# SEX-BIASED INFECTIONS SCALE TO POPULATION IMPACTS FOR AN EMERGING WILDLIFE DISEASE

Macy J. Kailing[1], Joseph R. Hoyt[1], J. Paul White[2], Heather M. Kaarakka[2], Jennifer A. Redell[2], Ariel E. Leon[3], Tonie E. Rocke[3], John E. DePue[4], William H. Scullon[5], Katy L. Parise[6], Jeffrey T. Foster[6], A. Marm Kilpatrick[7], and Kate E. Langwig[1]

1 Department of Biological Sciences, Virginia Polytechnic Institute, Blacksburg, VA, USA
2 Wisconsin Department of Natural Resources, Madison, WI, USA
3 U.S. Geological Survey, National Wildlife Health Center, Madison, WI, USA
4 Michigan Department of Natural Resources, Baraga, MI, USA
5 Michigan Department of Natural Resources, Norway, MI, USA
6 Pathogen and Microbiome Institute, Northern Arizona University, Flagstaff, AZ, USA
7 Department of Ecology and Evolutionary Biology, University of California, Santa Cruz, CA, USA

* Corresponding author: Macy Kailing, macykailing@gmail.com

Keywords: Sex biased infection, emerging infectious disease, white-nose syndrome, bats, Allee effects, fungal disease

**ABSTRACT**:
Demographic factors are fundamental in shaping infectious disease dynamics. Aspects of populations that create structure, like age and sex, can affect patterns of transmission, infection intensity and population outcomes. However, studies rarely link these processes from individual to population-scale effects. Moreover, the mechanisms underlying demographic differences in disease are frequently unclear. Here, we explore sex-biased infections for a multi-host fungal disease of bats, white-nose syndrome, and link disease-associated mortality between sexes, the distortion of sex ratios, and the potential mechanisms underlying sex differences in infection. We collected data on host traits, infection intensity, and survival of five bat species at 42 sites across seven years. We found females were more infected than males for all five species. Females also had lower apparent survival over winter and accounted for a smaller proportion of populations over time. Notably, female-biased infections were evident by early hibernation and likely driven by sex-based differences in autumn mating behavior. Male bats were more active during autumn, which likely reduced replication of the cool-growing fungus. Higher disease impacts in female bats may have cascading effects on bat populations beyond the hibernation season by limiting recruitment and increasing the risk of Allee effects.

## 1. INTRODUCTION

Emerging infectious diseases are a serious threat to wildlife health (1, 2). Population structure can shape epidemics by influencing spatial spread, outbreak size, and host impacts (2-4). Elements of populations that create structure such as classes of individuals of specific ages, sexes, or breeding stages, can have profound effects on disease dynamics (3, 5-9). Sex is an especially important factor because sex-biases in infections can contribute to differential transmission through populations due to behavior (10-15), amplify outbreaks due to seasonal changes in susceptibility (16-19), and modify population impacts through disproportionate mortality (20-22). Differences in infection and mortality can also modulate virulence evolution through sex-specific immune responses that affect pathogen replication and growth (23, 24). As such, determining patterns and mechanisms of sex-biases in infections can improve efforts to minimize outbreaks and manage impacts.

Behavioral and physiological traits are two mechanisms that can produce sex-biases in transmission, susceptibility, infection intensity, or disease-induced mortality (25-27). In most systems, males have an elevated risk of disease compared to females due to territory defense and

63 promiscuous mating, which increases contacts with conspecifics or increase pathogen
64 susceptibility due to physiological stress (9, 25, 28). Sex hormones can also have strong effects
65 on host immunity (29), such that testosterone suppresses immune responses whereas estrogens
66 enhance it, resulting in weaker immune responses in males, which can increase susceptibility to
67 pathogen infection (25, 30, 31). As such, the majority of empirical studies find that infections are
68 typically male-biased (28, 32, 33), although this generalization is sometimes reversed (34-40)
69 and may be linked to seasonal reproductive stress associated with pregnancy, parturition, or
70 parental care (16, 17). Thus, it is likely that the effects of sex-biased infections will be highly
71 pronounced in disease systems where host reproductive strategies differ seasonally among sexes
72 (19).
73 White-nose syndrome (WNS) is a highly seasonal fungal disease of bats that has caused
74 population collapse, with declines exceeding 95% in many populations of multiple species (41-
75 44). WNS is caused by the fungal pathogen, *Pseudogymnoascus destructans,* which invades the
76 epidermal tissue of bats during hibernation, and disrupts bat homeostasis, causing water and
77 electrolyte imbalances that increase arousals and deplete stored fat (45-52). Transmission of *P.*
78 *destructans* occurs through host-to-host contacts and contact between hosts and contaminated
79 environmental reservoirs inside winter hibernation sites (53-55). *Pseudogymnoascus destructans*
80 has a thermal growth range of 0-20ºC and optimal growth between 12 and 16 ºC (56). This limits
81 on-host growth to the periods when bats are in torpor and they lower their body temperature to
82 the ambient temperatures of their hibernation sites (57, 58). The restricted growth of *P.*
83 *destructans* below 20ºC drives the seasonal patterns of WNS, with infection only occurring
84 during winter hibernation and mortality peaking during late winter (59).
85 Temperate bat species have sex-based seasonally varying behaviors that may influence their
86 exposure, susceptibility, and mortality from WNS (60-63). Bats mate in autumn swarms, and
87 females store sperm over winter, delaying ovulation, and giving birth at maternity colonies in
88 spring (64). Male reproductive energy expenditures are highest during autumn when they
89 aggregate at hibernacula (subterranean sites where bats spend the winter) and mate
90 indiscriminately with females (65). Since autumn swarm coincides with the seasonal
91 transmission of *P. destructans* (59), breeding stress among males as well as exposure to the
92 environmental reservoirs could increase their susceptibility to infection. Female energy
93 expenditures are greatest during pregnancy and lactation in spring and summer when hosts

typically clear infection (66, 67) and transmission of *P. destructans* is low (59), suggesting females may be the less infected sex. However, female bats may spend more time torpid during winter than males to conserve energy for spring reproduction (62) which could influence the growth of *P. destructans*. Due to the differences in reproductive investment and seasonal energy expenditure, sex-based traits have the potential to affect exposure (e.g., contacting *P. destructans* from other hosts or in the environment) and pathogen growth (e.g., time spent at torpid temperatures that permit fungal growth). Thus, we predict sex-specific behaviors, and the highly seasonal pattern of WNS may drive intersex differences in infection, mortality, and population impacts. Here, we examined differences in *P. destructans* infection between females and males across five bat species in 42 sites across seven years. We then assessed the consequences of intersex differences in infection at both the individual and population-level. Lastly, we explored autumn activity patterns between sexes as a potential mechanism of sex-biased infections.

## 2. MATERIALS AND METHODS

### a. Study sites and sampling design

We sampled bats at hibernacula in Illinois, Massachusetts, Michigan, New York, Vermont, Virginia, and Wisconsin between 2011 and 2021 during an annual outbreak at two time points: early winter (November to December) and late winter (March to April) (Supplemental Table 1). During site visits, we walked each section of the hibernacula and counted all the bats of each species and resighted any bats that were previously banded. We sampled up to 20 bats of each species during each visit when possible and stratified sample collection throughout hibernacula to obtain a sample reflective of the spatial distribution of bats at each site. Bat species included little brown (*Myotis lucifugus*), northern long-eared (*Myotis septentrionalis*), tricolored (*Perimyotis subflavus*), big brown (*Eptesicus fuscus*), and Indiana (*Myotis sodalis*) bats. The animal sampling protocols were approved by the Virginia Tech Institutional Animal Care and Use Committee protocol 17-180. We followed the field decontamination procedures outlined by the U.S. Fish and Wildlife Service Decontamination Guidelines as well as the recommendations provided by individual states (68, 69).

