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Seasonality in Competence to Transmit West Nile Virus for a Widespread Reservoir

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Seasonality in Competence to Transmit West Nile Virus for a Widespread Reservoir

by

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of the requirements for the degree of
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Abstract

All organisms face a central conflict; a limited supply of resources must be optimally allocated to competing biological functions. Utilizing robust immune defenses can be energetically costly; thus, immune function is often diminished when other processes, such as reproduction, molt, or migration demand energy. As wild animals residing in temporally dynamic environments face seasonal patterns in resource availability, infectious disease risk, and environmental suitability, many species have evolved endogenous biological rhythms so that the performance of reproduction, molt, or migration and the associated trade-off with immune function be completed when doing so best promotes fitness. In turn, this demand to optimize physiology to intra-annually variable conditions should give rise to wild animals varying seasonally in host competence, or the capacity to transmit a pathogen to others. Seasonality in host competence could have broad implications for zoonotic disease spillover. However, few studies have characterized how zoonotic disease reservoirs vary seasonally in traits that directly reflect competence. Here, I performed a series of experimental West Nile virus (WNV) infections in a geographically widespread host, the house sparrow (HOSP; *Passer domesticus*), to determine whether endogenous biological rhythms are generating intra-annual variation in host competence over the course of the year. This work revealed that HOSP do indeed vary seasonally in competence, maintaining infectious WNV viral titers 51% longer in the fall when compared to other times of

the year. This prolonged infectious period was the result of a tendency for fall HOSP to possess higher WNV titers early in infection while tending to survive longer when compared to conspecifics at other times of the year. This period of increased competence in the fall may be the result of a delayed-tradeoff with molt, as the accumulation of the energetic demands imposed by molting and the inability to compensate for these demands by providing sufficient nutrients for a robust immune response leads to altered host-pathogen dynamics. Overall, the realization that HOSP are more competent during the peak period of WNV transmission suggests that zoonotic disease reservoir seasonality is important to consider when attempting to predict and prevent spillover. Further efforts to describe seasonality for other reservoir species in different environmental contexts could provide useful insights into zoonotic disease management.

Introduction

Most infectious diseases experience consistent seasonal patterns in prevalence (Altizer et al. 2006; Martinez 2018). Understanding the underlying ecological and evolutionary drivers of infectious disease seasonality can have implications for mitigation and management strategies, especially when considering zoonotic diseases transmitted from wild animal reservoirs to humans. For pathogens transmitted through direct contact with an infectious host, seasonal changes in host demographics, environmental conditions, parasite biology, or any combination of the above, can be impactful (Altizer et al. 2006; Martinez 2018). Most mathematical models for vector-borne zoonotic disease transmission tend to assume that seasonality is driven by oscillations in vector dynamics and/or do not allow for hosts to be heterogeneous among the susceptible, infectious, and resistant classes. Variation in the ability to maintain and transmit infection (i.e., competence) can have drastic effects on broader transmission dynamics (Dwyer et al. 1997; Barron et al. 2015; Gervasi et al. 2015; Martin et al. 2016). Indeed, an observation of fungal pathogen dynamics in nature found that epidemics were the outcome of both an increase in the rate susceptible hosts being exposed to the pathogen and a reduction in the ability of infected hosts to resist infection (Merrill et al. 2021). For West Nile virus (WNV), a zoonotic pathogen maintained in wild birds transmitted by mosquitoes, seasonality is primarily attributed to changes in

vector density or the influx of hosts into local areas. My thesis research asks: Does seasonality in host competence for house sparrows (*Passer domesticus*; HOSP), an urban WNV reservoir, play any consequential role in the seasonality of WNV? If zoonotic disease reservoirs were to vary over the course of the year in competence, then efforts to mitigate spillover could be oriented to when doing so would be most effective. For instance, if seasonality in competence were to arise through energetic stress associated with limited resource availability or the performance of energetically demanding life history processes, resource supplementation could allow hosts to maintain robust immune defenses (Ben-Hamo et al. 2017). However, few studies have characterized how competence varies seasonally in natural settings.

WNV is a nearly global pathogen responsible for human mortality and widespread population declines of several songbird species (LaDeau et al. 2007; Soverow et al. 2009; Harrigan et al. 2014; George et al. 2015; Semenza et al. 2016). As a vector-borne zoonotic disease, WNV is maintained in an enzootic cycle between wild birds and mosquitoes. Like other zoonotic pathogens, WNV risk varies over the year. In the United States, risk is low throughout the winter and spring, increasing during the summer and peaking in the fall (Centers for Disease Control and Prevention 2020; Florida Department of Health 2021; Figure 1). Most WNV reservoirs exhibit highly conserved seasonality in population size and physiology. However, when modeling WNV risk, individual members of the same species are assumed to respond similarly to infection regardless of environmental conditions, leaving the observed seasonal patterns in WNV risk being largely attributed to oscillations in the abundance of feeding mosquitoes (Reisen et al. 2008; Hartley et

al. 2012; Martinez 2018). Vector abundance is certainly important for WNV seasonality, though our understanding of WNV transmission dynamics can be improved by considering host competence in conjunction with vector demographics. For instance, blood meal analysis indicated that most mosquitoes carrying infectious WNV had fed on two exceptionally competent host species, HOSP and American robins (*Turdus migratorius*) (Kilpatrick et al. 2006a, 2006b). Therefore, depicting spatial heterogeneities in HOSP and American Robin abundance could facilitate more accurate evaluations of WNV risk. Indeed, another study found the spatial distribution of these competent WNV reservoirs to be predictive of human WNV cases (McKenzie and Goulet 2010). Therefore, seasonal heterogeneities in competence for WNV reservoirs could have similar implications as vector abundances, where outbreaks are the outcome of the interplay of both vector and host traits.

In addition to the ability of an infected host to eliminate a pathogen and prevent replication, transmission dynamics are also sensitive to heterogeneities in tolerance, or the capacity to mitigate damage brought about by a given pathogen burden (Råberg et al. 2009; Becker et al. 2019; Burgan et al. 2019). Tolerance is often measured as the relationship between a fitness metric of interest and pathogen burden over the course of infection, where a more tolerant host is able to maintain performance or fitness in a certain trait more effectively than others (Råberg et al. 2009). While the relationship between resistance and transmission is straightforward, where a poorly resistant host develops higher pathogen burdens and transmits to others at a higher rate, the epidemiological ramifications of tolerance are more dependent on the transmission mechanism of given pathogen

(Barron et al. 2015; Burgan et al. 2019). When considering a pathogen that is directly transmitted from host-to-host, given similar abilities to resist infection, a highly tolerant host that maintains activity and social engagements would be more competent as such hosts ought to have higher rates of contact with susceptible conspecifics than a poorly tolerant host displaying sickness behaviors (VanderWaal and Ezenwa 2016; Burgan et al. 2019). However, in the context of WNV, the opposite may be true, where a poorly tolerant bird is unable to maintain flight ability and perform defensive behaviors, being easier targets for vector feeding, contributing more to transmission than a highly tolerant host (Barron et al. 2015). Tolerance could further impact transmission through changes in mortality rates, as hosts that survive longer ought to transmit pathogen at higher rates if infectiousness is maintained until death (Bull and Lauring 2014). As with resistance, variation in tolerance can be correlated to resource availability and energetic demands. Although resistance and tolerance are not always inversely related to one-another, energetic stress often yields reduced resistance and enhanced tolerance (Cornet et al. 2014; Knutie et al. 2017; Ganeshan et al. 2019). Perhaps endogenous immunological rhythms are accompanied by a dynamic balance between resistance and tolerance, so that hosts are able to better tolerate greater pathogen burdens when resources are needed elsewhere. This interchange would have widespread impacts on pathogen transmission. If actively reproducing HOSP are more tolerant as a result of limited capacities to resist infection and the demand to defend one's nest and provide for nestlings, then such hosts would likely contact more susceptible conspecifics while surviving longer.

To better predict spillover risk and target mitigation efforts, it is necessary to describe how reservoirs vary seasonally in the traits that compose competence. Frustratingly, efforts to do such in the context of a zoonotic disease are rare. Doing so can improve our understanding of WNV seasonality. It is well known that seasonal variation in vector density is important, while the influence of endogenous host rhythms is much more obscure. Although tradeoffs are well documented, it remains vague on just how much this biological seasonality influences competence. The majority of prior investigations on immunological seasonality have described baseline immune parameters that do not necessarily reflect transmission. Classic immunological techniques, such as swelling in response to exposure to phytohemagglutinin, a benign indicator of infection, certainly can provide insights into host immune defenses, but it is not clear how a reduced response to a non-pathogenic immune challenge alters the duration or magnitude of infectiousness. By performing experimental infections in reservoir hosts across distinct life history states, insights directly relevant to transmission can be gained, though doing so is potentially hazardous and requires appropriate facilities and regulatory approval, rendering such efforts rare. My thesis work sought to fill this gap by characterizing seasonal variation in host competence for a widespread WNV reservoir. To accomplish this goal, I performed a series of experimental WNV infections in wild caught HOSP over a span of 18 months to determine if WNV competence varies over the course of the year. HOSP were captured from the Tampa Bay area and sub-cutaneously exposed to WNV inside a biosafety level 3 (BSL3) suite at the University of South Florida. Here, I sought to measure both resistance and tolerance. To measure resistance, I quantified WNV viral titers over a 10-day

period. For tolerance, I used three separate metrics: mass, capture avoidance, and mortality. A highly tolerant host would lose less body mass and maintain avoidance capacity when compared to others with similar WNV burdens. Additionally, I quantified the infectious period (IP) as duration of time that each bird possesses viral titers generally infectious for the most likely vector species. IP is sensitive to both resistance and tolerance, with mortality generally occurring during the midpoint of the IP (Kernbach et al. 2020). As HOSP exhibit highly conserved seasonal rhythmicity in the performance of reproduction and molt, I hypothesized that both resistance and tolerance to WNV would vary seasonally – when other biological processes demand energy, HOSP: (1) are less resistant, possessing elevated viral titers over the duration of the experiment, (2) are more tolerant, (3) maintain infectious viremia for longer.

