year period at the end of which, an annual renewal/amendment application must be submitted for years two (2) and three (3) of the protocol. A new registration application must be reviewed and approved by the Full Committee every three (3) years.

If you have any questions regarding the status of this project, please contact Research Integrity and Compliance at 974-0954.

Sincerely,

BA: dh
cc:DSR

Andrew Cannons, Ph.D.
Chairperson
Institutional Biosafety Committee

UNIVERSITY OF SOUTH FLORIDA

Lynn Martin, Ph.D.
Biology
SCA 110

7/24/2017

IBC Study number: **1323 Infectious Agent**

IBC Study Title: ***Stress hormone effects on host disease resistance, tolerance and transmission***

Dear Dr.Martin:

Your request to renew the above entitled Institutional Biosafety Committee (IBC) Study was reviewed and approved through an expedited process. The IBC acknowledges that this study is currently on going as previously approved. This action will be reported to the IBC at the next regularly scheduled meeting. In addition, please take note of the following:

- **Continuation of the project is approved for a one-year period beginning: 8/14/2017**
- The Principal Investigator shall not modify any research project approved by the IBC until that proposed modification has been registered with and approved by the IBC.
- All operations must be conducted at Biosafety containment level and practices as described in your application and in accordance with the NIH/CDC publication Biosafety in Microbiological and Biomedical Laboratories, 5th Edition.
- Report any significant problems, or any significant research-related accidents and illnesses to Research Integrity and Compliance at 974-0954.
- As a reminder to ensure security of the agent, entry doors to the lab must be shut and locked when the lab is left unattended.
- IBC registration application(s) are approved for a one-year period at the end of which, an annual renewal/amendment application must be submitted for years two (2) and three (3) of the protocol. A new registration application must be reviewed and approved by the Full Committee every three (3) years.

If you have any questions regarding the status of this project, please contact Research Integrity and Compliance at 974-0954.

Sincerely,

Andrew Cannons

BA: dc

cc:DSR

Andrew Cannons, Ph.D.
Chairperson
Institutional Biosafety Committee

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INSTITUTIONAL BIOSAFETY COMMITTEE
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Lynn Martin, Ph.D.
Biology
SCA 110

5/9/2018

IBC Study number: **1323 Infectious Agent**

IBC Study Title: ***Stress hormone effects on host disease resistance, tolerance and transmission***

Dear Dr.Martin:

Your request to renew the above entitled Institutional Biosafety Committee (IBC) Study was reviewed and approved through an expedited process. The IBC acknowledges that this study is currently on going as previously approved. This action will be reported to the IBC at the next regularly scheduled meeting. In addition, please take note of the following:

- **Continuation of the project is approved for a one-year period beginning: 8/13/2018**
- The Principal Investigator shall not modify any research project approved by the IBC until that proposed modification has been registered with and approved by the IBC.
- All operations must be conducted at Biosafety containment level and practices as described in your application and in accordance with the NIH/CDC publication Biosafety in Microbiological and Biomedical Laboratories, 5th Edition.
- Report any significant problems, or any significant research-related accidents and illnesses to Research Integrity and Compliance at 974-0954.
- As a reminder to ensure security of the agent, entry doors to the lab must be shut and locked when the lab is left unattended.
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If you have any questions regarding the status of this project, please contact Research Integrity and Compliance at 974-0954.

Sincerely,

BA: dc

cc:DSR

Andrew Cannons, Ph.D.
Chairperson
Institutional Biosafety Committee

APPENDIX E:

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Chapters 1 and 3:

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