Infectious disease as a cause and consequence of phenotypic responses to challenge in a songbird species

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Dissertation submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

In

Biological Sciences

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July 10th, 2024

Blacksburg, Virginia

Keywords: House finch, *Haemorhous mexicanus*, animal behavior, directly-transmitted pathogens, *Mycoplasma gallisepticum*, developmental stress, social behavior, behavioral tolerance

Infectious disease as a cause and consequence of phenotypic responses to challenge in a songbird species

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Academic Abstract

Throughout their lives, animals are faced with numerous ecological challenges stemming from abiotic and biotic conditions of their environment. Phenotypic shifts in response to one challenge can have cascading effects on other organismal systems, with downstream implications for individual fitness. Infectious disease presents a significant ecological challenge for most organisms on earth. Additionally, how an animal responds to disease can be shifted by exposure to other ecological challenges. Thus, infectious disease can both present an ecological challenge itself or shift as a consequence of another challenge. In this work, I used experimental captive studies on wild-caught house finches (*Haemorhous mexicanus*) to elucidate how an animal might shift its phenotypes when presented with an ecological challenge. In the first experiment, I examined how nutritional stress during nestling development impacted the magnitude of house finch responses to the bacterial pathogen *Mycoplasma gallisepticum* (MG). Although nutritional stress limited mass gain in nestlings, individual responses to MG did not vary with nutritional stress, possibly indicating that the development of immune responses is resilient even in the face of suboptimal nutritional conditions. Next, I investigated infectious disease as a challenge in itself and asked how individual social preferences were shifted by MG infection. I demonstrated that MG-infected house finches showed augmented sociality relative to control birds, choosing to spend more time with a group of conspecifics than alone. Because this increased social preference was no longer present once birds recovered, this phenotypic change in sociality may have specific benefits for actively infected birds. Finally, my last experiment expands upon these results, exploring whether group-living particularly benefits infected birds by offsetting two common fitness costs of infection: reduced foraging abilities and decreased anti-predator responses. Here we found that group-living provides all individuals with improved foraging and anti-predator behaviors, with the strongest benefits of group-living apparent for infected finches. This suggests that augmented sociality in infected house finches has important implications for surviving infection, and potentially, for the spread of MG within populations. As animals continue to face increasing and novel ecological challenges, it is vitally important to understand

individual responses to environmental challenges, which can have long-term effects for all levels of biological organization. In particular, my work highlights the role of social behavior as a potentially adaptive phenotypic response to infectious disease in wild animals. Taken together, my results demonstrate the importance of continuing to study infectious disease from multiple perspectives to better understand how animals will respond to a shifting world.

Infectious disease as a cause and consequence of phenotypic responses to challenge in a songbird species

Marissa Mae Langager

General Audience Abstract

All animals must respond to challenges in their environment, which can impact their lives in a variety of ways. Infectious disease is a significant challenge for most organisms on earth. Infection with a disease-causing pathogen must be met by the individual with behavioral, physiological, and immunological responses to increase the animal's likelihood of survival. Additionally, an animal's response to disease can be shifted by exposure to other adverse environmental conditions, such as reduced access to food. On the one hand, infectious disease can present a challenge in itself. Alternatively, how an animal responds to disease may shift as a consequence of another challenge. In this work, I brought wild-caught birds into a captive setting and performed three experiments to determine how an animal might respond to common ecological challenges. First, I studied how food shortages during early life impacted how strongly birds responded to infection with a disease-causing bacteria. In this study I found that host responses to disease did not shift, even when birds were given less food and experienced reduced mass growth during early life. Although young animals are developing rapidly and are particularly vulnerable to challenges in their environment, my results indicate that the development of responses to disease is resilient even in the face of suboptimal conditions. Next, I investigated how social behaviors were shifted due to disease. Here I demonstrated that diseased birds were more social than healthy birds, preferring to spend more time with a group of other birds than alone. In contrast, once these same birds had recovered from infection and were again healthy they became less social, which suggests that diseased birds in particular may benefit from being part of a group. My final experiment expanded upon these results, exploring whether group-living can help increase an individual's survival by compensating for two consequences of disease: reduced ability to acquire food and evade predators. Here I found that group-living provides individual benefits in terms of both acquiring food and evading predators, both of which have important implications for an individual's survival, especially while experiencing disease. As animals continue to face increasing and new challenges due to global change, it becomes vitally important to understand individual responses to environmental changes. While

the work highlighted here presents an important step in understanding individual responses, future work should use observational studies in the wild to determine how the social preferences and behaviors I demonstrated here are actually occurring in a natural habitat. Taken together, my results highlight the importance of continuing to study infectious disease from multiple perspectives to better understand how animals will respond to a shifting world.

Dedication

This work is dedicated to my parents, Steve and Paula Langager, who cultivated in me a voracious appetite for knowledge and a deep-rooted passion for animals and the natural world. Thank you for indulging all my persistent questions, for taking me outside, and for always supporting me on this journey.

Acknowledgements

I would like to start by thanking my advisor, Dr. Dana Hawley, for her unwavering support over the last 6 years. Working with an advisor who leads with kindness, respect, and grace has allowed me to rebound from my hardships and grow as a person and scientist. I'm so lucky and grateful to have had you in my corner during this journey. I would also like to thank Drs. Ignacio Moore, Kendra Sewall, and Jeff Walters, for their thoughtful guidance on my work. In addition to their academic insights, all of my committee members have provided me with opportunities to grow as a person, from teaching to writing departmental policy, for which I am extremely grateful.

I would also like to thank past and present Hawley lab members, who have supported me throughout this journey. I would especially like to thank Dr. Anna Perez-Umphrey, Sara Teemer Richards, Dr. Chava Weitzman, Alicia Arneson, Jesse Garrett-Larsen, Riley Meyers, and Allison Rowley for always being willing to give me help on all aspects of my time here. I could try to list the ways in which you all have helped and supported me over the years, but it would take up another 100 pages. I would also like to thank the amazing team of Hawley lab undergraduates throughout the years, but especially Cynthia Harrison for watching my behavioral videos, John Brule for all the expert nest searching, Drew Hynes for his help in feeding nestlings, and Danielle Alms for being my first undergraduate mentee. Thanks also to the many Hawley lab collaborators, but especially Dr. Jim Adelman and Dr. Ambi Henschen, whose patience and kindness towards a first-year graduate student in over her head will never be forgotten. Finally, none of this work would have been possible without our amazing animal care staff.

The friendships I have made as a graduate student have sustained and lifted me, even in the worst moments of my Ph.D. I am so grateful to all of the EEB graduate students, who are some of the kindest and most impressive people I have ever met. Special thanks go to Sara Teemer Richards who gets a second shout out because she's my SFAM, to Dr. Korin Jones for all the board games, to Taylor Fossett for loving birds just as much as me, and to Dr. Isaac Van Diest for the I&M "writing" "hours". Thanks also to my non-grad school friends, but especially Jenessa Albertson and Kelsey Dockter, who keep me grounded by reminding me that there is life outside of being a Ph.D. student. I also have to thank all my fellow Ornithology TAs, but especially my #noboysallowed ladies Sara, Taylor, and Bridget. Teaching with you all was one of

the highlights of my last 2 years. I'm so lucky to have such a stellar chosen family surrounding me. I'd also like to thank all of my dog friends (that is, friends, who are also dogs), who have given me support and sanity in the form of long walks, cuddles, and doggy kisses.

Finally, I would like to thank my family, Steve and Paula Langager. No one cheers harder for me or believes in me more than these two, which is why I have dedicated this work to them. As I complete this journey, I hope they know how much their love and support has allowed me to get to this point.

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Ch. 2: Nutritional stress during songbird development impacts nestling growth, but not responses to disease later in life

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Ch. 3: Let's stick together: infection enhances preferences for social grouping in a songbird species

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Ch. 4: In sickness and in health: Group-living augments behavioral responses to food and predation risk for sick house finches (*Haemorhous mexicanus*)

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Attributions

Ch. 2: Nutritional stress during songbird development impacts nestling growth, but not responses to disease later in life

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Ch. 4: In sickness and in health: Group-living augments behavioral responses to food and predation risk for sick house finches (*Haemorhous mexicanus*)

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Ch.1: Introduction

Wild animals face numerous ecological challenges during their lives, which can have both short- and long-term consequences for their fitness. Once an animal encounters an ecological challenge, the effects of any phenotypic shifts can impact various aspects of an individual's life, including host responses to disease (Harris & Seckl, 2011) and behaviors (Anisman et al., 1998). Cascading changes to both physiology and behavior in response to these challenges can affect individual homeostasis and possibly population-level dynamics (Tompkins et al., 2011; Warne et al., 2019). Infectious disease presents a unique ecological challenge, as host responses to disease can be altered due other environmental challenges, such as nutritional stress; however, disease itself can also act as a challenge to organismal homeostasis, thereby shifting host behavioral and physiological responses (Fig.1).

Infectious diseases are widespread and impact nearly every living organism, from bacteria to megafauna. This is especially true in the face of increasing anthropogenic change to habitats, as human behavior can be a driving force of disease spread (Funk et al., 2010). Due in part to human-mediated environmental changes, infectious diseases are emerging at an increasing frequency (Jones et al., 2008; Tompkins et al., 2015), so studying host-pathogen dynamics is important to understanding the full impact of infectious diseases. At an individual level, infectious diseases can lead to significant reductions in host fitness through several mechanisms, including reduced foraging ability (Tillman & Adelman, 2023), decreased anti-predator responses (Adelman et al., 2017), and lower mating success (Beltran-Bech & Richard, 2014). Ultimately, these disease-driven reductions in host fitness due to phenotypic shifts can drive changes in population trajectories and dynamics, with possible downstream effects on ecosystems as a whole (Nadler et al., 2023; Tompkins et al., 2011).