Methods

Capture and husbandry

A total of 75 HOSP were sampled across 6 experimental cohorts from November 2019 to May 2021. Cohort timing was designed to capture distinct phases in WNV prevalence (rare during winter, amplifying transmission in summer, and peak prevalence in fall) and HOSP life history stage (reproduction and molt; Figure 1). HOSP were captured using mist nets along bush lines in Tampa and St. Petersburg, Florida between the hours of 06:00 and 09:30. Immediately upon capture, each bird received a metal leg band, and a small blood sample (~50ul) was taken and stored in RNA Later at a ~1:100 ratio and stored at -80^{o}C. Thereafter, HOSP were placed in individual cloth bags and transferred to a temporary housing facility where they were housed individually in 13” x 15” x 18” cages. Due to limited availability of cages midway through the experiment, I was forced to switch to cages with the dimensions 14” x 11” x 17” for the final three cohorts. Following a brief acclimation period of 1-3 days, the birds were relocated to the University of South Florida Animal Biosafety Level 3 laboratory inside a BioBubble containment system at ~21^{o}C, 50% humidity, and lighting matching the current environmental photoperiod, with mixed seeds and water provided *ad libitum*, being monitored twice daily. All experimental procedures comply with protocol #6820 approved by the USF Institutional Animal Care and Use Committee.

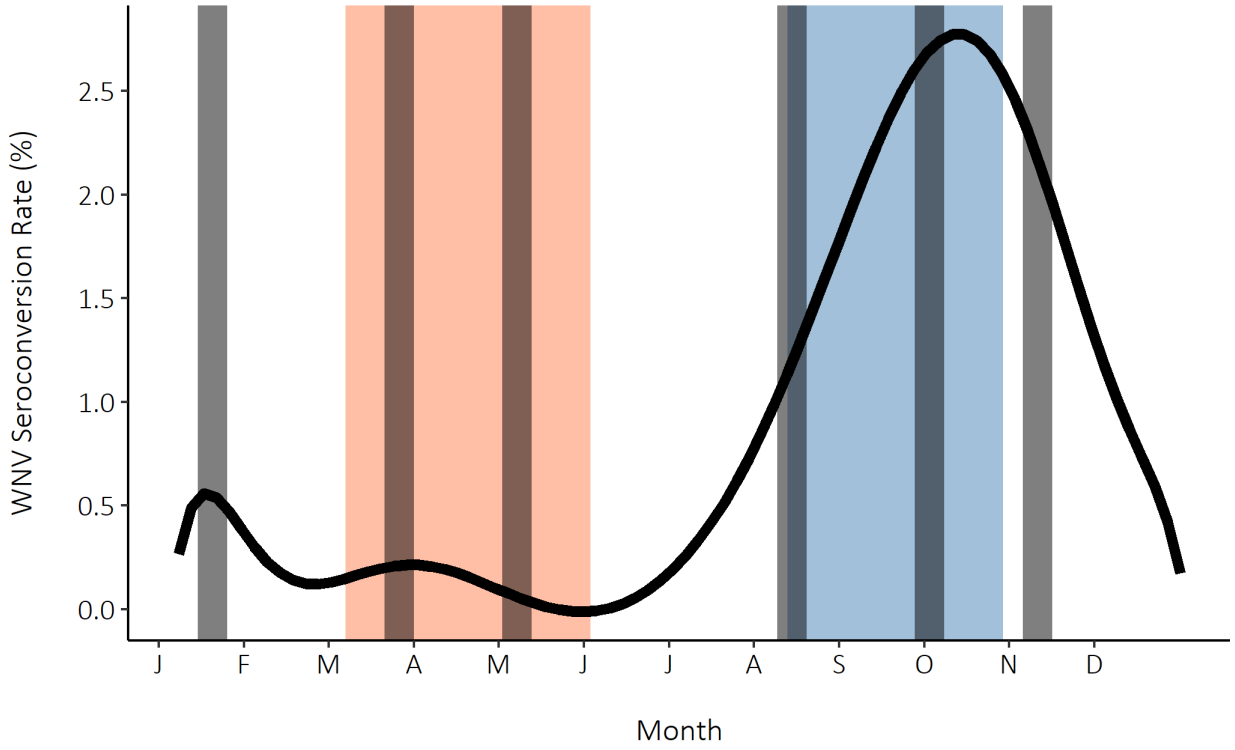

Figure 1: West Nile Virus seasonality, house sparrow annual cycle, and schedule of experimental cohorts. The black line represents WNV prevalence in Florida Sentinel Chickens, using data provided by the Florida Department of Health. The colored shaded areas represent the breeding season (orange) and molting period (blue) for Tampa area house sparrows. Meanwhile, the gray shaded areas signify the timing of the 6 experimental cohorts in this study. The timing of which was chosen to capture key phases in house sparrow life history and WNV transmission.

Experimental WNV infections and Sampling

I exposed each bird to 100 PFU of NY99 WNV via a subcutaneous injection following previously established lab protocols (Burgan et al. 2018; Kernbach et al. 2019). Prior to infection, and on days 2, 4, 6, and 10 post infection (DPI), I collected a blood sample of approximately 100 uL. Blood samples were centrifuged at 7200xg for 5 minutes and the plasma supernatant was removed and stored at -20^{o}C until extraction. Prior to sampling for each bird, I recorded body mass (to 0.1g) and the duration of time necessary for me to capture a bird from its cage as a

proxy for predator avoidance and mobility. Time to capture (TTC) was recorded on a stopwatch by a second investigator, beginning once my hand entered the bird's cage and stopping once the bird was in hand. Time to capture (TTC) was recorded for all cohorts but those sampled in November. At 10 DPI, all surviving birds were euthanized via an isoflurane overdose and cervical decapitation. Necropsies were performed to confirm sex and reproductive status. HOSP gonads can have over a 100-fold difference in mass during the breeding season (Anderson 2007). Gonads were removed and visually inspected, with all birds possessing enlarged gonads during the two spring cohorts.

RNA extractions and qRT-PCR

Using the QIAamp Viral RNA Mini Kit (Qiagen catalog #52904; Gervasi et al. 2017; Burgan et al. 2018; Kernbach et al. 2019), I extracted viral RNA from 10 uL serum isolates. Subsequently, viral load was measured by quantitative reverse transcription PCR using an iTaq Universal Probes One-Step Kit (Bio-Rad catalog #1725141; Gervasi et al. 2017). All samples were measured in duplicate alongside negative controls and standards ranging from 10^1 to 10^8 PFU/mL prepared from stock concentrations in which viral titer was confirmed using Vero cell plaque assay. Positive control values remained consistent with previously published work by the Martin lab (Gervasi et al. 2017; Burgan et al. 2018; Kernbach et al. 2019).

Statistical Analyses

All statistical analyses were performed in R version 4.0.2 using log10 transformed WNV titers. I utilized mixed models and type III analysis of variance (ANOVA) tests to assess each of my hypotheses. Preliminary analyses were conducted to determine whether capture site, age class (i.e., juvenile or adult), cage size, or sex impacted any measures. None of these potentially confounding variables were statistically significant and consequently they were excluded in all other models. To account for repeated sampling efforts, bird ID was used as a random effect. I used two iterations of models to analyze resistance. The first used DPI, season, and their interaction to predict the progression of WNV titer over the duration of the experiment (Formula 1). DPI was included as a second-order polynomial to accurately capture the quadratic shape of WNV infection. Post-hoc tests using the 'emmeans' package in R studio were used to compared viral titers between seasons at each day of sampling (Formula 2).

WNV Titer ~ Month * DPI + (~1|ID)

Formula 1: Model to assess whether the progression of WNV titers vary seasonally. Using the 'lme' function within the 'lme4' package in R Studio, the interaction of month and DPI were used to predict WNV titers. To accurately represent the progression of WNV titers over the duration of infection, DPI was included as a second-order polynomial. Bird ID was used as a random effect to account for repeated sampling efforts of the same individuals. A type III analysis of variance was used to test the significance of the interaction.

WNV Titer ~ Month|DPI

Formula 2: Comparing WNV titers to the population average on each sampling day. Using the 'emmeans' function, cohort averages for WNV titer on was compared to the population average on each day of sampling. The 'contrast' function was used to assess the significance of month.