### b. *P. destructans* sample collection and quantification

For every bat we sampled, we determined species and biological sex based on external morphology and attached an aluminum-lipped band (2.4, 2.9, and 4.2mm; Porzana Ltd.,

125 Icklesham, E. Sussex, U.K.) to the wing for individual identification and resighting. We
126 collected an epidermal swab from individuals to quantify infection prevalence (the fraction of
127 individuals positive for *P. destructans*) and severity (the quantity of *P. destructans* on infected
128 bats) using a standardized swabbing technique (44, 59). We placed swabs in RNAlater® for
129 storage before testing. We extracted DNA and tested for *P. destructans* presence and quantity by
130 quantitative PCR (59, 70).

131 **c. Activity data collection**

132 At three sites in Wisconsin in 2020, we installed radio frequency identification (RFID)
133 systems, consisting of a passive antenna and an automated data logger (IS10001; Biomark,
134 Boise, ID) at the entrances of each hibernaculum (2-3 per site). The systems at each entrance
135 were equipped with a solar or direct power source to run continuously. During autumn swarm,
136 we captured little brown bats swarming near hibernacula and injected 12.5-mm passive
137 integrative transponder (PIT) tags (Biomark APT12; Biomark, Boise, ID). Each RFID system
138 was programmed to record each time a unique individual passed through an entrance with a one-
139 minute delay to avoid duplicate detections of tags. We scored a detection as an active bat and
140 used it to characterize activity of females and males.

141 **d. Statistical analyses**

142 ***i. Infection***

143 We used generalized (GLMM) and linear (LMM) mixed effect models (71) to compare
144 differences in *P. destructans* prevalence and infection severity between males and females of
145 each of the five species. We defined prevalence as the fraction of bats testing positive for *P.*
146 *destructans* on qPCR (0|1), and infection severity as the log-transformed quantity of fungal DNA
147 (ng) on positive bats ($\log_{10}$ fungal loads). We used site as a random effect for all infection
148 analyses. We estimated differences in prevalence between sexes using a GLMM with a binomial
149 distribution and sex and species as fixed effects and compared models of additive and interactive
150 versions of these terms. For prevalence, we used data from early hibernation only as every bat
151 was infected at the end of hibernation. To estimate changes in fungal loads between sexes over
152 winter, we used a LMM with species, sex, and date as fixed effects. We also compared changes
153 in over winter fungal loads (the difference between late hibernation and early hibernation loads)
154 using a subset of recaptured individuals that were sampled in early and late hibernation of the
155 same year.

156 ***ii. Individual survival***

157 We examined differences between sexes in individual over winter survival using data from a
158 single species, little brown bats, which were abundant enough to obtain reasonable sample sizes.
159 We compared the probability of observing bats in late hibernation (March) that we had observed
160 in early hibernation (November). Bats affected by WNS often emerge prior to spring and die on
161 the landscape (72), enabling the use of recapture as a proxy for individual survival (73, 74). We
162 used a mixed effects model with site as a random effect with a binomial distribution and a logit
163 link to quantify how sex affected the probability of a bat being resighted over winter.

164 ***iii. Population-level impacts***

165 We estimated the proportion of female little brown bats sampled at each visit from a core set
166 of sites (N=14) that were sampled in multiple consecutive years to evaluate how female
167 proportions were changing over time. We estimated the probability of sampling a female (vs a
168 male) over time using generalized linear mixed model with a binomial distribution and a logit
169 link with years since *P. destructans* invasion as a fixed effect, and site as a random effect.

170 ***iv. RFID activity***

171 To explore differences in autumn activity of male and female bats, we used a generalized
172 linear mixed model with a binomial distribution and a logit link with sex as a fixed effect and
173 individual bat identification as a random effect. For our response variable, we treated each bat as
174 a series of binomial trials where it could be detected (=1) or not detected (=0) on a given night
175 throughout autumn swarm that any RFID system was operational at an entrance, as determined
176 by the detection of a programmed test tag. We determined the period of autumn swarm to be
177 between the dates in which more than 95% of all individual detections occurred (beginning of
178 swarm; August 19) and all individual detections concluded (end of swarm; October 01). Results
179 were qualitatively similar if we included all dates in which any bat was detected from early
180 August to November. Because bats are nocturnal and daily activity patterns span two calendar
181 days, we treated detections that occurred between hours 0:00 and 07:00 as a detection during the
182 previous night for consistency with bat ecology.

183

184 **3. RESULTS**

185 We examined the effects of sex on *P. destructans* infections of five bat species during winter.
186 We sampled a total of 665 females and 1071 males across all species, sites, years, and seasons

(Supplemental Table 1). On average, females had higher *P. destructans* prevalence than male bats in early winter (GLMM with site and species as random effects; female coef +/- SE: 0.630 +/- 0.221, P = 0.0043; Figure 1; Appendix 1.1) and suffered from higher fungal loads overall (LMM with site and species as random effects; female coef +/- SE: 0.300 +/- 0.072, P < 0.0001; Figure 2; Appendix 2.1). The best supported model (ΔAIC = -3.11) for predicting early winter *P. destructans* prevalence included species with the additive effect of sex, indicating female infections are generally higher across all bat species in our study (GLMM with site as random effect; female coef +/- SE: 0.637 +/- 0.223, P = 0.0043; Figure 1; Appendix 1.2).

The strong differences in infection prevalence during early winter paralleled higher fungal loads on female bats during the hibernation season. Across species, females began hibernation with higher fungal loads than males (LMM with site as random effect; female coef +/- SE: 0.490 +/- 0.093, P < 0.0001; Figure 2; Appendix 2.2). Fungal loads appeared to become more similar by the end of winter (Figure 2); however, this is likely a result of a mortality bias (Figure 3; Appendix 3.1) such that individuals with higher fungal loads are less likely to survive to late winter (Supplemental Figure 1A).

We did not detect a clear effect of early hibernation temperatures or body mass that explained the sex differences in prevalence, infection intensity, or mortality consistently across the species for which we had sufficient data to analyze (Supplemental Figure 2). Data from individual little brown bats sampled in both early and late winter indicated that the change in fungal loads over winter did not differ between sexes (LMM with site as a random effect; female coef +/- SE: -0.141 +/- 0.263, P = 0.593; Supplemental Figure 1B; Appendix 5.2).