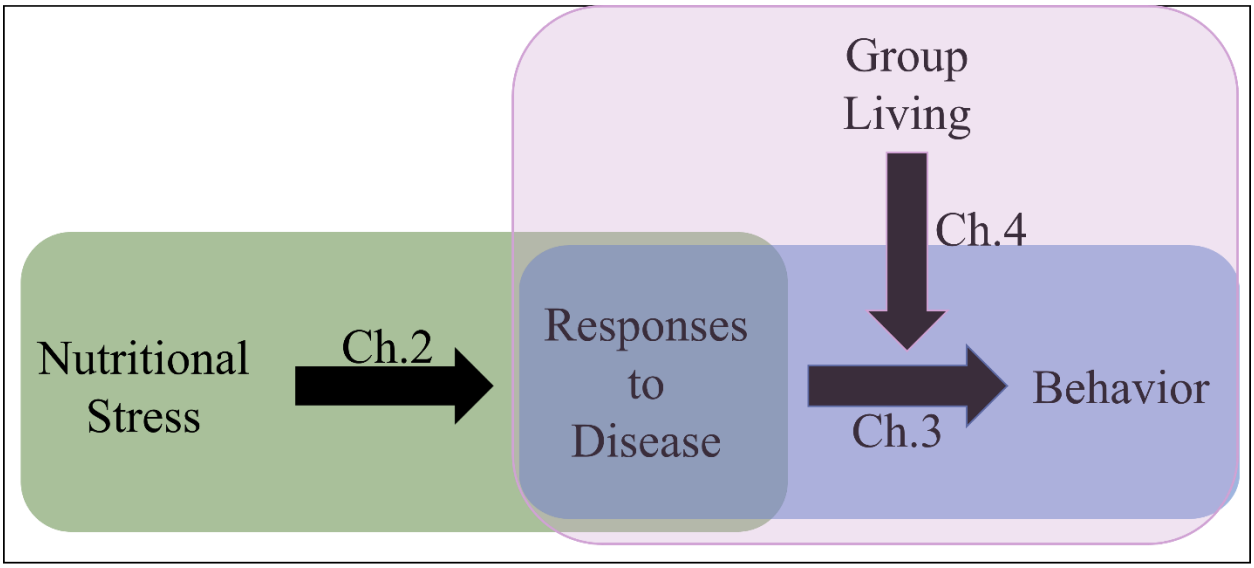

Figure 1. Conceptual framework for dissertation. I studied how host responses to disease shifts when individuals are exposed to the common ecological challenge of nutritional stress (Chapter 2, highlighted in green) as well as how disease acts as a challenge itself to infected hosts (Chapter 3, highlighted in blue). Additionally, I studied how the combined influence of disease and group-living impact behavioral responses crucial to survival (Chapter 3, highlighted in purple).

Effects of early-life nutritional stress on host responses to disease

Many environmental conditions during development can lead to shifts in how animals respond to challenges later in life. Loss of resource availability, especially food, due to environmental change can present a major hurdle for wild animals (Birnie-Gauvin et al., 2017). These new challenges may be particularly felt by young animals, as they are still developing many of the systems vital to responding to any shifts in their environment (Eyck et al., 2019). This may be especially true for species that produce altricial young which undergo a majority of their development while exposed to the outside environment (Wada & Coutts, 2021). In many altricial species, such as songbirds, parents are solely or primarily responsible for providing adequate amounts of nutrition to their offspring, which can vary depending on the number of offspring and availability of a resource (Nettle et al., 2016; Neuenschwander et al., 2003). Thus, any shifts in resource availability could have particularly dire consequences for young animals, as they are often dependent upon parental provisioning in order to develop key body systems, including immune responses, and survive this period of rapid development (Burley & Johnson, 2002). Any variation in food allocation, which might occur when young are reared during times

of less dense resource availability, likely causes developing animals to experience a wide range of environmental conditions that can have long-term impacts on overall fitness.

When food resources are limited and must be distributed across both parents and offspring, developing young may experience trade-offs in which the development of some systems are prioritized above others (Eyck et al., 2019). In young animals, the immune system is developing rapidly and, therefore, vulnerable to adverse ecological conditions which could alter how individuals respond to disease later in life (Avitsur et al., 2015; Killpack et al., 2013). Negative impacts on host responses to disease due to poor early-life conditions has been demonstrated in the young of several taxa including humans (e.g., Danese & McEwen, 2012; McDade et al., 2017), rodents (e.g., Avitsur et al., 2006; Bowers et al., 2008; O'Mahony et al., 2009; Shanks et al., 2000), and songbirds (Hõrak et al., 1999; Nettle et al., 2016; Saino et al., 1997; but see Chin et al., 2005; Killpack et al., 2015), whose young depend heavily on parental care to ensure success later in life. Differences in the development of the immune system may lead to long-term impacts on the way individuals respond to pathogen infection, which may have downstream effects on population dynamics and pathogen transmission.

Behavioral responses to disease

Disease presents a common challenge to all living organisms and can be energetically demanding and physically debilitating event that can lead to shifts in behavior through several possible mechanisms (Hart, 1988). While some pathogens appear to directly manipulate the behavior of their host to increase the likelihood of transmission (Klein, 2003; Poulin, 2010), other pathogens impact behavior through host-mediated mechanisms. When infected with a pathogen, hosts often shift energy reserves from more energetically costly activities, such as grooming or maintaining social groups, in order to accommodate the metabolic demands of immune responses and tissue repair (Adelman & Martin, 2009; Hart, 1988). Such behavioral shifts, collectively termed "sickness behaviors", include decreases in reproductive and other activities, and increases in lethargy (Dantzer & Kelley, 2007; Hart, 1988). While sickness behaviors are hypothesized to be adaptive for energy conservation, they can also have negative consequences for host fitness in the wild by decreasing foraging behaviors (e.g., D. J. McFarland & Hotchin, 1983; R. McFarland et al., 2021), decreasing time spent grooming (Yorinks &

Atkinson, 2000), and potentially lowering anti-predator defenses (Adelman et al., 2017), all of which may lead to decreased survival.

For many social species, sickness behaviors can also lead to decreased social behaviors and social affiliations (reviewed in Stockmaier et al., 2021). However, there is a growing body of work demonstrating that, in some systems, pathogen infection increases an individual's social behaviors (e.g., Langager et al., 2023; Siva-Jothy & Vale, 2019; Stephenson, 2019; Wu et al., 2023). Though social behaviors are costly, maintaining or increasing gregariousness may provide infected hosts with several fitness-related benefits, such as improved protection from predators and increased ability to acquire food (Almberg et al., 2015; Ezenwa & Worsley-Tonks, 2018). By reducing sickness behavior-induced fitness costs for infected hosts, augmented social behaviors may act as a form of behavioral tolerance, defined as using behavior to increase the likelihood of surviving infection (Ezenwa et al., 2016; Stockmaier et al., 2023). Elucidating the degree and direction that disease can shift social behaviors in infected hosts is important for understanding both individual fitness, as well as population-level pathogen transmission dynamics.

Study System

The house finch (*Haemorhous mexicanus*) presents an exciting system for studying the ways in which disease can lead to shifting host responses due an ecological challenge or act as an ecological challenge itself. House finches are gregarious songbirds that can now be found throughout North America (Badyaev et al., 2020). This species produces altricial young, which are especially vulnerable to challenges in early life, such as nutritional stress (Eyck et al., 2019). Parents feed their young extensively while in the nest, and continue to feed their offspring until nutritional independence, so any parental variation among or within nests could lead to differences in future phenotypes, such as their immunological, physiological, or behavioral responses to disease.

During the non-breeding fall and winter months, house finches flock in large groups, frequenting bird feeders, which provide a reliable, constant source of high-quality food. This social behavior and consistent use of common food sources has contributed to the spread of the pathogenic bacteria *Mycoplasma gallisepticum* (MG) among populations of house finches (Adelman et al., 2015; Altizer, Hochachka, et al., 2004). Due to its lack of a cell wall, MG has a limited survival period outside of its host, so bird feeders act as a fomite, allowing MG to survive

on surfaces (usually feeder ports) for up to 24-hour periods (Dhondt et al., 2007). Originally only affecting poultry, MG in wild finches was first documented in Northern Virginia in 1994 (Fischer et al., 1997) and has continued to spread throughout the house finch range across North America.

MG infection in house finches causes the disease mycoplasmal conjunctivitis, which includes swelling of the conjunctiva (sometimes to the point of temporary blindness), increased mucus secretion, and extreme lethargy (Ley et al., 1996). Infection with MG also leads to several energetic costs for infected hosts. House finches experimentally infected with MG display several physiological changes including increased levels of the primary avian glucocorticoid, corticosterone (Love et al., 2016) and increased metabolic rates (Hawley et al., 2012) relative to uninfected control birds. Further, host physiological responses to disease in this system seem to be mediated primarily by pro-inflammatory immune cytokines (Vinkler et al., 2018), which also mediate sickness behaviors in vertebrates (Dantzer, 2004). Overall, the physiological changes with MG infection likely contribute to the onset of sickness behaviors in infected birds (Adelman, Kirkpatrick, et al., 2013; Love et al., 2016).

The presence of sickness behaviors during infection likely also contributes directly to infection-mediated mortality in this system. While MG is not directly lethal to house finches, the presence of sickness behaviors, such as severe lethargy, can greatly increase the risk of predation for infected birds (Adelman et al., 2017). Due to increased likelihood of predation, mycoplasmal conjunctivitis is associated with significantly lower survival rates in the wild (Faustino et al., 2004) and population-level declines (Hochachka & Dhondt, 2000). Further, infected birds may struggle to keep up with flocks in the wild, as bird with conjunctivitis are observed alone or in smaller flocks more often than healthy individuals (Hawley et al., 2007; Hotchkiss et al., 2005). Because mortality from MG infection is indirect, gregariousness may have a protective effect for infected birds by increasing their ability to detect and evade predators. Studying the social preferences and benefits of group-living for infected house finches is key to addressing whether gregariousness can act as a benefit during infection in this system.

Current Study

My dissertation contains three studies exploring how host responses to infectious disease shift when exposed to other ecological challenges (Chapter 2) and how infectious disease acts as an ecological challenge in itself (Chapters 3 & 4) in a naturally-occurring host-pathogen system

(Fig.1). In this dissertation, I conducted three separate captive laboratory experiments to address three research objectives:

1. Investigate the relationship between early life nutritional stress, host responses to disease, and physiology.
2. Elucidate how infection with a directly-transmitted pathogen impacts social preferences of a naturally gregarious species, both during infection and following recovery.
3. Examine the ways in which group-living and disease interact to alter fitness-related behaviors during infection.