I used similar techniques to compare whether WNV tolerance varies seasonally. I used mortality and the change in body mass and TTC as metrics to quantify tolerance. Individual tolerance estimates were calculated using linear models with either body mass or TTC as the dependent variable and WNV burden as the predictor. The slope of the relationship in performance of a given trait and WNV titer for each bird represented a tolerance estimate (Burgan et al. 2019). This method allows for comparisons to be made between individuals relative to one's initial mass or capture avoidance. With the tolerance metrics in hand, I used linear models to assess whether either tolerance metric varied seasonally, where cohort was used to predict the slope of tolerance (Figure 3). For mortality, I used a Cox Proportional-Hazards regression in which whether an individual died and when this mortality occurred were modeled using season and peak WNV titer (Figure 4).

Tolerance ~ Month

Formula 3: Model used to determine whether tolerance to WNV varied seasonally for either time to catch or mass. Month was used to predict the calculated tolerance estimate. A type III analysis of variance test was used to assess the significance of the predictor variables.

Survival ~ Month * Peak titer

Formula 4: I used a cox proportional-hazards model to determine whether WNV-induced mortality varied seasonally for HOSP. The survival variable serves as a composite for both whether an individual died to infection and how long one survived until death. Month and maximum WNV titer sustained were used to predict survival. A type III analysis of variance test was used to test the significance of these predictor variables.

Finally, I sought to assess whether the duration of time an individual maintained an infectious viremia varied seasonally. For the most common WNV vectors, host viral titers exceeding 10^5 PFU/mL are considered as the threshold for infection to establish in a mosquito vector following feeding (Turell et al. 2005). Therefore, the duration of time an individual maintained WNV viremia above this threshold is considered as the IP (Figure 2). With this transmission threshold, assuming a linear relationship between sampling periods, I measured the point in time in which an individual's viremia intersected 10^5 PFU/mL. By subtracting the day in which viremia fell below the threshold by the day it had rose above, I quantified the IP. Then, I used a linear model to compare whether IP varied seasonally. In this model, I used season to predict IP (Formula 5).

Infectious Period ~ Month + Sex + Age class

Formula 5: A linear model used month and several potential confounding variables to predict the duration of time maintaining WNV viremia exceeding the transmission threshold. I used a type III analysis of variance test to determine the significance of month, sex, and age on infectious period.

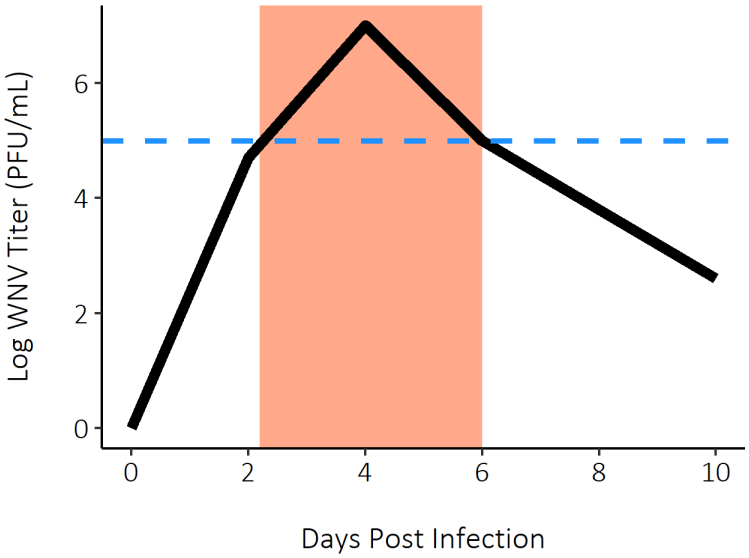

Figure 2: Graphical representation of WNV infectious period. Host viral titers (black line) exceeding 10^5 PFU/mL (blue dash) are generally infectious for common WNV vectors. The black line represents the typical course of WNV infection in house sparrows. The duration of time an individual maintains viremia above the transmission threshold is considered the infectious period (orange

Results

Infectious Period

The duration of time maintaining WNV titers above the 10^5 PFU/mL transmission threshold varied significantly across seasons (Season: $F_{5,67} = 3.37$, $P = 0.009$; Figure 3; Table 1). Infectious viremia was maintained longer in the fall, where in the October and November cohorts, HOSP possessed infectious WNV titers for an average of 4.33 days, compared to 2.86 days in the other four experimental cohorts, an increase of 51%.

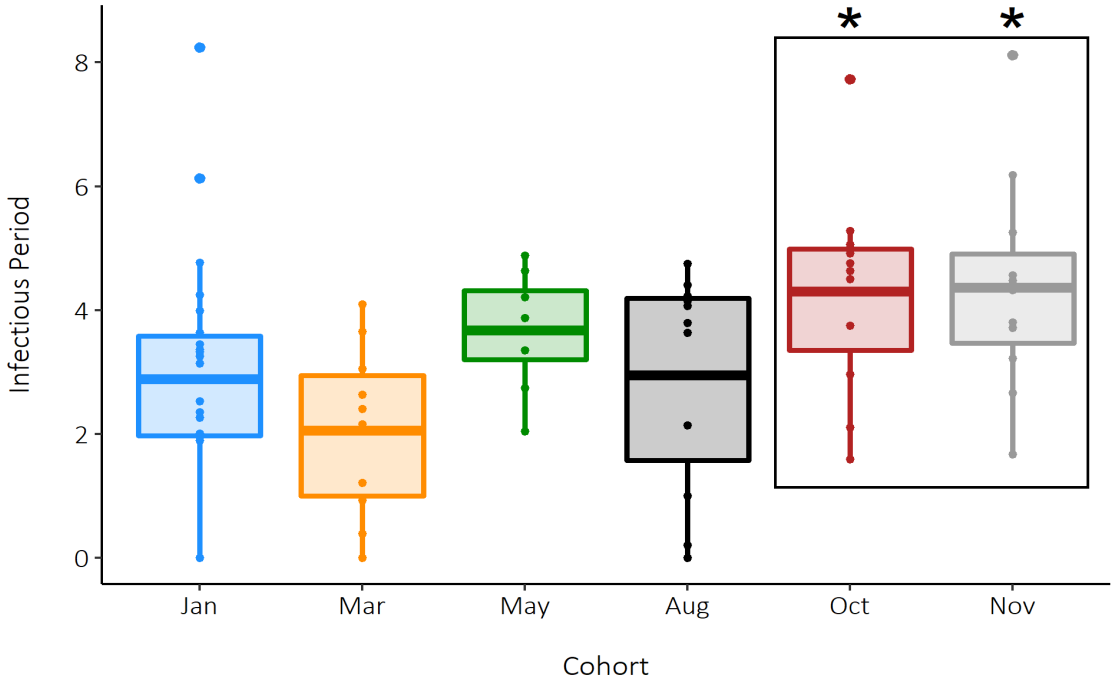

Figure 3: Seasonality in the duration of time maintaining infectious viremia. Dots represent the length of time maintaining viral titers above the WNV transmission threshold. House sparrows varied seasonally in infectious period ($F_{5,67} = 3.37$, $P = 0.009$), with infectious viremia being maintained longer in the fall.

Table 1: Effects of month, sex, and age on WNV infectious period. Significant terms are included in bold.

Term	Sum Sq	Df	F value	Pr(>F)
Month	**47.468**	**5**	**3.371**	**0.009**
Sex	8.741	1	3.104	0.083
Age class	0.909	1	0.323	0.572

West Nile Virus viremia

WNV titers increased rapidly following WNV exposure and subsequently declined across the duration of the experiment (DPI: $F_{2,220} = 311.42$, $P < 0.001$), in accordance with previous work using this experimental system (Burgan et al. 2018; Kernbach et al. 2019, 2020). All HOSP possessed detectable viremia over the duration of the experiment. 69 of the 75 HOSP included in this experiment developed WNV titers that exceeded the transmission threshold. Seasonal variation in the progression of WNV viremia was marginally non-significant (DPI x Season: $\chi^2 = 30.1$, $P = 0.068$; Figure 4; Table 2). Post-hoc analysis comparing viral titers across seasons at each day of sampling revealed that at 2 DPI, viral titers were elevated in the October cohort ($P = 0.023$; Figure 5; Table 3), with an average of 6.67 log PFU/mL, compared to a mean of 5.14 log PFU/mL across all other cohorts. This elevated viremia at 2DPI in October HOSP led to 89% of HOSP possessing infectious viremia, compared to 62% of the other cohorts.