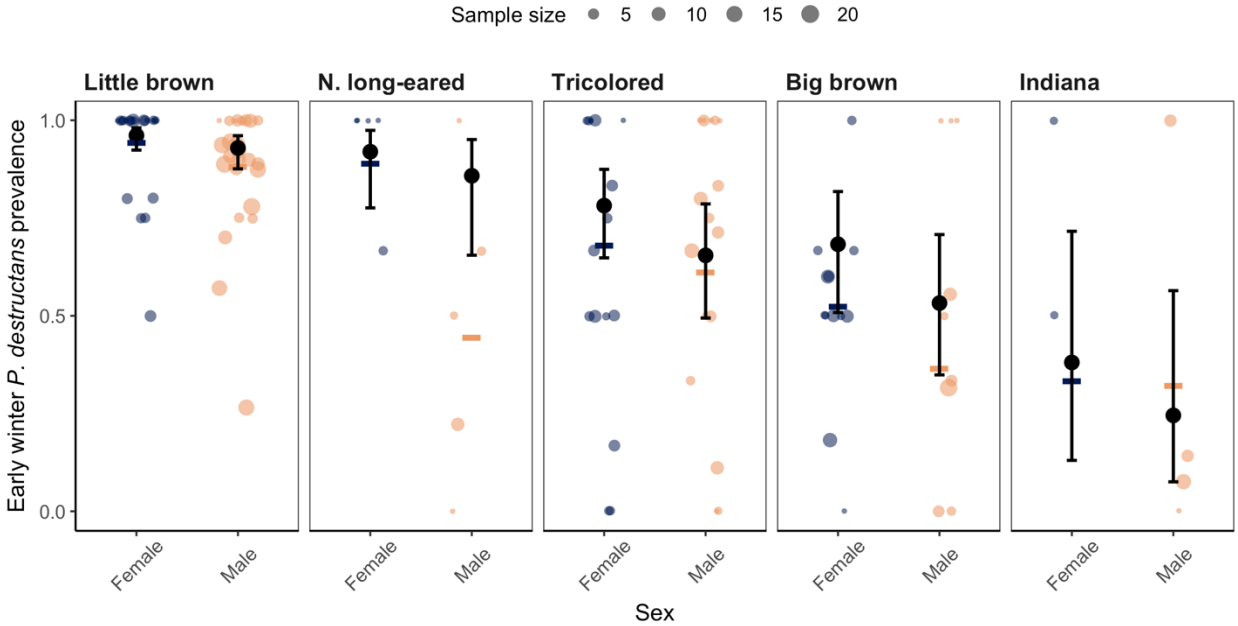


**Figure 1.** Early winter *Pseudogymnoascus destructans* prevalence by sex for five bat species. The colored points show the proportion of individuals that were infected at a given site and year. The size of colored points is scaled to the number of bats sampled during the site visit. The black circles show model-predicted prevalence with 95% confidence intervals denoted with error bars. Horizontal blue and orange lines show the grand mean prevalence for all females and males, respectively.

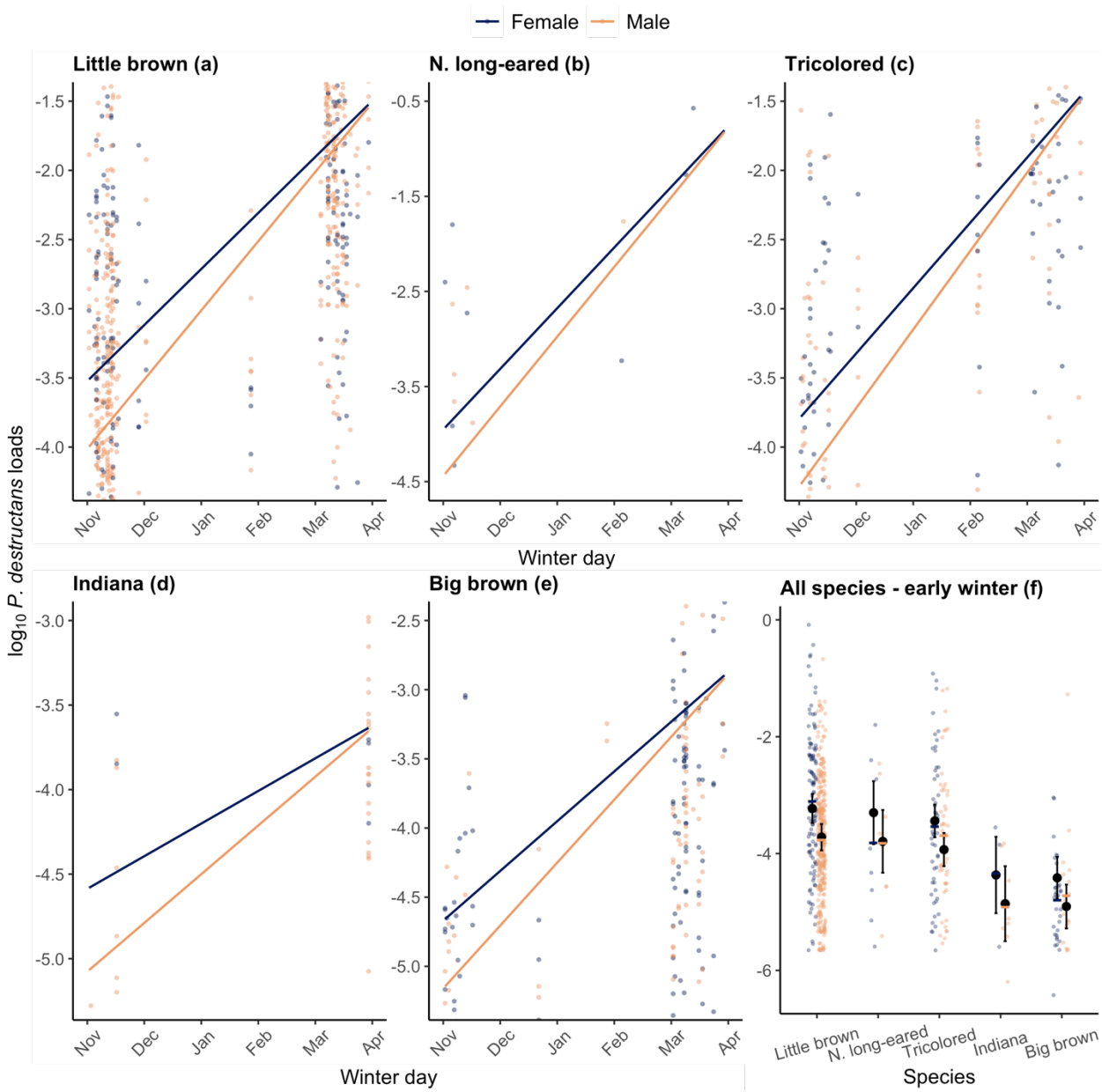


**Figure 2. (a-e)** Change in *Pseudogymnoascus destructans* infection severity (fungal loads) throughout winter by sex for each of the five host species. A point represents an individual bat, and females and males are shown in blue and orange, respectively. Lines represent model predicted fungal loads over winter. **(f)** Fungal loads in early winter by sex for each host species. Black points show model predicted fungal loads. Error bars show 95% confidence intervals. Blue and orange horizontal lines show grand mean fungal loads for all females and males, respectively.

At an individual-scale, using data from little brown bats that were banded in early hibernation (Supplemental Table 2), we found clear support that female little brown bats were less likely to be recaptured in late winter than males (GLMM with site as random effect; female coef +/- SE: -0.855 +/- 0.380, P = 0.0240; Figure 3A; Appendix 3.1). At a population-level, the proportion of females sampled in the same populations at the end of winter (Supplemental Table 3) decreased with time since WNS invasion (GLMM with site as random effect; years since invasion coef +/- SE: -0.363 +/- 0.173, P = 0.0365; Figure 3B; Appendix 3.2). Collectively, these data indicate that females have higher, more severe infections, and correspondingly experience higher mortality during winter from WNS.

Females begin hibernation with more severe infections than male bats and differential activity between sexes may influence infections. We found that the probability of a female being active on a given night was five-fold lower than males during autumn. (GLMM with individual bat as random effect; female coef +/- SE: -1.643 +/- 0.182, P < 0.0001; Figure 4; Supplemental Table 4; Appendix 4.1).