References

Adelman, J. S., Kirkpatrick, L., Grodio, J. L., & Hawley, D. M. (2013). House Finch Populations Differ in Early Inflammatory Signaling and Pathogen Tolerance at the Peak of *Mycoplasma gallisepticum* Infection. *The American Naturalist*, *181*(5), 674–689.

Adelman, J. S., & Martin, L. B. (2009). Vertebrate sickness behaviors: Adaptive and integrated neuroendocrine immune responses. *Integrative and Comparative Biology*, *49*(3), 202–214. https://doi.org/10.1093/icb/icp028

Adelman, J. S., Mayer, C., & Hawley, D. M. (2017). Infection reduces anti-predator behaviors in house finches. *Journal of Avian Biology*, *48*, 519–528.

Adelman, J. S., Moyers, S. C., Farine, D. R., & Hawley, D. M. (2015). Feeder use predicts both acquisition and transmission of a contagious pathogen in a North American songbird. *Proceedings of the Royal Society B*, *282*(20151429).

Almberg, E. S., Cross, P. C., Dobson, A. P., Smith, D. W., Metz, M. C., Stahler, D. R., & Hudson, P. J. (2015). Social living mitigates the costs of a chronic illness in a cooperative carnivore. *Ecology Letters*, *18*(7), 660–667. https://doi.org/10.1111/ele.12444

Altizer, S., Hochachka, W. M., & Dhondt, A. A. (2004). Seasonal dynamics of mycoplasmal conjunctivitis in eastern North American house finches. *Journal of Animal Ecology*, *73*, 309–322. www.birds.cornell.edu/hofi.

Anisman, H., Zaharia, M. D., Meaney, M. J., & Merali, Z. (1998). Do early-life events permanently alter behavioral and hormonal responses to stressors? *International Journal of Developmental Neuroscience*, *16*(3/4), 149–164.

Avitsur, R., Hunzeker, J., & Sheridan, J. F. (2006). Role of early stress in the individual differences in host response to viral infection. *Brain, Behavior, and Immunity*, *20*, 339–348.

Avitsur, R., Levy, S., Goren, N., & Grinshpahet, R. (2015). Early adversity, immunity and infectious disease. *Stress*, *18*(3), 289–296. https://doi.org/10.3109/10253890.2015.1017464

Badyaev, A. V., Belloni, V., & Hill, G. E. (2020). House Finch (Haemorhous mexicanus). In *Birds of the World,* A. F. Poole (Ed.). Cornell Lab of Ornithology. https://doi.org/10.2173/bow.houfin.01

Beltran-Bech, S., & Richard, F. J. (2014). Impact of infection on mate choice. *Animal Behaviour*, *90*, 159–170. https://doi.org/10.1016/j.anbehav.2014.01.026

Birnie-Gauvin, K., Peiman, K. S., Raubenheimer, D., & Cooke, S. J. (2017). Nutritional physiology and ecology of wildlife in a changing world. *Conservation Physiology*, *5*(1). https://doi.org/10.1093/conphys/cox030

Bowers, S. L., Bilbo, S. D., Dhabhar, F. S., & Nelson, R. J. (2008). Stressor-specific alterations in corticosterone and immune responses in mice. *Brain, Behavior, and Immunity*, *22*(1), 105–113. https://doi.org/10.1016/j.bbi.2007.07.012

Burley, N. T., & Johnson, K. (2002). The evolution of avian parental care. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *357*(1419), 241–250. https://doi.org/10.1098/rstb.2001.0923

Chin, E. H., Love, O. P., Clark, A. M., & Williams, T. D. (2005). Brood size and environmental conditions sex-specifically affect nestling immune response in the European starling *Sturnus vulgaris*. *Journal of Avian Biology*, *36*(6), 549–554.

Danese, A., & McEwen, B. S. (2012). Adverse childhood experiences, allostasis, allostatic load, and age-related disease. *Physiology and Behavior*, *106*(1), 29–39. https://doi.org/10.1016/j.physbeh.2011.08.019

Dantzer, R. (2004). Cytokine-induced sickness behaviour: A neuroimmune response to activation of innate immunity. *European Journal of Pharmacology*, *500*, 399–411.

Dantzer, R., & Kelley, K. W. (2007). Twenty years of research on cytokine-induced sickness behavior. *Brain, Behavior, and Immunity*, *21*(2), 153–160.

Dhondt, A. A., Dhondt, K. V., Hawley, D. M., & Jennelle, C. S. (2007). Experimental evidence for transmission of *Mycoplasma gallisepticum* in house finches by fomites. *Avian Pathology*, *36*(3), 205–208.

Eyck, H. J. F., Buchanan, K. L., Crino, O. L., & Jessop, T. S. (2019). Effects of developmental stress on animal phenotype and performance: a quantitative review. *Biological Reviews*, *94*(3), 1143–1160. https://doi.org/10.1111/brv.12496

Ezenwa, V. O., Ghai, R. R., McKay, A. F., & Williams, A. E. (2016). Group living and pathogen infection revisited. *Current Opinion in Behavioral Sciences*, *12*, 66–72. https://doi.org/10.1016/j.cobeha.2016.09.006

Ezenwa, V. O., & Worsley-Tonks, K. E. L. (2018). Social living simultaneously increases infection risk and decreases the cost of infection. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1892). https://doi.org/10.1098/rspb.2018.2142

Faustino, C. R., Jennelle, C. S., Connolly, V., Davis, A. K., Swarthout, E. C., Dhondt, A. A., & Cooch, E. G. (2004). *Mycoplasma gallisepticum* infection dynamics in a house finch population: seasonal variation in survival, encounter and transmission rate. *Journal of Animal Ecology*, *73*(4), 651–669.

Fischer, J. R., Stallknecht, D. E., Luttrell, M. P., Dhondt, A. A., & Converse, K. A. (1997). Mycoplasmal Conjunctivitis in Wild Songbirds: The Spread of a New Contagious Disease in a Mobile Host Population. *Emerging Infectious Diseases*, *3*(1), 69–72.

Funk, S., Salathe, M., & Jansen, V. A. A. (2010). Modelling the influence of human behaviour on the spread of infectious diseases: a review. *J. R. Soc. Interface*, *7*(50), 1247–1256.

Harris, A., & Seckl, J. (2011). Glucocorticoids, prenatal stress and the programming of disease. *Hormones and Behavior*, *59*(3), 279–289.

Hart, B. L. (1988). Biological basis of the behavior of sick animals. *Neuroscience and Biobehavioral Reviews*, *12*(2), 123–137. https://doi.org/10.1016/S0149-7634(88)80004-6

Hawley, D. M., Davis, A. K., & Dhondt, A. A. (2007). Transmission-relevant behaviours shift with pathogen infection in wild house finches (*Carpodacus mexicanus*). *Canadian Journal of Zoology*, *85*(6), 752–757.

Hawley, D. M., Durant, S. E., Wilson, A. F., Adelman, J. S., & Hopkins, W. A. (2012). Additive metabolic costs of thermoregulation and pathogen infection. *Functional Ecology*, *26*(3), 701–710. https://doi.org/10.1111/j.1365-2435.2012.01978.x

Hochachka, W. M., & Dhondt, A. A. (2000). Density-dependent decline of host abundance resulting from a new infectious disease. *PNAS*, *97*(10), 5303–5306.

Hõrak, P., Tegelmann, L., Ots, I., & Møller, A. P. (1999). Immune Function and Survival of Great Tit Nestlings in Relation to Growth Conditions. *Oecologia*, *121*(3), 316–322.

Hotchkiss, E. R., Davis, A. K., Cherry, J. J., & Altizer, S. (2005). Mycoplasmal conjunctivitis and the behavior of wild house finches (*Carpodacus mexicanus*) at bird feeders. *Bird Behavior*, *17*, 1–8.

Jones, K. E., Patel, N. G., Levy, M. A., Storeygard, A., Balk, D., Gittleman, J. L., & Daszak, P. (2008). Global trends in emerging infectious diseases. *Nature*, *451*(7181), 990–993. https://doi.org/10.1038/nature06536

Killpack, T. L., Carrel, E., & Karasov, W. H. (2015). Impacts of short-term food restriction on immune development in altricial house sparrow nestlings. *Physiological and Biochemical Zoology*, *88*(2), 195–207. https://doi.org/10.1086/680168

Killpack, T. L., Oguchi, Y., & Karasov, W. H. (2013). Ontogenetic patterns of constitutive immune parameters in altricial house sparrows. *Journal of Avian Biology*, *44*(6), 513–520. https://doi.org/10.1111/j.1600-048X.2013.00239.x

Klein, S. L. (2003). Parasite manipulation of the proximate mechanisms that mediate social behavior in vertebrates. *Physiology and Behavior*, *79*(3), 441–449. https://doi.org/10.1016/S0031-9384(03)00163-X

Langager, M. M., Adelman, J. S., & Hawley, D. M. (2023). Let's stick together: Infection enhances preferences for social grouping in a songbird species. *Ecology and Evolution*, *13*(10). https://doi.org/10.1002/ece3.10627

Ley, D. H., Berkhoff, J. E., & Mclaren, J. M. (1996). *Mycoplasma gallisepticum* Isolated from House Finches (*Carpodacus mexicanus*) with Conjunctivitis. *Avian Diseases*, *40*(2), 480–483.

Love, A. C., Foltz, S. L., Adelman, J. S., Moore, I. T., & Hawley, D. M. (2016). Changes in corticosterone concentrations and behavior during *Mycoplasma gallisepticum* infection in house finches (*Haemorhous mexicanus*). *General and Comparative Endocrinology*, *235*, 70–77. https://doi.org/10.1016/j.ygcen.2016.06.008

McDade, T. W., Ryan, C., Jones, M. J., MacIsaac, J. L., Morin, A. M., Meyer, J. M., Borja, J. B., Miller, G. E., Kobor, M. S., & Kuzawa, C. W. (2017). Social and physical environments early in development predict DNA methylation of inflammatory genes in young adulthood. *PNAS*, *114*(29), 7611–7616. https://doi.org/10.1073/pnas.1620661114

McFarland, D. J., & Hotchin, J. (1983). Host genetics and the behavioral sequelae to herpes encephalitis in mice. *Physiology & Behavior*, *30*(6), 881–884.