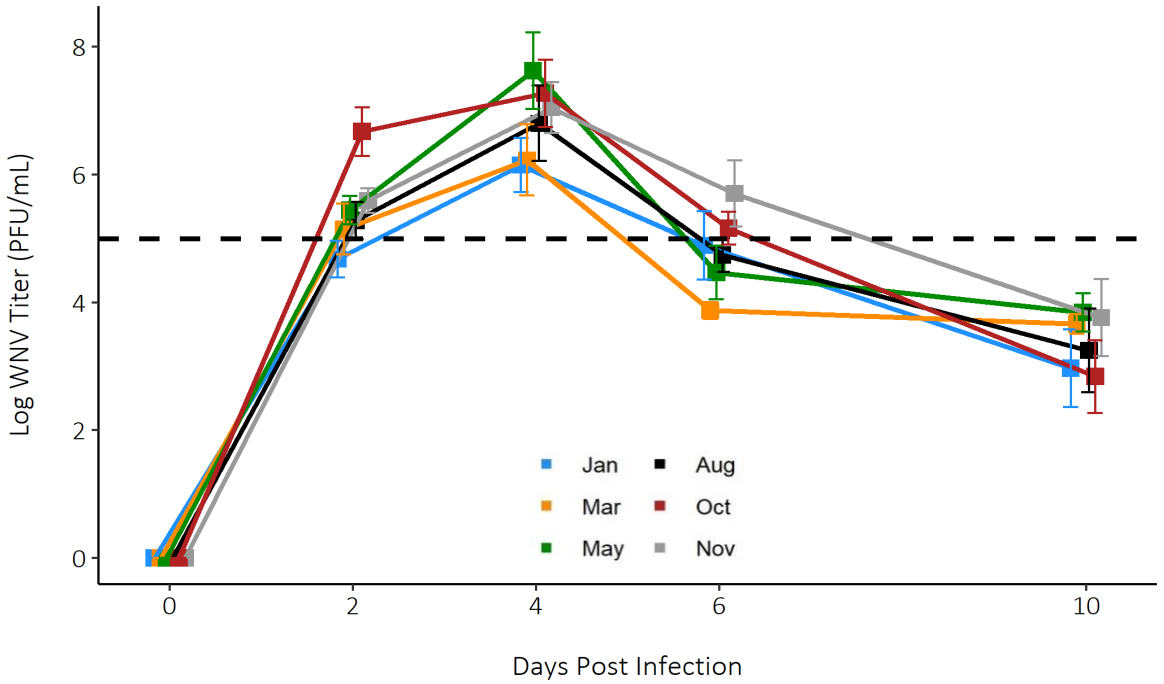

Figure 4: Viremia profiles for house sparrows exposed to West Nile Virus. WNV titers were measured over the course of infection for the six experimental cohorts spanning the annual cycle using RT-qPCR. Colored squares and error bars represent the average WNV titer +/- the standard error. The black dashed line at 10^5 PFU/mL signifies a conservative estimate for the WNV viral load required for infection to establish in *Culex* spp. mosquitos. The temporal course of WNV infection was marginally non-significant (χ^2 = 30.1, P = 0.068).

Table 2: Summary statistics of the linear mixed effects model to determine the effects of month and DPI on WNV titer. Significant terms are included in bold.

Term	χ^2	Df	Pr(>χ2)
Month	7.73	5	0.17
DPI	**393.25**	**4**	**0.0001**
Month*DPI	30.1	20	0.068

Table 3: Comparison of mean viral titers on each day of sampling to the population average. All significant values are reported in bold.

Month	Estimate	SE	T-ratio	P-value
2 DPI				
Jan	-0.74	0.28	-1.9	0.11
Mar	-0.32	0.37	-0.87	0.78
May	-0.02	0.4	-0.06	0.95
Aug	-0.19	0.34	-0.54	0.86
Oct	**1.14**	**0.38**	**3**	**0.02**
Nov	0.12	0.35	0.36	0.86
4 DPI				
Jan	-0.68	0.28	-2.46	0.1
Mar	-0.63	0.37	-1.71	0.19
May	0.77	0.4	1.92	0.18
Aug	-0.08	0.35	-0.23	0.82
Oct	0.42	0.35	1.19	0.36
Nov	0.19	0.35	0.55	0.7
6 DPI				
Jan	0.06	0.32	0.19	0.89
Mar	-0.87	0.41	-2.11	0.12
May	-0.37	0.49	-0.76	0.68
Aug	-0.06	0.43	-0.14	0.89
Oct	0.44	0.43	1.01	0.63
Nov	0.8	0.38	2.12	0.12
10 DPI				
Jan	-0.33	0.39	-0.86	0.68
Mar	0.21	0.44	0.47	0.68
May	0.42	0.62	0.68	0.68
Aug	-0.19	0.47	-0.41	0.68
Oct	-0.61	0.44	-1.38	0.68
Nov	0.51	0.5	1.02	0.68

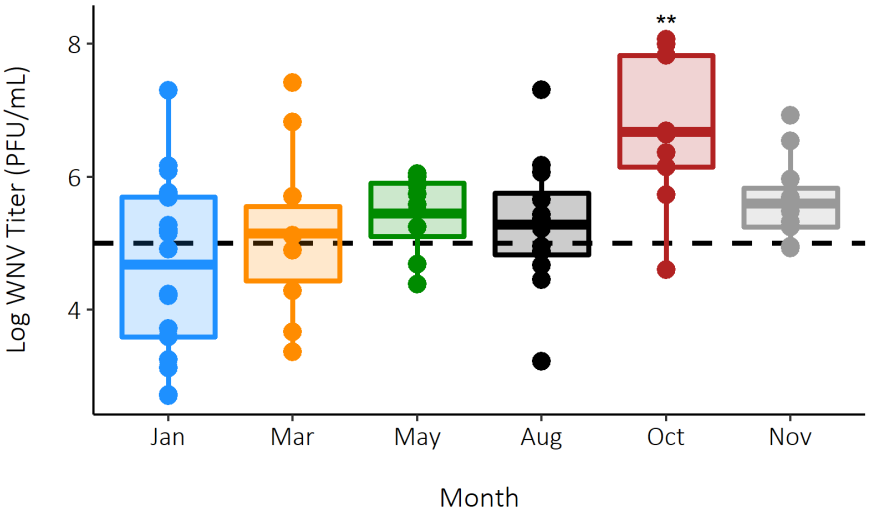

Figure 5: WNV viremia two days post infection. Each dot represents an individual sample measured by RT-qPCR, while the solid line signifies the group average. The 10^5 PFU/mL transmission threshold is indicated by the black dashed line. WNV titers were significantly elevated in the October cohort at 2 DPI (P = 0.023).

Host tolerance

I next asked whether HOSP varied seasonally in tolerance to WNV using body mass, capture avoidance, and mortality. Initial mass did not differ between groups (Season: $F_{5,69}$ = 1.6, P = 0.17). Over the duration of WNV infection, HOSP lost an average of 4.15% of body mass. For TTC, the initial TTC did vary seasonally in response to a change in cage sizes due to supply chain issues (Cage size: $F_{1,62}$ = 21.95, P = 0.001), though there were no differences among groups with the same cage size (Season: X^2 = 6.08, P = 0.193). Decreases in both mass (Titer: X^2 = 27.35, P = 0.001; Figure 6A) and TTC (Titer: X^2 = 5.99, P = 0.014; Figure 6C) were related to WNV titer, where birds with higher titers lost more mass and were easier

to catch. For each metric, I calculated a tolerance estimate as the rate of change in each trait over the range of WNV burden. With the tolerance estimates, I used a linear model in which season was used to predict tolerance to determine whether WNV tolerance varied seasonally. I found no seasonal effect for mass tolerance (Season: $F_{5,69} = 1.14$, $P = 0.35$, Figure 6B; Table 4) nor TTC Tolerance (Season: $F_{4,59} = 0.921$, $P = 0.458$, Figure 6D; Table 4).

Table 4: Effects of month on tolerance estimates for mass and time to catch.

Term	Sum Sq	Df	F value	Pr(>F)
Mass Tolerance				
Month	0.17	5	1.14	0.35
TTC Tolerance				
Month	23.14	4	0.92	0.46

Table 5: Results from Cox proportional-hazards model to assess the significance of maximum WNV titer and month on mortality. Significant effects are included in bold.

Term	Df	Pr(>χ2)
Month	5	0.56
Maximum Titer	**1**	**0.0001**
Month*Maximum Titer	5	0.59