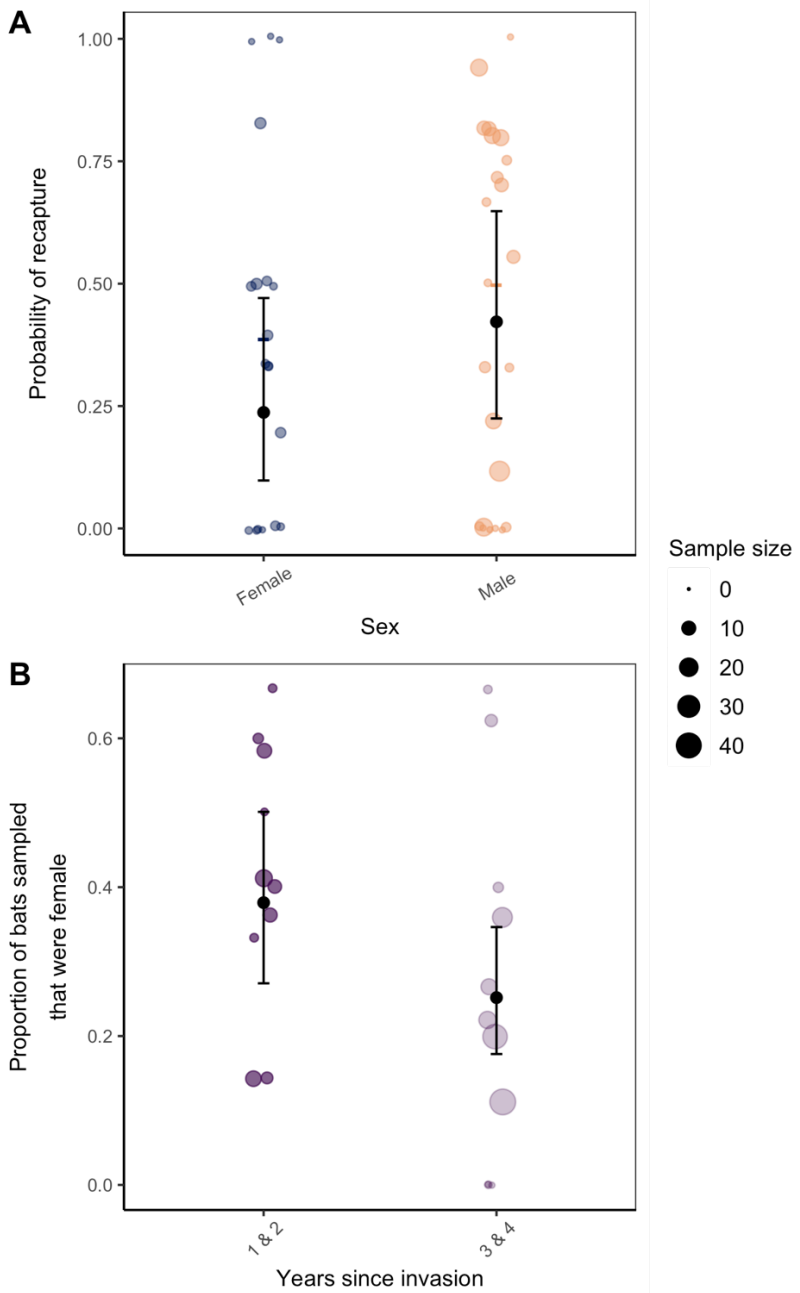


**Figure 3**. **(A)** Recapture probability of little brown bats (*Myotis lucifugus*) by sex. Blue and orange points show the mean probability of recapture from early (November) and late (March) winter from a given site and year for females and males, respectively. Colored horizontal bars show the grand mean recapture probabilities for each sex across all sites. **(B)** Proportional change in females with time since *Pseudogymnoascus destructans* invasion. Points show the proportion of sampled individuals that were female at the same sites sampled over time where *P. destructans* had been present for 1 & 2 years compared to years 3 & 4. Black points in both

panels show the model predicted means and error bars show 95% confidence intervals. The size of points in both panels is scaled to the number of bats sampled during the site visit.

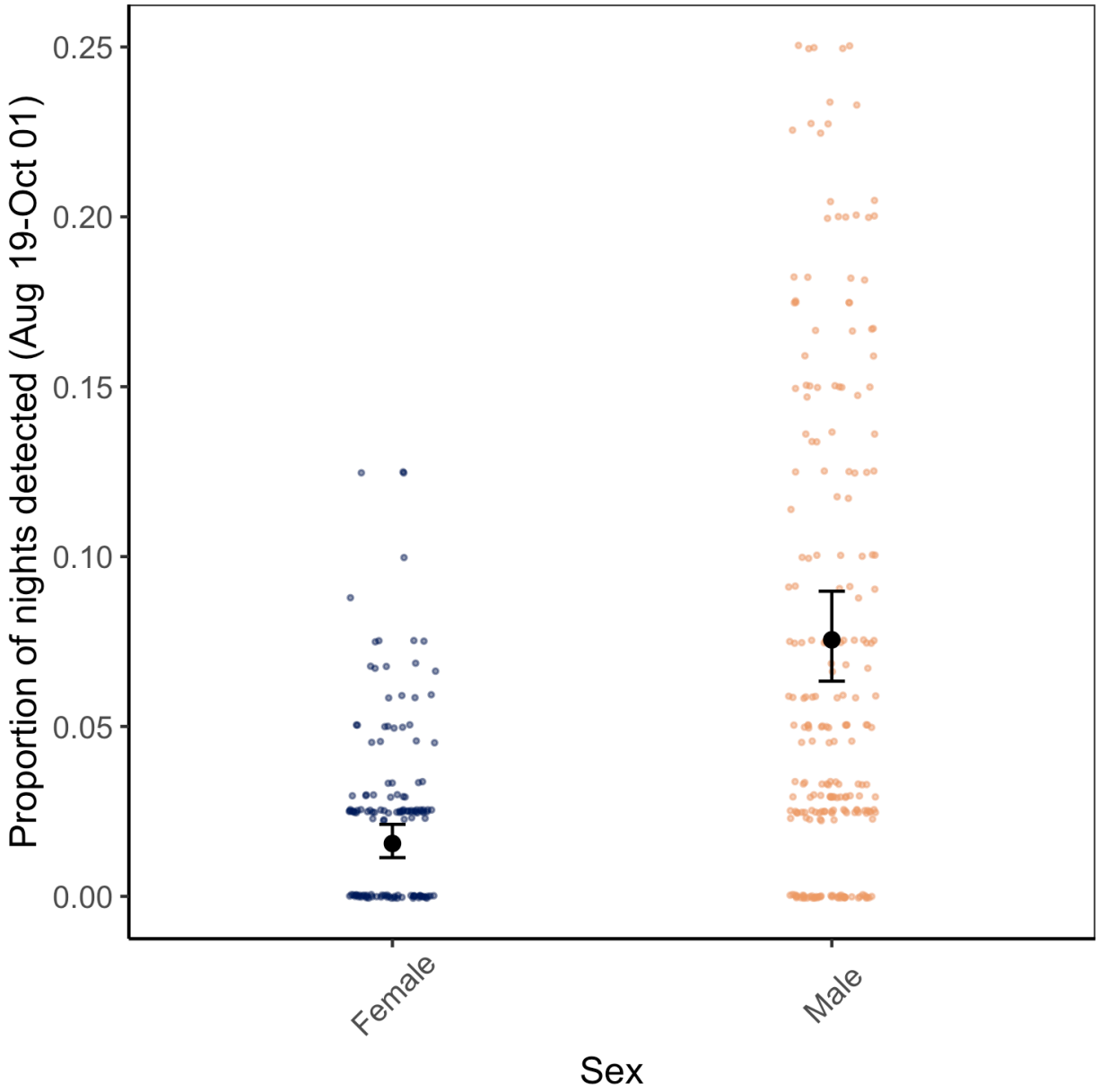


**Figure 4.** Differences in autumn activity of female and male little brown bats (*M. lucifugus*) at three hibernacula. Each data point represents the proportion of nights a bat was active at a site. The proportion of nights active was calculated as the number of nights an individual was detected at least once on the RFID systems divided by the total number of nights the RFID systems at each site were operating throughout autumn swarm. Points at 0 represent tagged bats