McFarland, R., Henzi, S. P., Barrett, L., Bonnell, T., Fuller, A., Young, C., & Hetem, R. S. (2021). Fevers and the social costs of acute infection in wild vervet monkeys. *PNAS*, *118*(44). https://doi.org/10.1073/pnas.2107881118/-/DCSupplemental

Nadler, L. E., Adamo, S. A., Hawley, D. M., & Binning, S. A. (2023). Mechanisms and consequences of infection-induced phenotypes. *Functional Ecology*, *37*(4), 796–800. https://doi.org/10.1111/1365-2435.14309

Nettle, D., Andrews, C., Reichert, S., Bedford, T., Gott, A., Parker, C., Kolenda, C., Martin-Ruiz, C., Monaghan, P., & Bateson, M. (2016). Brood size moderates associations between relative size, telomere length, and immune development in European starling nestlings. *Ecology and Evolution*, *6*, 8138–8148.

Neuenschwander, S., Brinkhof, M. W. G., Kölliker, M., & Richner, H. (2003). Brood size, sibling competition, and the cost of begging in great tits (*Parus major*). *Behavioral Ecology*, *14*(4), 457–462.

O'Mahony, S. M., Marchesi, J. R., Scully, P., Codling, C., Ceolho, A. M., Quigley, E. M. M., Cryan, J. F., & Dinan, T. G. (2009). Early Life Stress Alters Behavior, Immunity, and Microbiota in Rats: Implications for Irritable Bowel Syndrome and Psychiatric Illnesses. *Biological Psychiatry*, *65*(3), 263–267.

Poulin, R. (2010). Parasite Manipulation of Host Behavior: An Update and Frequently Asked Questions. In *Advances in the Study of Behavior* (Vol. 41, Issue C, pp. 151–186). https://doi.org/10.1016/S0065-3454(10)41005-0

Saino, N., Calza, S., & Moller, A. P. (1997). Immunocompetence of Nestling Barn Swallows in Relation to Brood Size and Parental Effort. *Journal of Animal Ecology*, *66*(6), 827–836.

Shanks, N., Windle, R. J., Perks, P. A., Harbuz, M. S., Jessop, D. S., Ingram, C. D., & Lightman, S. L. (2000). Early-life exposure to endotoxin alters hypothalamic-pituitary-adrenal function and predisposition to inflammation. *PNAS*, *97*(10), 5645–5650.

Siva-Jothy, J. A., & Vale, P. F. (2019). Viral infection causes sex-specific changes in fruit fly social aggregation behaviour. *Biology Letters*, *15*(9). https://doi.org/10.1098/rsbl.2019.0344

Stephenson, J. F. (2019). Parasite-induced plasticity in host social behaviour depends on sex and susceptibility. *Biology Letters*, *15*, 20190557. https://doi.org/10.1098/rsbl.2019.0557

Stockmaier, S., Stroeymeyt, N., Shattuck, E. C., Hawley, D. M., Meyers, L. A., & Bolnick, D. I. (2021). Infectious diseases and social distancing in nature. *Science*, *371*(6533). https://doi.org/10.1126/science.abc8881

Stockmaier, S., Ulrich, Y., Albery, G. F., Cremer, S., & Lopes, P. C. (2023). Behavioural defences against parasites across host social structures. *Functional Ecology*, *37*(4), 809–820. https://doi.org/10.1111/1365-2435.14310

Tillman, F. E., & Adelman, J. S. (2023). Searching while sick: How does disease affect foraging decisions and contact rates? *Functional Ecology*, *37*(4), 838–844. https://doi.org/10.1111/1365-2435.14207

Tompkins, D. M., Carver, S., Jones, M. E., Krkošek, M., & Skerratt, L. F. (2015). Emerging infectious diseases of wildlife: A critical perspective. *Trends in Parasitology*, *31*(4), 149–159. https://doi.org/10.1016/j.pt.2015.01.007

Tompkins, D. M., Dunn, A. M., Smith, M. J., & Telfer, S. (2011). Wildlife diseases: From individuals to ecosystems. *Journal of Animal Ecology*, *80*(1), 19–38. https://doi.org/10.1111/j.1365-2656.2010.01742.x

Vinkler, M., Leon, A. E., Kirkpatrick, L., Dalloul, R. A., & Hawley, D. M. (2018). Differing House Finch Cytokine Expression Responses to Original and Evolved Isolates of *Mycoplasma gallisepticum*. *Frontiers in Immunology*, *9*(13), 1–16.

Wada, H., & Coutts, V. (2021). Detrimental or beneficial? Untangling the literature on developmental stress studies in birds. *Journal of Experimental Biology*, *224*(19). https://doi.org/10.1242/jeb.227363

Warne, R. W., Baer, S. G., & Boyles, J. G. (2019). Community Physiological Ecology. *Trends in Ecology and Evolution*, *34*(6), 510–518. https://doi.org/10.1016/j.tree.2019.02.002

Wu, T., Ge, M., Wu, M., Duan, F., Liang, J., Chen, M., Gracida, X., Liu, H., Yang, W., Dar, A. R., Li, C., Butcher, R. A., Saltzman, A. L., & Zhang, Y. (2023). Pathogenic bacteria modulate pheromone response to promote mating. *Nature*, *613*(7943), 324–331. https://doi.org/10.1038/s41586-022-05561-9

Yorinks, N., & Atkinson, C. T. (2000). Effects of Malaria on Activity Budgets of Experimentally Infected Juvenile Apapane (*Himatione sanguinea*). *The Auk*, *117*(3), 731–738.

Ch. 2

Nutritional stress during songbird development impacts nestling growth, but not responses to disease later in life

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Abstract

Early life nutritional stress can affect an individual's future phenotype, including host responses to disease. Nestling songbirds are especially vulnerable to nutritional stress because they are reliant on food from their parents to develop. Because feeding rates for a given nestling can vary both within and among nests, developing songbirds face potential energetic trade-offs during development of body systems, including immune responses. Here we hand-raised nestling house finches (*Haemorhous mexicanus*) and tested how nutritional stress during early development affects their later responses to the pathogen *Mycoplasma gallisepticum* (MG), which commonly affects juveniles in the wild. We raised 10 nestlings from three total nests in captivity, with controls (n=5) receiving *ad libitum* food and nutritionally-stressed nestlings (n=5) receiving 70% the amount fed to controls. We weighed each nestling daily and measured tarsus length every other day. After reaching the juvenile stage (~60 days post-hatch), we inoculated all birds, along with 5 wild-caught juvenile house finches raised by their parents prior to capture, with equal doses of MG. We measured pathogen load over a period of 28 days and disease severity over 42 days. We also measured baseline plasma corticosterone levels on day 5 post-inoculation. We found that nutritionally stressed nestlings gained significantly less mass during the hand-rearing period than controls, but tarsus growth did not differ between the two groups. There were no significant differences in disease severity or pathogen load between nutritionally stressed and control birds. Interestingly, corticosterone levels were higher in MG-infected wild-caught finches compared to those raised in captivity and not nutritionally-stressed, which may be indicative of an effect of rearing environment on the development of stress responses to MG infection. Our results provide further understanding of the ways in which early life stressors can affect how individuals interact with and respond to stressors later in life.

Introduction

The conditions experienced in early life can vary, and this variation can affect many aspects of an individual's future phenotype. Young animals are particularly vulnerable to

stressors present within their environment, as they are still developing many of the systems that are vital for responding to these challenges (Eyck et al., 2019). Once a stressor occurs, effects from this early life exposure can have long-term consequences throughout an individual's lifetime. The scope of phenotypes that can be affected during early life development is wide-ranging and can include multiple systems within an individual, from behavior (Anisman et al., 1998; Smith & Pollak, 2020) to host responses to disease (reviewed in Avitsur et al., 2015). The way these varied bodily systems develop may also provide feedback on each other. For example, rodents that experience stress as pups exhibit an altered glucocorticoid stress response and are more likely to display abnormal adult behaviors (O'Mahony et al., 2009; Shanks et al., 2000), both of which may act to compromise responses to infection (Avitsur et al., 2006).

Altricial young are particularly susceptible to stressors within their environment, as they are rapidly developing outside of their mother's body. Though many different species produce altricial young, songbirds provide an excellent model for studying the effects of stressors during early life (Nowicki et al., 1998; Wada & Coutts, 2021), as, upon hatching, young are immediately exposed to a variety of developmental stressors within their home nest. Further, altricial young of songbirds are entirely reliant upon their parents to provide adequate levels of nutrition in order for them to undergo this rapid period of development, making parental care vital to songbird survival past the nestling stage of development (Burley & Johnson, 2002; Nowicki et al., 1998). Because altricial nestlings are provided finite nutritional resources, solely at the parent's discretion, nestlings may face trade-offs in allocating energy towards their numerous developing systems (Nowicki et al., 1998; Ricklefs, 1979; Wada & Coutts, 2021) .

The amount of food that altricial nestlings receive during development can vary both within and between nests. For example, while all songbird nestlings typically receive access to parental resources like food, these are often not equally distributed among nestlings within a brood. Many bird species begin incubation before the last egg of a clutch is laid, leading to hatching asynchrony that provides earlier-hatched young with more parental resources at the beginning of their lives, leading to significant competitive advantages against their later-hatching siblings (Magrath, 1990). Further, brood size can vary significantly among nests, with *per capita* feeding rates and amounts generally decreasing as the number of young in a nest increase (e.g., Saino et al., 1997). Overall, the extra time that earlier hatched young spend outside of the egg (anywhere from days to hours) with reduced sibling competition for parental food resources

likely leads to higher growth rate and increased body size compared to later-hatched offspring, both of which may impact the development of an individual's phenotype (Naguib et al., 2011; Nettle et al., 2016; Neuenschwander et al., 2003). Thus, nestlings that receive inadequate food allocation during development, such as those that hatch later in a nest or those in larger broods, may suffer fitness costs throughout their lifetime (Song et al., 2019).