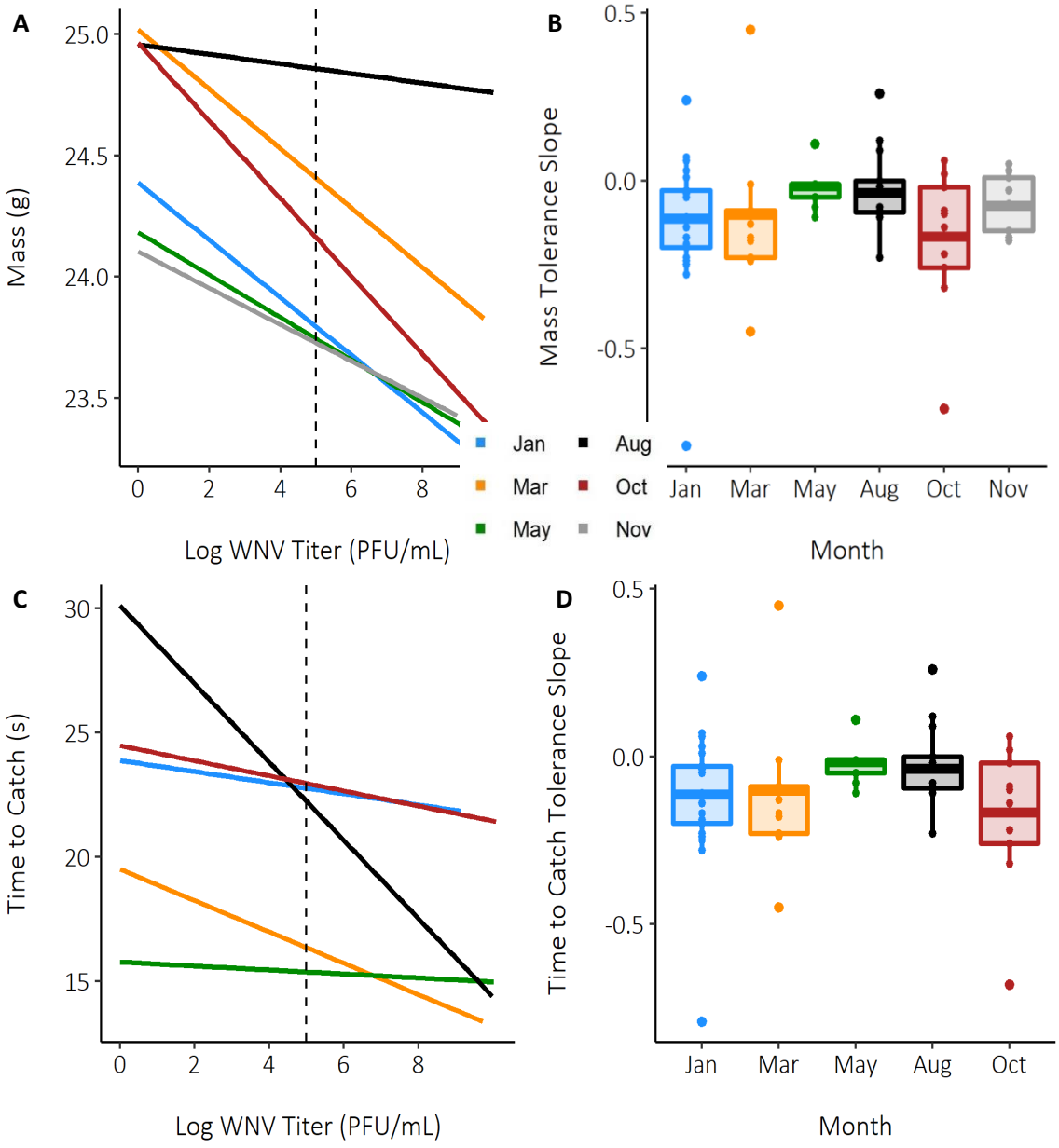

Figure 6: Proxies for tolerance in WNV-exposed house sparrows. (A) House sparrows lost body mass and (C) required less time for capture with greater viral burdens. The slope of this relationship represents the tolerance estimate for each method. Solid lines represent a linear model for each metric and WNV titer, while the dashed line signifies the transmission threshold at 10^5 PFU/mL. (B) House sparrows did not vary seasonally in tolerance when measured with mass ($F_{5,69}$ = 1.14, P = 0.35) or (D) TTC ($F_{4,59}$ = 0.921, P = 0.458).

Mortality

Overall, 42% of WNV-exposed HOSP died at some point during the study, mortality rates comparable to previous experimental WNV infections in house sparrows (Burgan et al. 2018; Kernbach et al. 2020). Death was related to WNV titer, where those birds with higher titers were more likely to perish (Max Titer: $P < 0.0001$; Table 5). Most deaths occurred between days 5 and 7, with the earliest death occurring at 4 DPI. WNV-induced mortality did not vary seasonally (Season: $P = 0.56$; Figure 7; Table 5), though the November cohort tended to survival slightly longer, with the first death not occurring until 6 DPI.

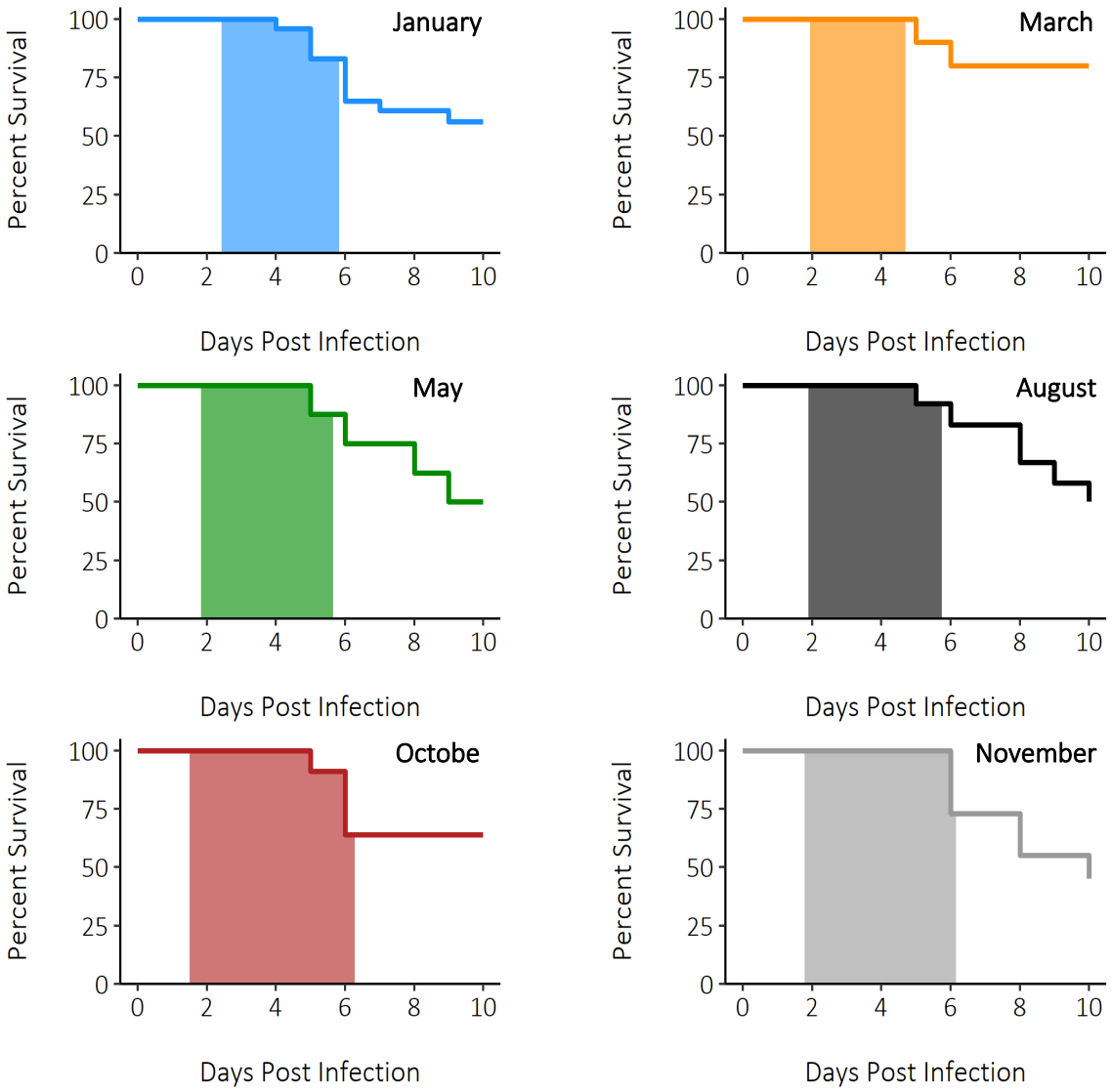

Figure 7: Seasonal comparisons in mortality and infectious period. Survival rate (colored lines) did not differ seasonally (P = 0.84). The colored shaded areas represent the period of time in which the average WNV titer exceeded the transmission threshold. This infectious period did vary seasonally ($F_{5,67}$ = 3.07, P = 0.015), with infectious WNV viremia being maintained longer in October and November.

Discussion

Seasonal variation in reservoir immune function has been widely documented and has long been suggested as a potential driver of zoonotic disease seasonality (Nelson 2004; Altizer et al. 2006; Martinez 2018), although depictions on how reservoirs vary in competence to transmit pathogens are rare. Here, I performed a series of experimental WNV infections in wild caught HOSP to describe seasonality in viremia, tolerance, and competence for a widespread avian reservoir. This work revealed that HOSP do indeed vary seasonally in competence to transmit WNV with infectious viral titers being maintained for 51% longer in the fall. Importantly, the apparent seasonality in competence coincides with the time of year when *Culex spp.* are most abundant. Not surprisingly, WNV prevalence in Florida is greatest during this period (Figure 8). This finding supports other work demonstrating that efforts to predict vector-borne zoonotic disease risk are improved by considering both reservoir and vector ecology (Childs et al. 2019).