254 that were never detected on a reader. Black points show model predicted activity by sex. Error
255 bars show 95% upper and lower confidence intervals.
256
257 **4. DISCUSSION**
258 Our results demonstrate that intersex differences in infection are an important driver of
259 disease dynamics. Across all species, females experienced higher infection prevalence and
260 fungal loads by the beginning of winter hibernation. In little brown bats, females were also less
261 likely to be recaptured over winter, indicating that more severe infections result in higher
262 mortality among females. Finally, female-biased mortality has likely resulted in a population-
263 level reduction in females over time. Higher impacts to female bats may mask even more severe
264 long-term impacts from WNS (75, 76) as a disproportionate loss of recruiting females will likely
265 have more severe population-level consequences than male-biased declines.
266 We consistently observed higher pathogen prevalence and intensity in females than males
267 across bat species. In other vertebrates, males maximize fitness at the cost of greater exposure
268 and susceptibility to pathogens (33, 77-79), in part through higher testosterone concentrations
269 which correlate negatively with immune defense (80-82). Given male bats undergo their most
270 substantial investment in reproduction during autumn swarm compared to females that invest
271 relatively little towards reproduction throughout autumn and over winter (64), we expected
272 infection to be higher in males. However, we found the opposite pattern with females having
273 more severe disease. Drivers of higher female infections likely include sex-based physiological
274 differences (25). Temperate female bats use fat stores acquired in autumn more slowly than
275 males suggesting a more substantial constraint on energy expenditure compared to males
276 throughout the autumn to spring period (62, 63). Therefore, different physiological strategies
277 (e.g., torpor patterns) between female and male bats could be shaping sex-biased infections.
278 Differences in sex-based physiology and behavior were supported by our data showing that
279 the proportion of nights males were active during autumn was substantially higher than females.
280 Differences in autumn activity patterns are likely motivated by differences in reproductive
281 energy allocation between sexes. For males, their fitness is enhanced by remaining active so they
282 can mate with as many females as possible. However, female bats, which store sperm and delay
283 ovulation until spring, may prefer to conserve energetic resources during fall and use torpor more
284 extensively (63). As bats arrive to contaminated hibernacula for autumn swarm and are exposed

285 to the pathogen, torpor use in autumn after exposure may permit pathogen growth when hosts
286 reduce their body temperatures to be within the thermal range of *P. destructans* growth. The
287 relationship between sex-based activity and fungal intensity may arise through two potential
288 pathways associated with torpor expression. First, less active females may provide favorable
289 conditions for pathogen growth for a longer period prior to hibernation compared to males, thus
290 resulting in the more severe female infections. Second, more active males may be able to inhibit
291 pathogen growth through euthermia compared to females, as euthermic mammals mount more
292 robust immune defenses than torpid or hibernating mammals (83, 84). Our activity estimates,
293 which are consistent with other studies (61, 85), suggest that vast differences between male and
294 female bats likely contribute to differences in infection.

295 We were less likely to recapture female little brown bats over winter than males, indicating
296 greater mortality in females affected by WNS. Generally, infections increase on bats overwinter
297 before reaching a threshold at which bats die from their infections (42). Female bats begin
298 hibernation with higher fungal loads than males but have similar fungal growth patterns over
299 winter (Supplemental Figure 1), indicating that females begin dying in mid-winter as they reach
300 high infection levels. Ultimately, the death of females with the highest infections early in winter
301 results in similar infections between males and the remaining females at the end of hibernation
302 (Figure 2). Our report of higher mortality in females with higher fungal loads is supported by
303 previous work demonstrating that WNS severity is positively correlated with mortality, and
304 mechanistically linked by more frequent arousals (42, 45, 51, 73). Several lines of evidence also
305 suggest that females did not leave hibernation sites early and survive elsewhere. In our study
306 region, emergence from hibernation in healthy populations does not begin until four to six weeks
307 after our sampling (86). Given that the proportion of females showed consistent declines each
308 year following WNS invasion, our results indicate that reductions in overwinter recaptures of
309 females are due to WNS mortality. Previous studies on intersex differences in survival associated
310 with WNS have found contrasting results. One study observed lower female survival in naturally
311 infected bats (87) whereas another reported higher female survival in experimental infections
312 where each sex was inoculated with the same dose of *P. destructans* at identical times (88). Our
313 results suggest that differences in disease outcomes are due to differences in pathogen growth
314 during autumn. Therefore, inoculating both sexes with the same dose simultaneously in the

experimental infection may have eliminated the underlying difference in early winter infections between female and males.

We provide the first evidence that sex-based survival of bats affected by WNS likely scales to population-level impacts. In the first year after *P. destructans* invasion, the mean proportion of females in late hibernation was 45%, but this percentage declined to 21% after four years of WNS. The reduction in the percent of females with time since pathogen invasion differs from previous studies of healthy bat populations that show relatively consistent interannual proportions of females (89-92). This suggests that that a comparable decline in females would not be typical in non-diseased populations. Losing females at higher rates than males will affect the ability of bat populations to respond to WNS. First, a reduction in the number of females will limit the recruitment potential of bat populations and is likely to be especially critical for imperiled populations that remain small and vulnerable to Allee effects. Many temperate bat species, including little brown bats, form maternity colonies during summer when they cooperatively rear offspring  and larger colony sizes afford females lower energy expenditure (93). If birth and offspring survival rates decline with density, as shown in other temperate bat species (94), the disproportionate loss of females could further impact recruitment. Second, distorted sex-ratios may negatively affect reproductive success during autumn. In other taxa, a significant increase in male to female sex-ratios resulted in increased stress to females from exacerbated pressure for males to mate with limited females, negatively affecting female fecundity (95, 96).

Our results include impacts to female bats up to four years after the arrival of WNS, which includes the epidemic phase of the disease, when the majority of mortality occurs (75). Females are likely under strong selection pressure to evolve mechanisms of survival given their increased mortality and will need to adapt in for populations to rebound. Future work focusing on the effects of female infection and mortality biases on bat population persistence and recovery could benefit conservation efforts, especially as the negative effects are likely to compound over time as sex ratios become increasingly distorted.

We find a novel example in which female-biased infections are shaping population-level impacts with an emerging disease. Our results provide a clear instance of an emerging pathogen that consistently affects females more than males regardless of host species. We describe a new mechanistic explanation to female-biased infections that links temperature-dependent fungal

growth to sex-specific seasonal physiology. Ultimately, disparate infections among demographic classes of hosts are fundamental for understanding and managing emerging infectious diseases, and cross-scale analyses can provide insights into the important consequences of demographic biases on disease systems.

**ACKNOWLEDGEMENTS**

We thank Steffany Yamada for data curation support, Skylar Hopkins for analysis support, Rick Reynolds and Carl Herzog for logistical support, and the many landowners for site access.

**FUNDING**

The research was funded by National Science Foundation grants DEB-1115895 to A.M.K. & J.T.F., DEB-1911853 to K.E.L., J.R.H., A.M.K. & J.T.F., the USFWS (F17AP00591) to K.E.L., and by Virginia Tech Institute for Critical Technology and Applied Science to M.J.K.