While individuals may incur lifelong fitness costs due to stressors during development, it is also possible that exposure to stressors early in life may act to prepare an animal for future challenges, priming them to respond to their environment as adults (Monaghan, 2008). For animals living in harsher environments, such as those simulated by experimental nutritional stress studies, it may actually benefit individuals to experience these conditions early in life, so that their developing systems are better attuned to suboptimal conditions. For birds facing nutritional stress during development, it may be that individuals still experience short-term negative impacts during early life, such as impeded mass growth; however, it may be that this allows an individual to invest more energy towards developing phenotypes that are positive in the long-term (reviewed in Crino & Breuner, 2015). Though seemingly negative, these developmental stressors may actually be adaptive to the individual, providing overall fitness benefits depending on their environment.

As with many other organismal body systems, the immune system undergoes rapid development during early life, and, thus, is susceptible to possible trade-offs facilitated by developmental nutritional stress (Soler et al., 2003). If nestlings are given differing amounts of food, each nestling will differ in the amount of resources allocated towards various developing systems, including the immune system. This trade-off has been documented in multiple species of songbirds. For example, captive-reared song sparrows (*Melospiza melodia*) that were food restricted mounted weaker immune responses than control individuals that were fed to satiation (Schmidt et al., 2015). Additionally, Nettle et al. (2016) found that, in European starling (*Sturnus vulgaris*) nests that had high sibling competition, individual nestlings experienced greater differences in immune responses. Further, these differences were related to body size, suggesting that larger nestlings were given more resources by the parents and, therefore, were able to invest more energy towards their immune system. Because altricial songbirds do not develop a full immune response until they reach adulthood, conditions within the nesting stage can ultimately determine immune function as adults (Hõrak et al., 1999). In particular, altered or compromised

development of immune systems as a nestling could lead to altered host responses to infectious disease as an adult. Although there is a large body of work examining the impacts of developmental stress on immune function through simulated immune challenges, few studies to date have examined individual responses to actual pathogen infection, which is critical to elucidating the downstream effects on host fitness. Further, to fully understand how host responses to disease are affected by early-life challenges in the wild, it is important to study the development of immune responses with actual pathogens, as in these studies the host immune system is continuously fighting off an actively replicating entity, rather than responding to a one-time challenge.

There are several ways in which the early life environment might influence host responses to infection. First, nutritional stress during early life development may decrease later-life pathogen resistance, defined here as the ability to prevent or minimize pathogen replication. However, in systems where immunopathology is the main fitness-reducing component of infection, as is common for many diseases that cause inflammation (Graham et al., 2005; Sears et al., 2011), nutritional stress may actually lead to increased disease tolerance for hosts, defined here as reducing overall fitness costs incurred during infection (Råberg, 2014; Råberg et al., 2007). The effects of early life stress may also act via other physiological mediators that can play a role in determining whether individuals will display a more resistant or more tolerant phenotype during infection. For some host-pathogen systems, the glucocorticoid stress response appears to be important in mediating host responses (e.g., Owen et al., 2012; Schoenle et al., 2018, 2019). Glucocorticoids, such as corticosterone in birds, are metabolic hormones that can shift physiological responses of animals, allowing them to react to changes within an environment and maintain their fitness (e.g., Breuner et al., 2008; Casto et al., 2001; Wingfield et al., 1998). The interaction between glucocorticoids and physiology can have important implications for an animal's response to pathogens, especially during the acute phase of a host's response to pathogens (Besedovsky & Del Rey, 1996), characterized by inflammatory responses and sickness behaviors often associated with fitness costs of infection. Animals with high glucocorticoid levels may actually show decreased pathology during the acute phase of infection by suppressing energy allocation to inflammatory responses of the immune system (Dantzer, 2004), which, in turn, may increase an individual's tolerance to a pathogen (Adelman & Hawley, 2017). On the other hand, high glucocorticoid levels can also be associated with decreased

resistance to disease (e.g., Belden & Kiesecker, 2005; Maule et al., 1989). Therefore, it is possible that increased glucocorticoid levels, such as those caused by developmental stress (reviewed in Anisman et al., 1998) may lead to a more tolerant or more resistant phenotype when facing infection in adulthood.

We investigated how early life nutritional stress impacted post-fledging responses to disease using a naturally-occurring songbird-pathogen system: house finches (*Haemorhous mexicanus*) infected with the bacterial pathogen *Mycoplasma gallisepticum* (MG). This pathogen causes mycoplasmal conjunctivitis in house finches (Kollias et al., 2004), an inflammatory disease that is largely driven by a bird's immune system (Vinkler et al., 2018). Therefore, any notable changes in inflammatory immune responses due to developmental stress would potentially influence the severity of conjunctivitis during infection. Throughout development, we hand-fed nestling house finches using one of two nutritional treatments, based on work done in a similarly sized songbird, the swamp sparrow (*Melospiza georgiana*) (Nowicki et al., 2002): nestlings were either fed to satiation (control treatment) or were restricted to 70% of the total food amount given to control nestlings (nutritional stress treatment). Two measures of nestling condition and growth, body mass and tarsus length, were recorded throughout the hand-rearing period. Approximately 60 days post-hatch, when birds were considered juveniles, we inoculated birds with MG (infected treatment) to determine host responses to infection. Additionally, because being raised in captivity and exposed to repeated handling by humans may constitute a stressor in itself (Lynn et al., 2013; Sagar et al., 2019) we controlled for any differences we might observe due to hand-rearing by inoculating wild-caught finches (who had been fully raised in the wild by their parents) with either MG or a sham control (sterile media). To assess differences in disease severity in response to MG, we measured conjunctivitis severity, pathogen load in the conjunctiva, and mass weekly during infection. Further, because we know that the glucocorticoid response interacts with host responses to pathogens (O'Dwyer et al., 2020), we also collected blood to measure corticosterone, the primary avian glucocorticoid, on day 5 post-inoculation.

Given prior work demonstrating effects of developmental stress on immune function in altricial birds (Chin et al., 2013; Fargallo et al., 2002; Musgrove et al., 2017; Schmidt et al., 2015), we predicted that nutritional stress during development will cause energetic trade-offs that ultimately compromise a house finch's immune response to MG infection later in life. However, suppression of immune function in this system could manifest as higher pathogen loads and

disease severity, or potentially as reduced disease severity during infection, given the apparent role of immune over-reactivity in mediating disease severity in this system (Vinkler et al. 2018).

Methods

Nest searching, nest collection, intake, and captive housing

House finch nests were found across the Virginia Tech campus (Blacksburg, VA, USA) using parental behavioral cues and surveying likely habitats. Out of all identified nests, three nests met our two main collection criteria: 1) surviving until nestlings were at least 3 days old and 2) being relatively synchronous, which we defined as the youngest nestlings in each nest hatching within 3-4 days of one another. Having synchronous nests allowed for simplicity in feeding schedules, as all nests would reach developmental milestones, such as fledging, at similar times. Once nests were located, we monitored them every other day, which allowed us to keep a record of the hatch day of each nestling with minimal disturbance to the nest. As each nestling hatched, we assigned it a hatch order and gave nestlings a corresponding distinct painted toenail. If two nestlings hatched on a day the nest was not checked, we determined hatch order by mass, with the heavier bird being assumed to be the older sibling (Table S1).

Once all nestlings were at least 3 days old, but not older than 5 days, we collected the whole nest and all nestlings within a clear, ventilated plastic tupperware container and transported it to our captive facility. Collection of nests and nestlings was permitted under the Virginia Department of Game and Inland Fisheries (VDGIF 061440) and United States Fish and Wildlife Service (USFWS MB-158404-0). Nests were collected April 27-29, 2021 between 6:30AM and 8:00AM. Upon intake, nestlings were given unique markings with a permanent marker on the top of their heads to denote hatch order and nutritional stress treatment (see *Experimental design* for how treatments were assigned). Placing the markings on top of each nestling's head allowed the person feeding birds to easily identify individuals and their feeding treatment as they were begging, without having to pick up each nestling, which may have impacted their begging response. We also measured each nestling's mass and the length of their tarsus at intake. When nestlings were 7-9 days old, they were given a unique captive metal band and color band combination.

In captivity, nestlings were kept within their home nest for the duration of their nestling period (with one exception noted below). Additionally, each nest was kept in the small plastic

container that had been used during transport. This unit (nest and small container) was then placed in a larger container, which would allow birds to fledge safely. On day 4 in captivity, one nestling was pushed out of the nest by its siblings overnight; to prevent this, we then placed cardboard barriers around each of the nests. These barriers did not appear to impact begging behaviors in any of the nestlings and were removed 2 days before expected date of fledge (when oldest nestlings were ~15 days old), to allow birds to perch on the side of the nest to practice wing flapping.

Nutritional stress treatments

Three wild-collected nests containing a total of 11 nestlings were hand-reared in captivity (n=3 nestlings in nest 1; n=4 nestlings in nests 2 and 3). Our experiment varied nutritional stress in two separate treatments (with treatments alternated within nest to account for non-independence of siblings): a control group, where nestlings were fed to satiation, and a nutritional stress treatment group that was fed 70% the amount fed to controls. This protocol was based on previous nutritional stress studies in a similarly-sized songbird, that saw effects of restricting nestlings to 70% of control food on various aspects of behavior and neurodevelopment (Nowicki et al., 2002). Nutritional stress treatments were alternated within a nest based on intake mass to control for nestling size, with the heaviest nestling in nest 1 being randomly assigned to a nutritional stress treatment, then nutritional stress treatments alternating for other nestlings within that nest. To control for nest origin, we also alternated nutritional stress treatments between nests, so that in some nests the heaviest nestling was in the control treatment and others the heaviest nestling was nutritionally stressed. One nestling in nest 3 hatched much earlier than its nestmates (3 days before closest sibling), which aligned with the ages of nest 1. Thus, while we brought this nestling in and hand-reared it with nest 1, it was assigned a treatment based on the alternating pattern of nest 3 (see Table S1 for more information).