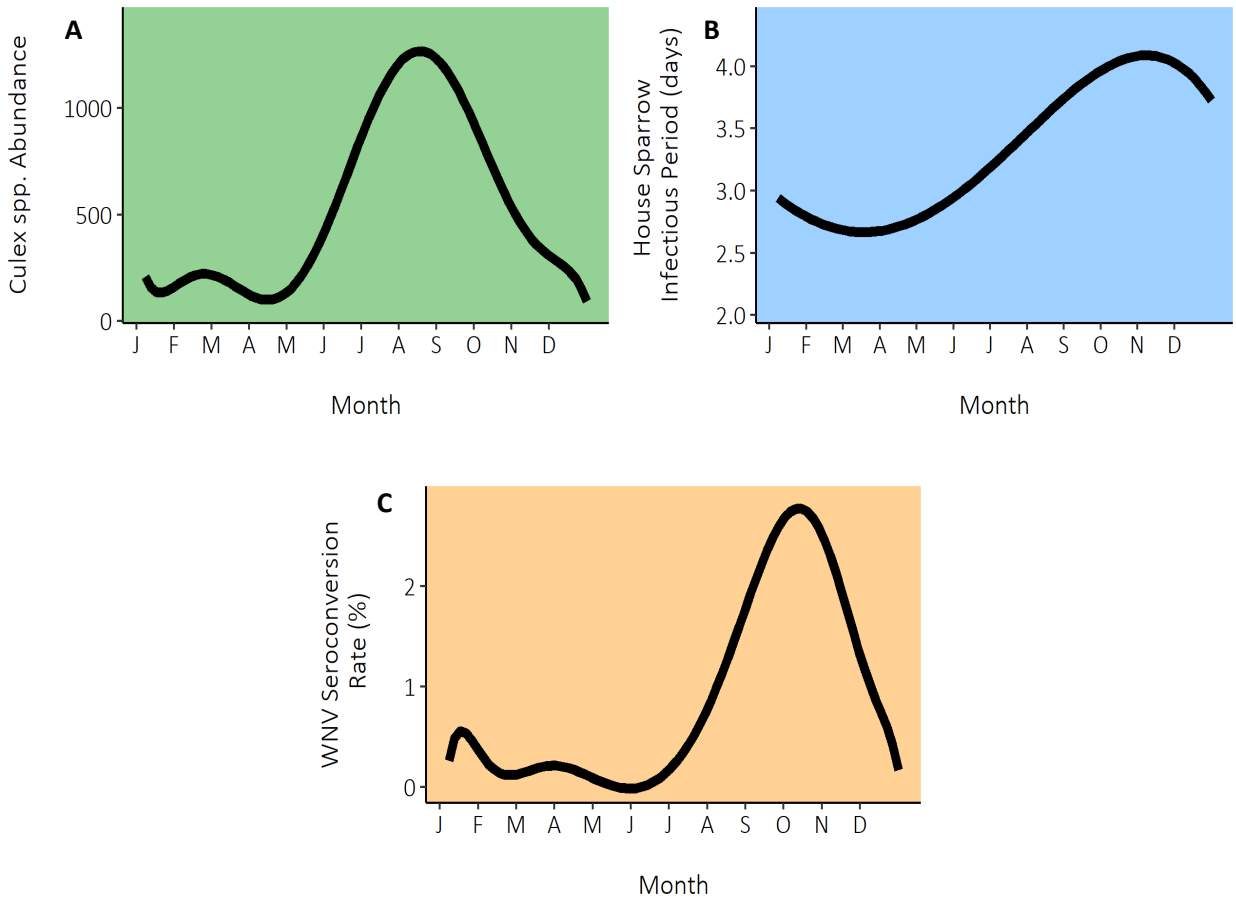

Figure 8: Mosquito density and reservoir competence dynamics. (A) *Culex spp.* abundance in Manatee County, Florida (VectorBase, 2021) is greatest during the late summer, while (B) a widespread reservoir, the house sparrow, is most competent during in the weeks directly following. (C) Consequently, WNV seropositivity rates in Florida sentinel chickens reaches its peak in this late summer/early fall period when vectors are most abundant, and reservoirs are most competent.

Intra-annual variation in competence may arise through evolutionary demands to optimize resource allocation to climatic conditions, resource availability, and parasite exposure, all of which vary over the course of the year. All organisms face a fundamental conflict: limited resources must be optimally allocated to several biological functions that compete for energy. Life history theory posits that organisms able to allocate resources in a way that best promotes fitness will be

more likely to survive and reproduce (Sheldon and Verhulst 1996; Norris and Evans 2000; Schmid-Hempel 2003). As the maintenance and usage of immune responses is energetically and nutritionally expensive, investments into immune defenses are reduced when other demands persist (Lochmiller and Deerenberg 2000; Nelson 2004; Martin et al. 2008). In response, natural selection has favored the evolution of endogenous biological seasonality to optimize fitness over the year (Lincoln et al. 2006; Stevenson et al. 2015). Using environmental cues, such as photoperiod and precipitation, physiology and behavior are modified in accordance with historical patterns in environmental suitability (Altizer et al. 2006; Martinez-Bakker and Helm 2015). For instance, increasing day lengths that indicate for upcoming favorable conditions stimulate gonadal recrudescence in preparation for breeding in the springtime when resources are plentiful (Prendergast et al. 2003; Walton et al. 2011). Immunological tradeoffs have been widely demonstrated for reproduction (Christe et al. 2000; Greenman et al. 2005), molt (Martin 2005; Moreno-Rueda 2010), and migration (Eikenaar and Hegemann 2016).

Contrary to findings from other observations of seasonality in immune function, I did not find any evidence for a tradeoff between WNV resistance and reproduction. Although well documented, most studies demonstrating diminished immune function during reproduction have utilized parameters that serve as proxies for immune function, but not directly relate to traits the influence transmission. My experimental design allowed me to assess this potential tradeoff using a trait central to WNV epidemiology. Rather than a tradeoff, HOSP were actually least competent during the breeding season. In the early spring, a growing photoperiod stimulates gonadal recrudescence and a reduction in immune function (Anderson

2007; Walton et al. 2011). Necropsies confirmed that reproductive tissues were indeed modulated as expected, though this cohort was among the most resistant at each sampling event and had the lowest overall IP. One potential reason for why I found such a result that seems to contradict a wealth of literature is that this work was performed in a sub-tropical environment with significantly less intra-annual climatic variation when compared to other efforts in more temperate locations where muted climatic seasonality might require less significant behavioral and physiological adaptions across seasons. In essence, the window of time in which favorable conditions exist for reproduction is longer in sub-tropical climates and rather than going 'all-in' to produce as many offspring as possible in a brief timeframe, reproductive effort could be more feasibly spread over a longer period of time with a larger number of small clutches (Martin et al. 2000; Mcnamara et al. 2008). Birds in tropical and sub-tropical environments experience less seasonality in resource availability and a greater risk of nest predation, making more, smaller clutches advantageous (Ricklefs 1980; Martin et al. 2006b). Indeed, songbirds in tropical and sub-tropical environments often spawn smaller clutches over a longer period of time when compared to their temperate counterparts (Baker 1995; Anderson 2007; Cox and Martin 2009). This suggestion is supported by a common garden experiment in which seasonal variation in immune responses for temperate HOSP were not apparent in neo-tropical conspecifics, despite being maintained under identical conditions (Martin et al. 2004).

In October, resistance to WNV was diminished during early infection; at 2 DPI, this group possessed elevated viral titers, and consequently breached the transmission threshold sooner, where 89% of this cohort possessed titers exceeding

10^5 PFU/mL, compared to 62% across all other groups. Throughout the remaining experimental period, October HOSP possessed WNV burdens similar to all other groups. Conversely, the elevated IP in the November cohort was driven by subtle differences in mortality. In November, the first death did not occur until 6 DPI, whereas mortality began at 4 DPI and 5 DPI for the other cohorts. As all birds that perished to WNV-infection in this experiment maintained infectious viremia until death, the date of death served as the endpoint for infectiousness. This period in which HOSP maintain infectiousness longer during the fall is potentially indicative of a tradeoff between molt and immune function. Molt is the annual renewal of feathers to improve flight and thermoregulatory capacity in anticipation of cooler temperatures during winter. As with reproduction, molt is energetically demanding and necessitates adjustments in physiology and foraging behavior (Pap et al. 2008; Ben-Hamo et al. 2017). In regard to life history tradeoffs, molt has not received as much attention as other life history processes, though an antagonistic relationship between molt and immunity has been documented (Martin 2005; Moreno-Rueda 2010); however, increasing evidence suggests that this tradeoff occurs in a delayed fashion, rather than instantaneously (Greenman et al. 2005; Martin et al. 2006a). Rather than an active redistribution of energy and reduction in immune efficacy during the performance of molt, the tradeoff has shown to occur in the weeks directly following molt as physiological and energetic costs accumulate. Comparisons of the inflammatory response in female HOSP across various distinct physiological states found a significant reduction in inflammation in the period of time directly following the completion of molt, compared to a much more modest decrease during reproduction and while actively molting (Martin et al. 2006a). In

my work, most HOSP captured in August were actively undergoing molt, while all sampled in October and November had new feathers. In support of the delayed-tradeoff hypothesis, WNV IP was elevated in post-molt HOSP, suggesting that the delayed tradeoff between molt and immunity is important to consider when regarding seasonal dynamics of avian zoonotic diseases.

I predicted that the balance between resistance and tolerance across seasons would change as energetic constraints select for elevated tolerance when other biological process demand resources. Here, I measured tolerance using two separate tolerance metrics: body mass and predator avoidance. A highly tolerant host would see little to no change in body mass or the ability to avoid predators when compared to others with similar viral burdens. Mass is perhaps the most often used metric of tolerance to represent body condition (Råberg et al. 2007; Adelman et al. 2015; Gervasi et al. 2017; Burgan et al. 2019), while predator avoidance was used to serve as a more ecologically and epidemiologically relevant indicator for host performance. I measured avoidance as the duration of time required for capture prior to each sampling event. Tolerant individuals maintained the ability to avoid capture using a combination of defensive behaviors, movement, and cognitive perception better than others. As a neuroinvasive pathogen, severe WNV is frequently associated with a loss of flight ability and locomotor coordination (Suthar et al. 2013). Thus, the ability to use skills that are disrupted by WNV infection is directly indicative of tolerance. Ecologically, I am interested in this trait as it facilitates valuable insights into HOSP ability to avoid being preyed upon by a predator, which is a less common, yet documented form of transmission among wild birds (Vidaña et al. 2020). The same skills used to avoid predators might also

important when defending against vectors, as flight ability and defensive behavior are important determinants of feeding success and blood meal size (Klowden and Lea 1979). Unsurprisingly, I found a reciprocal relationship between viral burden and capture time and mass – sicker birds lost more weight and were easier to catch. Contrary to my initial hypothesis, I did not find any evidence for seasonality in WNV tolerance. Although population heterogeneities in tolerance are important to consider when exploring infectious disease dynamics, my results suggest that tolerance does not vary over the course of the year.