**DISCLAIMER**

Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

**AUTHOR CONTRIBUTIONS**

M.J.K.: conceptualization, investigation, methodology, visualization, data curation, writing-original draft, writing-review and editing, formal analysis; J.R.H.: conceptualization, investigation, methodology, visualization, funding acquisition, resources, project administration, data curation, writing-review and editing; J.P.W.: investigation, writing-review and editing; H.M.K.: investigation, writing-review and editing; J.A.R.: investigation; A.E.L.: resources, data curation, writing-review and editing; T.E.R.: resources, writing-review and editing; J.E.D.: investigation, writing-review and editing; W.H.S.: investigation; K.L.P.: investigation, writing-review and editing; J.T.F.: investigation, funding acquisition, writing-review and editing; A.M.K.: investigation, funding acquisition, writing-review and editing; K.E.L.: conceptualization, investigation, visualization, methodology, funding acquisition, resources, project administration, data curation, writing-original draft, writing-review and editing, supervision

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

## DATA, CODE, AND MATERIALS

The datasets and code generated in this study will be deposited in Dryad Digital Repository upon final submission. Exact site locations are not disclosed to protect endangered species and landowners.

## CONFLICT OF INTEREST DECLARATION

The authors declare no competing interests.

## ETHICS

All bat handling procedures were reviewed and approved by Virginia Tech Institute for Animal Care and Use Committee protocol 17-180.

## LITERATURE CITED


1. Daszak P, Cunningham AA, Hyatt AD. Emerging infectious diseases of wildlife - Threats to biodiversity and human health. Science. 2000;287(5452):443-9.
2. De Castro F, Bolker B. Mechanisms of disease-induced extinction. Ecology Letters. 2004;8(1):117-26.
3. Silk MJ, Hodgson DJ, Rozins C, Croft DP, Delahay RJ, Boots M, McDonald RA. Integrating social behaviour, demography and disease dynamics in network models: Applications to disease management in declining wildlife populations. Philosophical Transactions of the Royal Society B: Biological Sciences. 2019;374(1781).
4. Lloyd-Smith JO, Schreiber SJ, Kopp PE, Getz WM. Superspreading and the effect of individual variation on disease emergence. Nature. 2005;438(7066):355-9.
5. VanderWaal KL, Ezenwa VO. Heterogeneity in pathogen transmission: mechanisms and methodology. Functional Ecology. 2016;30(10):1606-22.
6. Rogers W, Brandell EE, Cross PC. Epidemiological differences between sexes affect management efficacy in simulated chronic wasting disease systems. Journal of Applied Ecology. 2022;59(4):1122-33.
7. Silk MJ, Croft DP, Delahay RJ, Hodgson DJ, Boots M, Weber N, McDonald RA. Using Social Network Measures in Wildlife Disease Ecology, Epidemiology, and Management. Bioscience. 2017;67(3):245-57.

8. Gorsich EE, Ezenwa VO, Cross PC, Bengis RG, Jolles AE. Context-dependent survival, fecundity and predicted population-level consequences of brucellosis in African buffalo. Journal of Animal Ecology. 2015;84(4):999-1009.
9. Perkins SE, Ferrari MF, Hudson PJ. The effects of social structure and sex-biased transmission on macroparasite infection. Parasitology. 2008;135(13):1561-9.
10. Rogers LM, Delahay R, Cheeseman CL, Langton S, Smith GC, Clifton-Hadley RS. Movement of badgers (Meles meles) in a high–density population: individual, population and disease effects. Proceedings of the Royal Society of London Series B: Biological Sciences. 1998;265(1403):1269-76.
11. Graham J, Smith GC, Delahay RJ, Bailey T, McDonald RA, Hodgson D. Multi-state modelling reveals sex-dependent transmission, progression and severity of tuberculosis in wild badgers. Epidemiology and Infection. 2013;141(7):1429-36.
12. Ferrari N, Cattadori IM, Nespereira J, Rizzoli A, Hudson PJ. The role of host sex in parasite dynamics: field experiments on the yellow-necked mouse Apodemus flavicollis. Ecology Letters. 2003;7(2):88-94.
13. Grear DA, Luong LT, Hudson PJ. Sex-biased transmission of a complex life-cycle parasite: Why males matter. Oikos. 2012;121(9):1446-53.
14. Stephenson JF. Parasite-induced plasticity in host social behaviour depends on sex and susceptibility. Biology Letters. 2019;15(11).
15. Ezenwa VO, Snider MH. Reciprocal relationships between behaviour and parasites suggest that negative feedback may drive flexibility in male reproductive behaviour. Proceedings of the Royal Society B: Biological Sciences. 2016;283(1831):20160423.
16. Sweeny AR, Corripio-Miyar Y, Bal X, Hayward AD, Pilkington JG, McNeilly TN, Nussey DH, Kenyon F. Longitudinal dynamics of co-infecting gastrointestinal parasites in a wild sheep population. Parasitology. 2022;149(5):593-604.
17. Cattadori IM, Boag B, Bjørnstad ON, Cornell SJ, Hudson PJ. Peak shift and epidemiology in a seasonal host-nematode system. Proceedings of the Royal Society B: Biological Sciences. 2005;272(1568):1163-9.
18. Altizer S, Davis AK, Cook KC, Cherry JJ. Age, sex, and season affect the risk of mycoplasmal conjunctivitis in a southeastern house finch population. Canadian Journal of Zoology. 2004.
19. Altizer S, Dobson A, Hosseini P, Hudson P, Pascual M, Rohani P. Seasonality and the dynamics of infectious diseases. Ecology Letters. 2006;9(4):467-84.
20. Miller MR, White A, Wilson K, Boots M. The Population Dynamical Implications of Male-Biased Parasitism in Different Mating Systems. PLOS ONE. 2007;2(7):e624.
21. Rosa GM, Bosch J, Martel A, Pasmans F, Rebelo R, Griffiths RA, Garner TWJ. Sex-biased disease dynamics increase extinction risk by impairing population recovery. Animal Conservation. 2019;22(6):579-88.
22. Härkönen T, Harding K, Rasmussen TD, Teilmann J, Dietz R. Age- and Sex-Specific Mortality Patterns in an Emerging Wildlife Epidemic: The Phocine Distemper in European Harbour Seals. PLOS ONE. 2007;2(9):e887.
23. Úbeda F, Jansen VAA. The evolution of sex-specific virulence in infectious diseases. Nature Communications. 2016;7.
24. Hall MD, Mideo N. Linking sex differences to the evolution of infectious disease life-histories. Philosophical Transactions of the Royal Society B: Biological Sciences. 2018;373(1757).