Hand-rearing, growth tracking, and fledging

All nestlings were fed a room temperature slurry of Katyee™ Exact Handfeeding Baby Bird food, in 1cc syringes. Feedings occurred every 30 minutes from 6:00AM-9:00PM, to mimic feeding times in the wild. All control birds within a nest were fed first until their crops were full and they slowed begging. Within each nest, the total amount of food given to each control

nestling was averaged and then multiplied by 70% to determine the amounts to be given to birds in the nutritional stress treatment. Except on intake day, daily food amounts were calculated at the 7AM feeding and stayed consistent throughout the remainder of the day. Mass was taken daily at 1:30PM, and we skipped this feeding in order to ensure the recorded mass did not include any mass added by having food in the crop. In addition to mass, we measured tarsus length every other day using calipers. These two measurements were used to document growth rates of the nestlings over the entirety of hand-rearing.

Nestlings fledged at approximately 17 days old, and, in our nests, all birds fledged within ~30 minutes of each other. Once fledged, we moved each bird from the large plastic tub containing the nest into a small, flat-bottomed cage. Each fledgling cage had 2 perches, a fake nest in one corner, and a small water dish. After a bird had fledged, hand-feeding occurred every 1 hour and was supplemented by several *ad libitum* food choices (crumbled hard boiled eggs, ground sunflower seeds, and soaked sunflower seeds with fruit) to encourage birds to begin self-feeding. Birds were video recorded in between hand-feeding times to detect when they began to eat on their own. Once a bird was confirmed to be self-feeding, they were moved to an every-3-hour hand-feeding schedule, as long as they were still begging for the syringe. Hand-rearing was stopped for all birds after the youngest in the nest reached 25 days old, and all of our birds had begun eating *ad libitum* by this time. Once they were 25 days old, birds were pair-housed in flight cages and provided food (a 4:1 mix of Roudybush Maintenance Nibbles and hulled sunflower seed) and water *ad libitum.*

After hand-raised birds were at least 60 days old, we inoculated them with MG to determine the effects of nutritional stress on individual response to MG. One nestling died after being pushed out of the nest by its siblings, resulting in a total of 10 captive-raised birds in our experiment (n=5 per each nutritional stress treatment). Additionally, to control for any differences in host responses to disease due to being hand-reared in the lab, we captured and inoculated 9 wild-caught juvenile birds (who had been raised by their house finch parents and captured post-fledging) with either MG (n=5) or sterile media as a sham control (n=4).

Inoculation and disease monitoring

After the 10 hand-raised birds were approximately 60 days old (considered juveniles; Badyaev et al., 2020), they were individually housed in wire-mesh flight cages (76 x 46 x 46

cm). We inoculated them with MG to determine the effects of nutritional stress on individual response to MG. The death of one nestling (see above) resulted in a sample size of 10 captive-raised birds in our experiment (n=5 per each nutritional stress treatment).

Additionally, to control for any differences in host responses to disease due to being hand-reared in the lab, we captured and inoculated 9 wild-caught juvenile birds (who had been raised by their house finch parents and captured post-fledging) with either MG (n=5) or sterile media as a sham control (n=4). These birds were captured under the same permits listed above on the Virginia Tech campus (Blacksburg, VA) in June 2021 using baited wire traps placed over a bird feeder. We determined all birds were within their hatch year through plumage, the presence of a yellow gape line, and the lack of either a brood patch or a cloacal protuberance. Because we were examining responses to experimental MG inoculation, we ensured that all birds had not had prior MG exposure by catching all nine wild-caught birds in their captive cages three times over a 14-day period post-capture to examine them for clinical signs of MG. None of the nine birds developed signs of MG before experimental inoculation.

We moved our wild-caught birds to be single-housed in the same flight cages as captive-reared birds. All 19 birds were housed in the same housing room, with constant daylength and temperature. On 30 June 2021, birds were inoculated bilaterally in the conjunctiva with 70 µL of either MG in Frey's media (infection treatment: captive-raised n=10, wild-caught n=5) or with the same volume of sterile media alone (sham-inoculated treatment: wild-caught n=4). We used a strain of MG (NC2006) known to be highly virulent in this population (Fleming-Davies et al., 2018; Langager et al., 2023).

We scored disease severity weekly for all birds for up to 64 days post-inoculation (DPI) on a 0-3 scale, with a score of 0 for birds displaying no signs of conjunctivitis and a score of 3 indicating severe conjunctivitis (Hawley et al., 2011). Though we started to see a reduction in conjunctival swelling after 35 DPI, we scored disease severity until 64 DPI to detect any differences present in recovery rate. We also swabbed the conjunctiva weekly until 28 DPI to quantify MG load. Swabs were stored in 300 µL of tryptose phosphate broth (TPB) at -20˚ C until DNA extraction using Qiagen 96 DNeasy Blood and Tissue Kit. The amount of MG present in each bird's conjunctiva was determined using a probe-based qPCR as outlined in previous work (Hawley et al., 2011). Mass was taken for each bird weekly until 35 DPI.

Blood samples were collected for baseline corticosterone concentrations on 5 DPI, a time point that has been shown in previous work to represent peak corticosterone concentration shifts in captive birds infected with MG (Love et al., 2016). Approximately 100 µL of blood was collected from the brachial vein using heparinized micro-hematocrit capillary tubes within 3 minutes of entering the rooms where birds were housed, a period of time that allows for the detection of baseline corticosterone levels before concentrations begin to rise in response to capture (Romero & Romero, 2002). After collection, all samples were placed immediately on ice and then centrifuged for 7 minutes to separate blood plasma from red blood cells. Plasma samples were stored at -20˚ C until corticosterone extraction and quantification using radioimmunoassay (RIA) and final plasma corticosterone concentrations were corrected based on individual extraction efficiency, based on a standard RIA protocol (Schoenle et al., 2018). One sample was obtained 4 minutes after capture (wild-caught sham control), however, because this bird's baseline was within range of other samples and its inclusion did not shift our results in any way, we included this bird in our analyses.

Statistical analysis

All analyses were done in R v. 4.3.2 (R Core Team, 2023) and all plots were made in ggplot2 (Wickham, 2016).

Nestling growth

To determine whether our nutritional stress treatment had an effect on nestling growth in our hand-reared birds, we fit two separate three-parameter (3P) logistic growth curves to our mass growth and tarsus length growth data, taken over the nutritional stress period. To fit the 3P logistic growth curve, we used the equation $f(x) = \frac{L}{1+e^{-k(x-x_0)}}$, where L is the maximum mass or tarsus length value, x_0 is the time point where growth rate is maximized, and k is the logistic scaling parameter that represents how quickly the growth rate changes over time. We then compared the parameters L, x_0, and k in both our mass and tarsus 3P logistic growth curves by treatment, using a binary indicator variable.

Disease Severity and Pathogen Load

Within the MG-inoculated treatment only (n=15 birds total: n=5 captive-raised nutritionally-stressed, n=5 captive-raised nutritional controls, n=5 wild-raised), we tested whether nutritional treatment (control or nutritional stress) and captive raising influenced either disease severity or pathogen load using separate GLMMs (in the package glmmTMB; Brooks et al., 2017). None of the 4 wild-raised sham-control individuals were included in our analyses of disease severity or pathogen load. We included nutritional treatment and rearing environment (captive-raised nutritionally-stressed, captive-raised controls, and wild-raised juveniles), days post-inoculation, and the interaction of nutritional treatment and days post-inoculation as predictor variables. Because both pathogen load and disease severity were taken over multiple sampling periods for each individual, bird ID was added to each model as a random effect. Interactions were removed from final models if $p>0.1$. The Anova function (car package; Fox & Weisberg, 2019) was used to test the significance of the predictor variables in our models by generating Type II Wald χ^2 tests.

Corticosterone

To assess any differences in baseline plasma corticosterone levels between our four treatment groups (MG-inoculated captive-raised controls, MG-inoculated captive-raised nutritionally-stressed, MG-inoculated wild-raised, and sham-inoculated wild-raised), we used generalized linear models, using Gamma error distribution linked with the log function. We then tested for pairwise differences between each group using contrasts produced in the emmeans package (Lenth, 2023), which then produced a Tukey-adjusted p-value for each family of contrasts.

Results

Nestling growth

Our non-linear, 3P logistic growth curve analyzing mass growth during the nutritional stress period (nestling and fledgling stage) showed that nutritional treatment produced significantly different values for all three measured parameters: the maximum mass value (L), the time where growth rate was maximized (x_0), and the scale parameter of how quickly growth rate changed over time (k). For our nutritional stress group, the maximum mass achieved during

the early development period was lower, the point of maximal growth occurred later, and the growth rate did not change as rapidly (Fig. 1A; Table 1), compared to our nutritional control group. This suggests that growth in terms of body mass was impaired overall for nutritionally stressed birds.

In contrast, none of our model parameters for tarsus length differed between nutritionally stressed and nutritional control birds, indicating no overall differences in the growth of tarsus length due to nutritional stress (Fig. 1B; Table 2).

Disease severity and pathogen load

There was no significant effect of nutritional treatment on either disease severity (Fig. 2A; Wald chi-squared=0.389, d.f.=2, p=0.82) nor pathogen load (Fig. 2B; Wald chi-squared=0.202, d.f. = 2, p=0.90) within MG-inoculated birds. However, as expected, days post-inoculation did significantly predict differences in both pathogen load (Wald chi-squared= 35.079, d.f.=1, p<0.0001) and disease severity (Wald chi-squared=18.97, d.f.=1, p<0.0001).

Baseline corticosterone concentrations

Wild-raised, MG-inoculated birds were found to have significantly higher baseline plasma corticosterone levels than captive-raised nutritional control birds that had also been inoculated with MG (Fig. 3; $\beta=1.25 \pm 0.437$, t=2.87, p=0.01). However, there were no detected differences between our captive-raised nutritional stressed and nutritional control birds (Fig. 3; $\beta=0.71 \pm 0.437$, t=1.63, p=0.12). *Post-hoc* pairwise confirm a significant difference between only the wild-caught, MG-inoculated birds and the captive-raised nutritional control birds inoculated with MG (t ratio=-2.87, p=0.05). There was a moderate, but non-significant pairwise difference detected between our wild-raised sham control birds and our wild-raised MG-inoculated birds, with the MG-inoculated birds displaying higher corticosterone levels than sham controls (t ratio=-2.78, p=0.06). All other pairwise comparisons were not significant.