My findings should motivate further efforts to describe how endogenous seasonal rhythms in zoonotic disease reservoirs influences spillover. As the theoretical basis for seasonal variation in immune function revolves around the notion that wild animals are balancing biological demands in response to environmental conditions and resource availability, it might be expected that the extent of seasonal variation in immune function varies across space (i.e., urban vs rural, temperate vs tropical, presence of predators). Performing similar experiments across distinct ecological contexts could yield further insights that are not evident by either just spatial or temporal efforts. For instance, urban environments feature several elements that can influence immunity and life history, including altered diets (Gavett and Wakeley 1986), increased oxidative stress (Herrera-Dueñas et al. 2017), and exposure to light pollution. Light pollution, in particular, represents a compelling area in which seasonality should be examined under, as exposure to artificial light at night is associated with desynchronized maintenance of reproductive tissues and initiation of the breeding season (Kempenaers et al. 2010; Dominoni et al. 2013; Robert et al. 2015; Russ et al. 2015), as well as diminished

immune responses to WNV (Kernbach et al. 2019, 2020). These efforts ought to also extend to other species known to host zoonotic pathogens. Observed seasonal patterns for many pathogens harbored by bats and rodents have been tightly linked to reproductive cycles (Pourrut et al. 2007; Plowright et al. 2008; Tersago et al. 2011; Hranac et al. 2019). Illustrating the seasonal dynamics of competence for these reservoir species could allow more effective targeting of surveillance and management efforts. For instance, the identification of both vector abundance and reservoir coalescing at similar times for WNV suggests that resource provisioning for wild birds and insecticide spraying would be most beneficial during this period of time, although more research is needed on how resource provisioning would influence competence and pathogen transmission (Becker and Hall 2014).

Although a central foundation of my experimental design was to thoroughly illustrate how wild HOSP vary in competence seasonally by limiting time in captivity prior to infection and maintaining natural light cycles when in captivity, awareness must be given to the fact that all studies occurring in laboratory environments should be interpreted with caution. Regardless, my study is among the first of its kind, demonstrating a relationship between endogenous biological seasonality and WNV reservoir competence. Further descriptions of how endogenous biological rhythms in reservoirs interact with population dynamics, environmental conditions, and vector ecology can improve our ability to predict spillover risk.

Works Cited

Adelman J.S., L. Kirkpatrick, J.L. Grodio, and D.M. Hawley. 2015. House Finch Populations Differ in Early Inflammatory Signaling and Pathogen Tolerance at the Peak of Mycoplasma gallisepticum Infection. https://doi.org/101086/670024 181:674–689.

Altizer S., A. Dobson, P. Hosseini, P. Hudson, M. Pascual, and P. Rohani. 2006. Seasonality and the dynamics of infectious diseases. Ecology Letters 9:467–484.

Anderson T. 2007. Biology of the Ubiquitous House Sparrow: From Genes to Populations. Biology of the Ubiquitous House Sparrow: From Genes to Populations. Oxford University Press.

Baker M. 1995. Environmental Component of Latitudinal Clutch-Size Variation in House Sparrows (Passer domesticus). The Auk 112:249–252.

Barron D.G., S.S. Gervasi, J.N. Pruitt, and L.B. Martin. 2015. Behavioral competence: How host behaviors can interact to influence parasite transmission risk. Current Opinion in Behavioral Sciences. Elsevier Ltd.

Becker D.J., C.J. Downs, and L.B. Martin. 2019. Multi-Scale Drivers of Immunological Variation and Consequences for Infectious Disease Dynamics. Pp. 1129–1137 in Integrative and Comparative Biology. Oxford University Press.

Becker D.J. and R.J. Hall. 2014. Too much of a good thing: resource provisioning alters infectious disease dynamics in wildlife. Biology Letters 10.

Ben-Hamo M., C.J. Downs, D.J. Burns, and B. Pinshow. 2017. House sparrows offset the physiological trade-off between immune response and feather growth by adjusting foraging behavior. Journal of Avian Biology 48:837–845.

Bull J.J. and A.S. Lauring. 2014. Theory and Empiricism in Virulence Evolution. PLOS Pathogens 10:e1004387.

Burgan S.C., S.S. Gervasi, L.R. Johnson, and L.B. Martin. 2019. How individual variation in host tolerance affects competence to transmit parasites. Physiological and Biochemical Zoology. University of Chicago Press.

Burgan S.C., S.S. Gervasi, and L.B. Martin. 2018. Parasite Tolerance and Host Competence in Avian Host Defense to West Nile Virus. EcoHealth 15:360–371.

Centers for Disease Control and Prevention. 2020. Final Cumulative Maps and Data for 1999-2019.

Childs M.L., N. Nova, J. Colvin, and E.A. Mordecai. 2019. Mosquito and primate ecology predict human risk of yellow fever virus spillover in Brazil. Philosophical Transactions of the Royal Society B: Biological Sciences 374.

Christe P., R. Arlettaz, and P. Vogel. 2000. Variation in intensity of a parasitic mite (Spinturnix myoti) in relation to the reproductive cycle and immunocompetence of its bat host (Myotis myotis). Ecology Letters 3:207–212.

Cornet S., C. Bichet, S. Larcombe, B. Faivre, and G. Sorci. 2014. Impact of host nutritional status on infection dynamics and parasite virulence in a bird-malaria system. Journal of Animal Ecology 83:256–265.

Cox W.A. and T.E. Martin. 2009. Breeding biology of the Three-striped warbler in Venezuela: A contrast between tropical and temperate parulids. Wilson Journal of Ornithology 121:667–678.

Dominoni D., M. Quetting, and J. Partecke. 2013. Artificial light at night advances avian reproductive physiology. Proceedings of the Royal Society B: Biological Sciences 280.

Dwyer G., J.S. Elkinton, and J.P. Buonaccorsi. 1997. HOST HETEROGENEITY IN SUSCEPTIBILITY AND DISEASE DYNAMICS: TESTS OF A MATHEMATICAL MODEL 150.

Eikenaar C. and A. Hegemann. 2016. Migratory common blackbirds have lower innate immune function during autumn migration than resident conspecifics. Biology Letters 12.

Florida Department of Health. 2021. Mosquito-Borne Disease Surveillance.

Ganeshan K., J. Nikkanen, K. Man, Y.A. Leong, Y. Sogawa, J.A. Maschek, T. van Ry, et al. 2019. Energetic Trade-Offs and Hypometabolic States Promote Disease Tolerance. Cell 177:399-413.e12.

Gavett A. and J. Wakely. 1986. Diets of House Sparrows in Urban and Rural Habitats. Wilson Ornithological Society 98:137–144.

George T.L., R.J. Harrigan, J.A. Lamanna, D.F. Desante, J.F. Saracco, and T.B. Smith. 2015. Persistent impacts of West Nile virus on North American bird populations. Proceedings of the National Academy of Sciences of the United States of America 112:14290–14294.

Gervasi S.S., S.C. Burgan, E. Hofmeister, T.R. Unnasch, and L.B. Martin. 2017. Stress hormones predict a host superspreader phenotype in the west nile virus system. Proceedings of the Royal Society B: Biological Sciences 284.

Gervasi S.S., D.J. Civitello, H.J. Kilvitis, and L.B. Martin. 2015. The context of host competence: A role for plasticity in host-parasite dynamics. Trends in Parasitology. Elsevier Ltd.

Greenman C.G., L.B. Martin, and M. Hau. 2005. Reproductive state, but not testosterone, reduces immune function in male house sparrows (Passer domesticus). Physiological and Biochemical Zoology 78:60–68.

Harrigan R.J., H.A. Thomassen, W. Buermann, and T.B. Smith. 2014. A continental risk assessment of West Nile virus under climate change. Global Change Biology 20:2417–2425.

Hartley D.M., C.M. Barker, A. le Menach, T. Niu, H.D. Gaff, and W.K. Reisen. 2012. Effects of temperature on emergence and seasonality of West Nile virus in California. American Journal of Tropical Medicine and Hygiene 86:884–894.

Herrera-Dueñas A., J. Pineda-Pampliega, M.T. Antonio-García, and J.I. Aguirre. 2017. The Influence of Urban Environments on Oxidative Stress Balance: A Case Study on the House Sparrow in the Iberian Peninsula. Frontiers in Ecology and Evolution 0:106.

Hranac C., J.C. Marshall, A. Monadjem, and D.T.S. Hayman. 2019. Predicting Ebola virus disease risk and the role of African bat birthing. Epidemics 29:100366.

Kempenaers B., P. Borgström, P. Loës, E. Schlicht, and M. Valcu. 2010. Artificial night lighting affects dawn song, extra-pair siring success, and lay date in songbirds. Current Biology 20:1735–1739.

Kernbach M.E., V.M. Cassone, T.R. Unnasch, and L.B. Martin. 2020. Broad-spectrum light pollution suppresses melatonin and increases West Nile virus-induced mortality in House Sparrows (Passer domesticus). Condor 122:1–13.