459 25. Guerra-Silveira F, Abad-Franch F. Sex Bias in Infectious Disease Epidemiology: Patterns
460 and Processes. PLOS ONE. 2013;8(4).
461 26. McDonald JL, Smith GC, McDonald RA, Delahay RJ, Hodgson D. Mortality trajectory
462 analysis reveals the drivers of sex-specific epidemiology in natural wildlife–disease interactions.
463 Proceedings of the Royal Society B: Biological Sciences. 2014;281(1790):20140526-.
464 27. Zuk M. The Sicker Sex. PLoS Pathogens. 2009;5(1):e1000267-e.
465 28. Zuk M, McKean KA. Sex Differences in Parasite Infections: Patterns and Processes.
466 International Journal for Parasitology. 1996;26(10):1009-24.
467 29. Ezenwa VO, Stefan Ekernas L, Creel S. Unravelling complex associations between
468 testosterone and parasite infection in the wild. Functional Ecology. 2012;26(1):123-33.
469 30. Foo YZ, Nakagawa S, Rhodes G, Simmons LW. The effects of sex hormones on immune
470 function: a meta-analysis. Biological Reviews. 2017;92(1):551-71.
471 31. Klein SL. Hormonal and immunological mechanisms mediating sex differences in
472 parasite infection. Parasite Immunology. 2004;26(6-7):247-64.
473 32. Klein SL. The effects of hormones on sex differences in infection: From genes to
474 behavior. Neuroscience & Biobehavioral Reviews. 2000;24(6):627-38.
475 33. Klein SL. Hormones and mating system affect sex and species differences in immune
476 function among vertebrates. Behavioural Processes. 2000;51(1-3):149-66.
477 34. Morales-Montor J, Chavarria A, León MAD, Castillo LID, Escobedo EG, Sánchez EN,
478 Vargas JA, Hernández-Flores M, Romo-González T, Larralde C. Host gender in parasitic
479 infections of mammals: an evaluation of the female host supremacy paradigm. Journal of
480 Parasitology. 2004;90(3):531-46.
481 35. Christe P, Glaizot O, Evanno G, Bruyndonckx N, Devevey G, Yannic G, Patthey P,
482 Maeder A, Vogel P, Arlettaz R. Host sex and ectoparasites choice: preference for, and higher
483 survival on female hosts. Journal of Animal Ecology. 2007;76(4):703-10.
484 36. Sanchez A, Devevey G, Bize P. Female-biased infection and transmission of the
485 gastrointestinal nematode Trichuris arvicolae infecting the common vole, Microtus arvalis.
486 International Journal for Parasitology. 2011;41(13-14):1397-402.
487 37. Kiffner C, Stanko M, Morand S, Khokhlova IS, Shenbrot GI, Laudisoit A, Leirs H,
488 Hawlena H, Krasnov BR. Sex-biased parasitism is not universal: Evidence from rodent-flea
489 associations from three biomes. Oecologia. 2013;173(3):1009-22.
490 38. Hillegass MA, Waterman JM, Roth JD. The influence of sex and sociality on parasite
491 loads in an African ground squirrel. Behavioral Ecology. 2008;19(5):1006-11.
492 39. McCurdy DG, Shutler D, Mullie A, Forbes MR. Sex-Biased Parasitism of Avian Hosts:
493 Relations to Blood Parasite Taxon and Mating System. Oikos. 1998;82(2):303-12.
494 40. Duneau D, Luijckx P, Ruder LF, Ebert D. Sex-specific effects of a parasite evolving in a
495 female-biased host population. BMC Biology. 2012;10(1):104.
496 41. Frick WF, Pollock JF, Hicks AC, Langwig KE, Reynolds DS, Turner GG, Butchkoski
497 CM, Kunz TH. An Emerging Disease Causes Regional Population Collapse of a Common North
498 American Bat Species. Science. 2010;329(5992):679-82.
499 42. Langwig KE, Frick WF, Hoyt JR, Parise KL, Drees KP, Kunz TH, Foster JT, Kilpatrick
500 AM. Drivers of variation in species impacts for a multi-host fungal disease of bats. Philosophical
501 Transactions of the Royal Society B: Biological Sciences. 2016;371(1709).
502 43. Langwig KE, Frick WF, Bried JT, Hicks AC, Kunz TH, Marm Kilpatrick A. Sociality,
503 density-dependence and microclimates determine the persistence of populations suffering from a
504 novel fungal disease, white-nose syndrome. Ecology Letters. 2012;15(9):1050-7.

44. Frick WF, Puechmaille SJ, Hoyt JR, Nickel BA, Langwig KE, Foster JT, Barlow KE, Bartonička T, Feller D, Haarsma A-J, et al. Disease alters macroecological patterns of North American bats. Global Ecology & Biogeography. 2015;24(7):741-9.
45. Warnecke L, Turner JM, Bollinger TK, Lorch JM, Misra V, Cryan PM, Wibbelt G, Blehert DS, Willis CKR. Inoculation of bats with European Geomyces destructans supports the novel pathogen hypothesis for the origin of white-nose syndrome. Proceedings of the National Academy of Sciences of the United States of America. 2012;109(18):6999-7003.
46. Warnecke L, Turner JM, Bollinger TK, Misra V, Cryan PM, Blehert DS, Wibbelt G, Willis CKR. Pathophysiology of white-nose syndrome in bats: a mechanistic model linking wing damage to mortality. Biology Letters. 2013;9(4).
47. Verant ML, Meteyer CU, Speakman JR, Cryan PM, Lorch JM, Blehert DS. White-nose syndrome initiates a cascade of physiologic disturbances in the hibernating bat host. 2014.
48. Cryan PM, Meteyer CU, Boyles JG, Blehert DS. Wing pathology of white-nose syndrome in bats suggests life-threatening disruption of physiology. BMC Biology. 2010;8:135-.
49. McGuire LP, Mayberry HW, Willis CKR. White-nose syndrome increases torpid metabolic rate and evaporative water loss in hibernating bats. American Journal of Physiology - Regulatory Integrative and Comparative Physiology. 2017;313(6):R680-R6.
50. Wilcox A, Warnecke L, Turner JM, McGuire LP, Jameson JW, Misra V, Bollinger TC, Willis CKR. Behaviour of hibernating little brown bats experimentally inoculated with the pathogen that causes white-nose syndrome. Animal Behaviour. 2014;88:157-64.
51. Reeder DM, Frank CL, Turner GG, Meteyer CU, Kurta A. Frequent Arousal from Hibernation Linked to Severity of Infection and Mortality in Bats with White-Nose Syndrome. PLoS ONE. 2012;7(6):38920-.
52. Lilley TM, Johnson JS, Ruokolainen L, Rogers EJ, Wilson CA, Schell SM, Field KA, Reeder DAM. White-nose syndrome survivors do not exhibit frequent arousals associated with Pseudogymnoascus destructans infection. Frontiers in Zoology. 2016;13(1).
53. Lorch JM, Meteyer CU, Behr MJ, Boyles JG, Cryan PM, Hicks AC, Ballmann AE, Coleman JTH, Redell DN, Reeder DM, et al. Experimental infection of bats with Geomyces destructans causes white-nose syndrome. Nature. 2011;480(7377):376-8.
54. Hoyt JR, Langwig KE, Sun K, Parise KL, Li A, Wang Y, Huang X, Worledge L, Miller H, White JP, et al. Environmental reservoir dynamics predict global infection patterns and population impacts for the fungal disease white-nose syndrome. Proceedings of the National Academy of Sciences. 2020:201914794-.
55. Hoyt JR, Langwig KE, White JP, Kaarakka HM, Redell JA, Kurta A, Depue JE, Scullon WH, Parise KL, Foster JT, et al. Cryptic connections illuminate pathogen transmission within community networks. Nature. 2018;563:710-3.
56. Verant ML, Boyles JG, Waldrep W, Wibbelt G, Blehert DS. Temperature-Dependent Growth of Geomyces destructans, the Fungus That Causes Bat White-Nose Syndrome. PLoS ONE. 2012;7(9):46280-.
57. Perry RW. A review of factors affecting cave climates for hibernating bats in temperate North America. Environmental Reviews 21: 28-39. 2013;21:28-39.
58. Webb PI, Speakman JR, Racey PA. How hot is a hibernaculum? A review of the temperatures at which bats hibernate. Canadian Journal of Zoology. 1996;74(4):761-5.
59. Langwig KE, Frick WF, Reynolds R, Parise KL, Drees KP, Hoyt JR, Cheng TL, Kunz TH, Foster JT, Marm Kilpatrick A. Host and pathogen ecology drive the seasonal dynamics of a