Discussion

In this study, we find that house finch responses to disease appear resilient to developmental food restriction. Specifically, we found that nestlings exposed to nutritional stress experienced impairments in body mass, but not skeletal growth, while they are nutritionally

dependent. Yet despite these differences in mass accumulation during early life, these same nutritionally stressed birds did not show detectable differences in their responses to experimental infection with *Mycoplasma gallisepticum* (MG) when compared to both captive-raised, nutritional control birds and wild-raised birds. These results indicate that, when faced with energetic trade-offs, house finches prioritize development of some bodily systems above others, notably immune responses and bone growth. While there were no differences in either disease severity or pathogen load across our treatments, our wild-raised, MG-infected birds did have higher baseline corticosterone levels than birds in our captive-raised, nutritional control treatment, which may demonstrate the importance of rearing environment in the development of endocrine responses during infection.

Our nutritional stress treatment had detectable effects on overall rates of daily mass gained throughout the nestling growth period, suggesting that nestling nutrition was constrained as intended. However, we found no differences in the growth rate of the tarsus bone across nutritional stress treatments. This finding suggests that allocation of the limited energetic resources in nestlings receiving the nutritional stress treatment was focused more on skeletal growth than mass gain, which has been observed in previous studies of nutritionally stressed nestlings (Killpack & Karasov, 2012; Lepczyk & Karasov, 2000; Moe et al., 2004). Alternatively, it is possible that while we were limiting overall caloric intake in our nutritionally stressed birds, the limited food provided may have still been meeting minimum requirements for other vital nutrients required for skeletal growth, such as calcium, which can greatly impact nestling growth when limited in the diet (Dawson & Bidwell, 2005). Therefore, even though nutritionally-stressed birds may suffer lower mass gain throughout the nestling period, by meeting other nutritional requirements, such as calcium, nestlings may be able to counteract the impacts on their growth over time.

After birds began to eat on their own, we observed a period of rapid growth in which the birds in our nutritional stress treatment were able to "catch up" to control birds' mass by the time they were nutritionally independent through compensatory growth (Wada & Coutts, 2021). Such compensatory growth has been observed in species of birds that produce altricial young (Criscuolo et al., 2008; Hsu et al., 2017; Killpack et al., 2014; Kriengwatana et al., 2013). Though undergoing this rapid period of compensatory growth provides individuals with short-term, size-related benefits, undergoing development at such a fast rate can also have long-term

consequences for many different body systems within an individual (Mangel & Munch, 2005; Metcalfe & Monaghan, 2001) and could lead to lower survival later in life (Dmitriew & Rowe, 2007). Notably, long-term consequences of developmental stress on immune responses have been documented in other studies (Hõrak et al., 1999; Murillo-Rincón et al., 2017; Schmidt et al., 2015; Soler et al., 2003), though there are several additional studies in which nutritional stress does not produce changes in immune response (Carmona-Isunza et al., 2013; Tschirren et al., 2009). Though we did not find any effects of nutritional stress treatment on measured responses to infection, including baseline corticosterone, these systems are complex and multifaceted and, therefore, the effects of compensatory growth, if they are present, may be in aspects of the immune system or endocrine system outside the scope of this project. For example, it is possible that there are impacts of nutritional stress on components of host defense against pathogens that were not measured or detected in our experiment, which focused specifically on pathogen loads and disease severity in response to a pathogen of particular impact for this host species. Despite previous research demonstrating the consequences of developmental stress on immune responses, our results suggest that nutritional stress during development does not notably influence host responses to MG as juveniles, a time period in which free-living house finches commonly experience MG infections (Altizer, Davis, et al., 2004). While this may indicate that the immune system is resilient to stressors in early life, it could also be that, in this system, the immune system is not effective against MG directly, so that if there are any shifts to immune responses due to nutritional stress, we would not have detected them using our metrics of disease severity or pathogen load. Further work with other common pathogens may illuminate whether development of the immune system is conserved during times of energetic trade-off or if our results are simply due to house finch immune systems being ineffective against MG infection.

One limitation of our study is that we did not directly measure immune components of the adaptive immune response, such as post-inoculation antibody responses. While the immune system in altricial birds is not fully developed until adulthood, studies have demonstrated that nestlings can mount an adaptive immune response quite early in development (Grindstaff et al., 2006; Killpack et al., 2013). However, adaptive responses in nestlings can be weaker than those observed in adults (Killpack et al., 2013), suggesting that adaptive responses to infection may still be vulnerable to effects of early-life nutritional stress. In a study of lab-raised mice, those individuals that had been exposed to nutritional stress during development (through maternal

separation) had altered adaptive responses to viral infection and reduced host resistance, which led to higher viral titers, and therefore more harm, to the host (Avitsur et al., 2006). However, development of the adaptive immune system may be robust even in suboptimal early life conditions, as was observed in nutritionally stressed zebra finches (*Taeniopygia guttata*) that showed no differences in adaptive antibody responses compared to control birds (Killpack et al., 2014). Further work is needed to determine if different components of the immune system, such as antibody responses, are impacted by nutritional stress in house finches, and wild animals more broadly.

We also examined how nutritional stress and rearing environment affected levels of the primary avian glucocorticoid, corticosterone, during infection. Previous work has shown that levels of circulating corticosterone can be impacted by developmental nutritional stress (Buchanan et al., 2003; Honarmand et al., 2010; Kriengwatana et al., 2014), which can, in turn, affect host responses to disease (Owen et al., 2012; Schoenle et al., 2018, 2019). Although we hypothesized that nutritional stress would alter corticosterone responses to MG infection in this system, we did not detect any differences between our captive-raised nutritionally-stressed and control treatments in the baseline corticosterone concentrations detected during infection. However, we did detect significantly higher baseline corticosterone concentrations in our wild-raised birds that were infected with MG compared to our infected birds that were captive-raised nutritional controls. Further, wild-raised infected birds have marginally higher circulating baseline corticosterone concentrations than wild-caught, sham-inoculated birds, which mirrors previous work in this system that found a significant difference between MG- and sham-infected birds on day 5 post-inoculation (Love et al. 2006). Further, prior work on free-living house finches that were clinically healthy at the time of capture found baseline levels of corticosterone (range 0.03-6.95 ng/mL; Moyers et al., 2018) that align with our wild-caught, sham-inoculated birds (0.86-6.64 ng/mL). In contrast, the corticosterone levels for our captive-raised, MG-infected birds are lower than expected based on prior work in this system on wild-raised birds only (Love et al., 2016), suggesting a potential influence of captive rearing on baseline corticosterone concentrations in house finches. Though our small sample size limits the inference that can be drawn with respect to potential corticosterone differences, this would be an interesting avenue for future research.

Overall, we found that, although our nutritionally stressed house finches did have lower mass gain throughout early life development (nestling and fledgling) period, they did not differ in their responses to infection with a virulent pathogen, *Mycoplasma gallisepticum*. The overlap in both disease severity scores and pathogen load in our MG-inoculated birds (captive-raised nutritionally-stressed and captive-raised controls) indicates that development of the immune system components used for MG responses may be resilient to many environmental conditions, including nutritional limitation. While our study did not find changes due to nutritional stress in either baseline corticosterone concentrations or disease metrics, it is possible that other bodily systems were impacted by the early-life stress, so that animals that suffer from early life nutritional stress may experience fitness costs due to deficiencies in other areas, such as trouble finding mates (Hsu et al., 2017; Spencer & MacDougall-Shackleton, 2011) or a lower likelihood of survival (Metcalfe & Monaghan, 2001). With increasing amounts of environmental change, it is possible that many species will experience decreases or developmental mismatches in their food sources, which would likely impact rapidly developing young most severely. Therefore, it is vital to understand how fluctuating environmental conditions, particularly reduced food availability, might impact individual fitness as a whole.

Acknowledgements

We thank John Brule, Andrew Hynes, Sara Teemer Richards, and Dr. Chava Weitzman for their assistance during the experiment. We also thank Susan Peters for sharing her extensive knowledge of hand-rearing nestling songbirds with us. We thank Dr. Jeff Walters for his helpful input. Our experiments were conducted under approved Virginia Tech Institutional Animal Care and Use Committee protocol. All experimental methods were carried out in accordance with the guidelines and regulations set forth by the Virginia Tech IACUC and Virginia Department of Game and Inland Fisheries. This work was funded by a Sigma Xi Grant-in-Aid of Research (to M.M.L), the Virginia Tech Graduate Student Assembly Graduate Research Development Program (to M.M.L), and NSF grant IOS-1754872 (to D.M.H).

References

Adelman, J. S., & Hawley, D. M. (2017). Tolerance of infection: A role for animal behavior, potential immune mechanisms, and consequences for parasite transmission. *Hormones and Behavior*, *88*, 79–86.

Altizer, S., Davis, A. K., Cook, K. C., & Cherry, J. J. (2004). Age, sex, and season affect the risk of mycoplasmal conjunctivitis in a southeastern house finch population. *Canadian Journal of Zoology*, *82*, 755–763.

Anisman, H., Zaharia, M. D., Meaney, M. J., & Merali, Z. (1998). Do early-life events permanently alter behavioral and hormonal responses to stressors? *International Journal of Developmental Neuroscience*, *16*(3/4), 149–164.

Avitsur, R., Hunzeker, J., & Sheridan, J. F. (2006). Role of early stress in the individual differences in host response to viral infection. *Brain, Behavior, and Immunity*, *20*, 339–348.