Kernbach M.E., D.J. Newhouse, J.M. Miller, R.J. Hall, J. Gibbons, J. Oberstaller, D. Selechnik, et al. 2019. Light pollution increases West Nile virus competence of a ubiquitous passerine reservoir species. Proceedings of the Royal Society B: Biological Sciences 286.

Kilpatrick A., P. Daszak, M.J. Jones, P.P. Marra, and L.D. Kramer. 2006a. Host heterogeneity dominates West Nile virus transmission. Proceedings of the Royal Society B: Biological Sciences 273:2327–2333.

Kilpatrick A.M., L.D. Kramer, M.J. Jones, P.P. Marra, and P. Daszak. 2006b. West Nile Virus Epidemics in North America Are Driven by Shifts in Mosquito Feeding Behavior. (R. Ostfeld, ed.)PLoS Biology 4:e82.

Klowden M.J. and A.O. Lea. 1979. Effect of defensive host behavior on the blood meal size and feeding success of natural populations of mosquitoes (Diptera: Culicidae). Journal of Medical Entomology 15:514–517.

Knutie S.A., C.L. Wilkinson, · Qiu, C. Wu, · C Nicole Ortega, and J.R. Rohr. 2017. Host resistance and tolerance of parasitic gut worms depend on resource availability. Oecologia 183:1031–1040.

LaDeau S.L., A.M. Kilpatrick, and P.P. Marra. 2007. West Nile virus emergence and large-scale declines of North American bird populations. Nature 2007 447:7145 447:710–713.

Lincoln G.A., I.J. Clarke, R.A. Hut, and D.G. Hazlerigg. 2006. Characterizing a Mammalian Circannual Pacemaker. Science 314:1941–1944.

Lochmiller R.L. and C. Deerenberg. 2000. Trade-offs in evolutionary immunology: Just what is the cost of immunity? Oikos 88:87–98.

Martin L.B. 2005. Trade-offs between molt and immune activity in two populations of house sparrows (Passer domesticus). Canadian Journal of Zoology 83:780–787.

Martin L.B., S.C. Burgan, J.S. Adelman, and S.S. Gervasi. 2016. Host competence: An organismal trait to integrate immunology and epidemiology. Pp. 1225–1237 in Integrative and Comparative Biology. Oxford University Press.

Martin L.B., P. Han, J. Kwong, and M. Hau. 2006a. Cutaneous immune activity varies with physiological state in female house sparrows (Passer domesticus). Physiological and Biochemical Zoology 79:775–783.

Martin L.B., M. Pless, J. Svoboda, and M. Wikelski. 2004. Immune activity in temperate and tropical House Sparrows: A common-garden experiment. Ecology 85:2323–2331.

Martin L.B., Z.M. Weil, and R.J. Nelson. 2008. Seasonal changes in vertebrate immune activity: Mediation by physiological trade-offs. Philosophical Transactions of the Royal Society B: Biological Sciences.

Martin T.E., R.D. Bassar, S.K. Bassar, J.J. Fontaine, P. Lloyd, H.A. Mathewson, A.M. Niklison, et al. 2006b. LIFE-HISTORY AND ECOLOGICAL CORRELATES OF GEOGRAPHIC VARIATION IN EGG AND CLUTCH MASS AMONG PASSERINE SPECIES. Evolution 60:390–398.

Martin T.E., P.R. Martin, C.R. Olson, B.J. Heidinger, and J.J. Fontaine. 2000. Parental Care and Clutch Sizes in North and South American Birds. Science 287:1482–1485.

Martinez M.E. 2018. The calendar of epidemics: Seasonal cycles of infectious diseases. PLoS Pathogens. Public Library of Science.

Martinez-Bakker M. and B. Helm. 2015. The influence of biological rhythms on host-parasite interactions. Trends in Ecology and Evolution. Elsevier Ltd.

McKenzie V.J. and N.E. Goulet. 2010. Bird community composition linked to human West Nile virus cases along the Colorado front range. EcoHealth 7:439–447.

Mcnamara J.M., Z. Barta, M. Wikelski, and A.I. Houston. 2008. A Theoretical Investigation of the Effect of Latitude on Avian Life Histories. Source: The American Naturalist 172:3.

Merrill T.E.S., S.R. Hall, and C.E. Cáceres. 2021. Parasite exposure and host susceptibility jointly drive the emergence of epidemics. Ecology 102:e03245.

Moreno-Rueda G. 2010. Experimental test of a trade-off between moult and immune response in house sparrows Passer domesticus. Journal of Evolutionary Biology 23:2229–2237.

Nelson R.J. 2004. Seasonal immune function and sickness responses. Trends in Immunology 25:187–192.

Norris K. and M.R. Evans. 2000. Ecological immunology: Life history trade-offs and immune defense in birds. Behavioral Ecology 11:19–26.

Pap P.L., C.I. Vágási, G.Á. Czirják, and Z. Barta. 2008. Diet quality affects postnuptial molting and feather quality of the house sparrow (Passer domesticus): Interaction with humoral immune function? Canadian Journal of Zoology 86:834–842.

Plowright R.K., H.E. Field, C. Smith, A. Divljan, C. Palmer, G. Tabor, P. Daszak, et al. 2008. Reproduction and nutritional stress are risk factors for Hendra virus infection in little red flying foxes (Pteropus scapulatus). Proceedings of the Royal Society B: Biological Sciences 275:861–869.

Pourrut X., A. Délicat, P.E. Rollin, T.G. Ksiazek, J. -P. Gonzalez, and E.M. Leroy. 2007. Spatial and Temporal Patterns of *Zaire ebolavirus* Antibody Prevalence in the Possible Reservoir Bat Species. The Journal of Infectious Diseases 196:S176–S183.

Prendergast B.J., A.K. Hotchkiss, S.D. Bilbo, S.G. Kinsey, and R.J. Nelson. 2003. Photoperiodic Adjustments in Immune Function Protect Siberian Hamsters from Lethal Endotoxemia.

Råberg L., A.L. Graham, and A.F. Read. 2009. Decomposing health: Tolerance and resistance to parasites in animals. Philosophical Transactions of the Royal Society B: Biological Sciences 364:37–49.

Råberg L., D. Sim, and A.F. Read. 2007. Disentangling Genetic Variation for Resistance and Tolerance to Infectious Diseases in Animals. Science 318:812–814.

Reisen W.K., R.M. Takahashi, B.D. Carroll, and R. Quiring. 2008. Delinquent mortgages, neglected swimming pools, and West Nile virus, California. Emerging Infectious Diseases 14:1747–1749.

Ricklefs R.E. 1980. Geographical Variation in Clutch Size among Passerine Birds: Ashmole's Hypothesis. The Auk 97:38–49.

Robert K.A., J.A. Lesku, J. Partecke, and B. Chambers. 2015. Artificial light at night desynchronizes strictly seasonal reproduction in a wild mammal. Proceedings of the Royal Society B: Biological Sciences 282.

Russ A., S. Reitemeier, A. Weissmann, J. Gottschalk, A. Einspanier, and R. Klenke. 2015. Seasonal and urban effects on the endocrinology of a wild passerine. Ecology and Evolution 5:5698–5710.

Schmid-Hempel P. 2003. Variation in immune defence as a question of evolutionary ecology. Proceedings of the Royal Society of London Series B: Biological Sciences 270:357–366.

Semenza J.C., A. Tran, L. Espinosa, B. Sudre, D. Domanovic, and S. Paz. 2016. Climate change projections of West Nile virus infections in Europe: implications for blood safety practices. Environmental Health 2016 15:1 15:125–136.

Sheldon B.C. and S. Verhulst. 1996. Ecological immunology: Costly parasite defences and trade-offs in evolutionary ecology. Trends in Ecology and Evolution. Trends Ecol Evol.

Soverow J.E., G.A. Wellenius, D.N. Fisman, and M.A. Mittleman. 2009. Infectious Disease in a Warming World: How Weather Influenced West Nile Virus in the United States (2001–2005). Environmental Health Perspectives 117:1049.

Stevenson T.J., M.E. Visser, W. Arnold, P. Barrett, S. Biello, A. Dawson, D.L. Denlinger, et al. 2015. Disrupted seasonal biology impacts health, food security and ecosystems. Proceedings of the Royal Society B: Biological Sciences 282.

Suthar M.S., M.S. Diamond, and M. Gale. 2013. West Nile virus infection and immunity. Nature Reviews Microbiology. Nat Rev Microbiol.

Tersago K., R. Verhagen, O. Vapalahti, P. Heyman, G. Ducoffre, and H. Leirs. 2011. Hantavirus outbreak in Western Europe: Reservoir host infection dynamics related to human disease patterns. Epidemiology and Infection 139:381–390.

VanderWaal K.L. and V.O. Ezenwa. 2016. Heterogeneity in pathogen transmission: mechanisms and methodology. (D. Hawley, ed.)Functional Ecology 30:1606–1622.

Vidaña B., N. Busquets, S. Napp, E. Pérez-Ramírez, M.Á. Jiménez-Clavero, and N. Johnson. 2020. The role of birds of prey in west nile virus epidemiology. Vaccines. MDPI AG.

Walton J.C., Z.M. Weil, and R.J. Nelson. 2011. Influence of photoperiod on hormones, behavior, and immune function. Frontiers in Neuroendocrinology 32:303–319.