fungal disease, white-nose syndrome. Proceedings of the Royal Society B: Biological Sciences. 2015;282(1799).
60. Burns LE. Dynamics of Autumn Swarming and Population Structure of Little Brown and North Myotis Bats (Myotis lucifugus and M. septentrionalis): Dalhousie University; 2014.
61. Burns LE, Broders HG. Maximizing mating opportunities: higher autumn swarming activity in male versus female Myotis bats. Journal of Mammalogy. 2015;96(6):1326-36.
62. Czenze ZJ, Jonasson KA, Willis CKR. Thrifty females, frisky males: Winter energetics of hibernating bats from a cold climate. Physiological and Biochemical Zoology. 2017;90(4):502-11.
63. Jonasson KA, Willis CKR. Changes in Body Condition of Hibernating Bats Support the Thrifty Female Hypothesis and Predict Consequences for Populations with White-Nose Syndrome. PLoS ONE. 2011;6(6).
64. Racey P, Entwistle A, Crichton E, Krutzsch P. Reproductive biology of bats. Academic Press, London; 2000.
65. Thomas DW, Brock Fenton M, Barclay RMR. Social behavior of the little brown bat, Myotis lucifugus. Behavioral Ecology and Sociobiology. 1979;6(2):129-36.
66. Kurta A, Kunz TH. Size of bats at birth and maternal investment during pregnancy. Symposia of the Zoological Society of London. 1987;57:79-106.
67. Wimsatt WA. Notes on Breeding Behavior, Pregnancy, and Parturition in Some Vespertilionid Bats of the Eastern United States. Journal of Mammalogy. 1945;26(1):23-.
68. Shelley V, Kaiser S, Shelley E, Williams T, Kramer M, Haman K, Keel K, Barton HA. Evaluation of strategies for the decontamination of equipment for Geomyces destructans, the causative agent of white-nose syndrome (WNS). Journal of Cave and Karst Studies. 2013;75(1):1-10.
69. Group W-nSDMW. National White-Nose Syndrome Decontamination Protocol - October 2020. 2020.
70. Muller LK, Lorch JM, Lindner DL, O'Connor M, Gargas A, Blehert DS. Bat white-nose syndrome: a real-time TaqMan polymerase chain reaction test targeting the intergenic spacer region of Geomyces destructans. Mycologia. 2013;105(2):253-9.
71. Bates D, Mächler M, Bolker B, Walker S. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software. 2015;67(1):1 - 48.
72. Frick WF, Puechmaille SJ, Willis CKR. White-Nose Syndrome in Bats. In: Voigt CC, Kingston T, editors. Bats in the Anthropocene: Conservation of Bats in a Changing World. Cham: Springer International Publishing; 2016. p. 245-62.
73. Hoyt JR, Langwig KE, White JP, Kaarakka HM, Redell JA, Parise KL, Frick WF, Foster JT, Kilpatrick AM. Field trial of a probiotic bacteria to protect bats from white-nose syndrome. Scientific Reports. 2019;9(1):9158.
74. Hopkins SR, Hoyt JR, White JP, Kaarakka HM, Redell JA, DePue JE, Scullon WH, Kilpatrick AM, Langwig KE. Continued preference for suboptimal habitat reduces bat survival with white-nose syndrome. Nature Communications. 2021;12(1):166.
75. Hoyt JR, Kilpatrick AM, Langwig KE. Ecology and impacts of white-nose syndrome on bats. Nature Reviews Microbiology. 2021;19(3):196-210.
76. Maslo B, Valent M, Gumbs JF, Frick WF. Conservation implications of ameliorating survival of little brown bats with white-nose syndrome. Ecological Applications. 2015;25(7):1832-40.

595 77. Luong LT, Perkins SE, Grear DA, Rizzoli A, Hudson PJ. The relative importance of host
596 characteristics and co-infection in generating variation in Heligmosomoides polygyrus fecundity.
597 Parasitology. 2010;137(6):1003-12.
598 78. Jenkins HE, Cox DR, Delahay RJ. Direction of Association between Bite Wounds and
599 Mycobacterium bovis Infection in Badgers: Implications for Transmission. PLoS ONE.
600 2012;7(9).
601 79. Delahay RJ, Walker NJ, Forrester GJ, Harmsen B, Riordan P, Macdonald DW, Newman
602 C, Cheeseman CL. Demographic correlates of bite wounding in Eurasian badgers, Meles meles
603 L., in stable and perturbed populations. Animal Behaviour. 2006;71(5):1047-55.
604 80. Folstad I, Karter AJ. Parasites, bright males, and the immunocompetence handicap.
605 American Naturalist. 1992;139(3):603-22.
606 81. Edler R, Goymann W, Schwabl I, Friedl TWP. Experimentally elevated testosterone
607 levels enhance courtship behaviour and territoriality but depress acquired immune response in
608 Red Bishops Euplectes orix. Ibis. 2011;153(1):46-58.
609 82. Roberts M, Peters A. Is testosterone immunosuppressive in a condition-dependent
610 manner? An experimental test in blue tits. Journal of Experimental Biology. 2009;212(12):1811-
611 8.
612 83. Prendergast BJ, Freeman DA, Zucker I, Nelson RJ. Periodic arousal from hibernation is
613 necessary for initiation of immune responses in ground squirrels. American Journal of
614 Physiology - Regulatory Integrative and Comparative Physiology. 2002;282(4 51-4).
615 84. Bouma HR, Carey HV, Kroese FGM. Hibernation: the immune system at rest? Journal of
616 Leukocyte Biology. 2010;88(4):619-24.
617 85. Meier F, Grosche L, Reusch C, Runkel V, van Schaik J, Kerth G. Long-term
618 individualized monitoring of sympatric bat species reveals distinct species- and demographic
619 differences in hibernation phenology. BMC Ecology and Evolution. 2022;22(1):1-12.
620 86. Meyer GA, Senulis JA, Reinartz JA. Effects of temperature and availability of insect prey
621 on bat emergence from hibernation in spring. Journal of Mammalogy. 2016;97(6):1623-33.
622 87. Grieneisen LE, Brownlee-Bouboulis SA, Johnson JS, Reeder DM. Sex and hibernaculum
623 temperature predict survivorship in white-nose syndrome affected little brown myotis (Myotis
624 lucifugus). Royal Society Open Science. 2015;2.
625 88. Johnson JS, Reeder DM, McMichael JW, Meierhofer MB, Stern DWF, Lumadue SS,
626 Sigler LE, Winters HD, Vodzak ME, Kurta A, et al. Host, pathogen, and environmental
627 characteristics predict white-nose syndrome mortality in captive little brown myotis (Myotis
628 lucifugus). PLoS ONE. 2014;9(11).
629 89. Davis WH, Hitchcock HB. Biology and migration of the bat, Myotis lucifugus, in New
630 England. Journal of Mammalogy. 1965;46(2):296-313.
631 90. Mohr CE. Sex ratios of bats in Pennsylvania. Proceedings of the Pennsylvania Academy
632 of Science. 1945;19:65-9.
633 91. Tinkle DW, Milstead WW. Sex Ratios and Population Density in Hibernating Myotis.
634 The American Midland Naturalist. 1960;63(2):327-34.
635 92. Lynda MSL, William C. Sex ratio variation of Myotis velifer (Chiroptera:
636 Vespertilionidae) in Oklahoma The Southwestern Naturalist. 2007;52(1):67-74.
637 93. Willis CKR, Brigham RM. Social thermoregulation exerts more influence than
638 microclimate on forest roost preferences by a cavity-dwelling bat. Behavioral Ecology and
639 Sociobiology. 2007;62(1):97-108.

640 94. Linton DM, Macdonald DW. Spring weather conditions influence breeding phenology
641 and reproductive success in sympatric bat populations. Journal of Animal Ecology.
642 2018;87(4):1080-90.
643 95. Le Galliard J-F, Fitze Patrick S, Ferrière R, Clobert J. Sex ratio bias, male aggression,
644 and population collapse in lizards. Proceedings of the National Academy of Sciences.
645 2005;102(50):18231-6.
646 96. Johanos TC, Becker BL, Baker JD, Ragen TJ, Gilmartin WG, Gerrodette T. Impacts of
647 sex ratio reduction on male aggression in the Critically Endangered Hawaiian monk seal
648 Monachus schauinslandi. Endangered Species Research. 2010;11(2):123-32.

649