Avitsur, R., Levy, S., Goren, N., & Grinshpahet, R. (2015). Early adversity, immunity and infectious disease. *Stress*, *18*(3), 289–296. https://doi.org/10.3109/10253890.2015.1017464

Badyaev, A. V., Belloni, V., & Hill, G. E. (2020). House Finch (*Haemorhous mexicanus*). In *Birds of the World,* A. F. Poole (Ed.). Cornell Lab of Ornithology. https://doi.org/10.2173/bow.houfin.01

Belden, L. K., & Kiesecker, J. M. (2005). Glucocorticosteroid Hormone Treatment of Larval Treefrogs Increases Infection by *Alaria* Sp. Trematode Cercariae. *Journal of Parasitology*, *91*(3), 686–688.

Besedovsky, H., & Del Rey, A. (1996). Immune-Neuro-Endocrine Interactions: Facts and Hypotheses. *Endocrine Reviews*, *17*(1), 64–102.

Breuner, C. W., Patterson, S. H., & Hahn, T. P. (2008). In search of relationships between the acute adrenocortical response and fitness. *General and Comparative Endocrinology*, *157*, 288–295.

Brooks, M. E., Kristensen, K., van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug, H. J., Maechler, M., & Bolker, B. M. (2017). *glmmTMB* Balances speed and

flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal*, *9*(2), 378–400.

Buchanan, K. L., Spencer, K. A., Goldsmith, A. R., & Catchpole, C. K. (2003). Song as an honest signal of past developmental stress in the European starling (*Sturnus vulgaris*). *Proceedings of the Royal Society B: Biological Sciences*, *270*(1520), 1149–1156. https://doi.org/10.1098/rspb.2003.2330

Burley, N. T., & Johnson, K. (2002). The evolution of avian parental care. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *357*(1419), 241–250. https://doi.org/10.1098/rstb.2001.0923

Carmona-Isunza, M. C., Núñez-de la Mora, A., & Drummond, H. (2013). Chronic stress in infancy fails to affect body size and immune response of adult female blue-footed boobies or their offspring. *Journal of Avian Biology*, *44*(4), 390–398. https://doi.org/10.1111/j.1600-048X.2013.00057.x

Casto, J. M., Nolan, Jr., V., & Ketterson, E. D. (2001). Steroid Hormones and Immune Function: Experimental Studies in Wild and Captive Dark-Eyed Juncos (*Junco hyemalis*). *The American Naturalist*, *157*(4), 408–420.

Chin, E. H., Quinn, J. S., & Burness, G. (2013). Acute stress during ontogeny suppresses innate, but not acquired immunity in a semi-precocial bird (*Larus delawarensis*). *General and Comparative Endocrinology*, *193*, 185–192. https://doi.org/10.1016/j.ygcen.2013.08.007

Crino, O. L., & Breuner, C. W. (2015). Developmental stress: evidence for positive phenotypic and fitness effects in birds. *Journal of Ornithology*, *156*(1), 389–398. https://doi.org/10.1007/s10336-015-1236-z

Criscuolo, F., Monaghan, P., Nasir, L., & Metcalfe, N. B. (2008). Early nutrition and phenotypic development: “Catch-up” growth leads to elevated metabolic rate in adulthood. *Proceedings of the Royal Society B: Biological Sciences*, *275*(1642), 1565–1570. https://doi.org/10.1098/rspb.2008.0148

Dantzer, R. (2004). Cytokine-induced sickness behaviour: A neuroimmune response to activation of innate immunity. *European Journal of Pharmacology*, *500*, 399–411.

Dawson, R. D., & Bidwell, M. T. (2005). Dietary calcium limits size and growth of nestling tree swallows *Tachycineta bicolor* in a non-acidified landscape. *Journal of Avian Biology*, *36*(2), 127–134. https://doi.org/10.1111/j.0908-8857.2005.03406.x

Dmitriew, C., & Rowe, L. (2007). Effects of early resource limitation and compensatory growth on lifetime fitness in the ladybird beetle (*Harmonia axyridis*). *Journal of Evolutionary Biology*, *20*(4), 1298–1310. https://doi.org/10.1111/j.1420-9101.2007.01349.x

Eyck, H. J. F., Buchanan, K. L., Crino, O. L., & Jessop, T. S. (2019). Effects of developmental stress on animal phenotype and performance: a quantitative review. *Biological Reviews*, *94*(3), 1143–1160. https://doi.org/10.1111/brv.12496

Fargallo, J. A., Laaksonen, T., Pöyri, V., & Korpimäki, E. (2002). Inter-sexual differences in the immune response of Eurasian kestrel nestlings under food shortage. *Ecology Letters*, *5*(1), 95–101. https://doi.org/10.1046/j.1461-0248.2002.00290.x

Fleming-Davies, A. E., Williams, P. D., Dhondt, A. A., Dobson, A. P., Hochachka, W. M., Leon, A. E., Ley, D. H., Osnas, E. E., & Hawley, D. M. (2018). Incomplete host immunity favors the evolution of virulence in an emergent pathogen. *Science*, *359*, 1030–1033. https://www.science.org

Fox, J., & Weisberg, S. (2019). *An R Companion to Applied Regression*. Sage. https://socialsciences.mcmaster.ca/jfox/Books/Companion/

Graham, A. L., Allen, J. E., & Read, A. F. (2005). Evolutionary causes and consequences of immunopathology. *Annual Review of Ecology, Evolution, and Systematics*, *36*, 373–397. https://doi.org/10.1146/annurev.ecolsys.36.102003.152622

Grindstaff, J. L., Hasselquist, D., Nilsson, J. Å., Sandell, M., Smith, H. G., & Stjernman, M. (2006). Transgenerational priming of immunity: Maternal exposure to a bacterial antigen enhances offspring humoral immunity. *Proceedings of the Royal Society B: Biological Sciences*, *273*(1600), 2551–2557. https://doi.org/10.1098/rspb.2006.3608

Hawley, D. M., Grodio, J., Frasca, S., Kirkpatrick, L., & Ley, D. H. (2011). Experimental infection of domestic canaries (*Serinus canaria domestica*) with *Mycoplasma gallisepticum:*

A new model system for a wildlife disease. *Avian Pathology*, *40*(3), 321–327. https://doi.org/10.1080/03079457.2011.571660

Honarmand, M., Goymann, W., & Naguib, M. (2010). Stressful dieting: Nutritional conditions but not compensatory growth elevate corticosterone levels in zebra finch nestlings and fledglings. *PLoS ONE*, *5*(9). https://doi.org/10.1371/journal.pone.0012930

Hõrak, P., Tegelmann, L., Ots, I., & Møller, A. P. (1999). Immune Function and Survival of Great Tit Nestlings in Relation to Growth Conditions. *Oecologia*, *121*(3), 316–322.

Hsu, B. Y., Dijkstra, C., & Groothuis, T. G. G. (2017). Organizing effects of adverse early-life condition on body mass, compensatory growth and reproduction: experimental studies in rock pigeons. *Journal of Avian Biology*, *48*(8), 1166–1176. https://doi.org/10.1111/jav.01172

Killpack, T. L., & Karasov, W. H. (2012). Growth and development of house sparrows (*Passer domesticus*) in response to chronic food restriction throughout the nestling period. *Journal of Experimental Biology*, *215*(11), 1806–1815. https://doi.org/10.1242/jeb.066316

Killpack, T. L., Oguchi, Y., & Karasov, W. H. (2013). Ontogenetic patterns of constitutive immune parameters in altricial house sparrows. *Journal of Avian Biology*, *44*(6), 513–520. https://doi.org/10.1111/j.1600-048X.2013.00239.x

Killpack, T. L., Tie, D. N., & Karasov, W. H. (2014). Compensatory growth in nestling Zebra Finches impacts body composition but not adaptive immune function. *The Auk*, *131*(3), 396–406. https://doi.org/10.2307/90008555

Kollias, G. V., Sydenstricker, K. V., Kollias, H. W., Ley, D. H., Hosseini, P. R., Connolly, V., & Dhondt, A. A. (2004). Experimental infection of house finches with *Mycoplasma gallisepticum*. *Journal of Wildlife Diseases*, *40*(1), 79–86. https://doi.org/10.7589/0090-3558-40.1.79

Kriengwatana, B., Wada, H., Macmillan, A., & MacDougall-Shackleton, S. A. (2013). Juvenile nutritional stress affects growth rate, adult organ mass, and innate immune function in zebra finches (*Taeniopygia guttata*). *Physiological and Biochemical Zoology*, *86*(6), 769–781. https://doi.org/10.1086/673260

Kriengwatana, B., Wada, H., Schmidt, K. L., Taves, M. D., Soma, K. K., & MacDougall-Shackleton, S. A. (2014). Effects of nutritional stress during different developmental periods on song and the hypothalamic-pituitary-adrenal axis in zebra finches. *Hormones and Behavior*, *65*(3), 285–293. https://doi.org/10.1016/j.yhbeh.2013.12.013

Langager, M. M., Adelman, J. S., & Hawley, D. M. (2023). Let's stick together: Infection enhances preferences for social grouping in a songbird species. *Ecology and Evolution*, *13*(10). https://doi.org/10.1002/ece3.10627

Lenth, R. V. (2023). *emmeans: Estimated Marginal Means, aka Least-Squares Means*. https://CRAN.R-project.org/package=emmeans

Lepczyk, C. A., & Karasov, W. H. (2000). Effect of Ephemeral Food Restriction on Growth of House Sparrows. *The Auk*, *117*(1), 164–174. https://www.jstor.org/stable/4089556?seq=1&cid=pdf-reference#references_tab_contents

Love, A. C., Foltz, S. L., Adelman, J. S., Moore, I. T., & Hawley, D. M. (2016). Changes in corticosterone concentrations and behavior during *Mycoplasma gallisepticum* infection in house finches (*Haemorhous mexicanus*). *General and Comparative Endocrinology*, *235*, 70–77. https://doi.org/10.1016/j.ygcen.2016.06.008

Lynn, S. E., Kern, M. D., & Phillips, M. M. (2013). Neonatal handling alters the development of the adrenocortical response to stress in a wild songbird (eastern bluebird, *Sialia sialis*). *General and Comparative Endocrinology*, *186*, 157–163. https://doi.org/10.1016/j.ygcen.2013.03.004

Magrath, R. D. (1990). Hatching asynchrony in altricial birds. *Biological Reviews*, *65*(4), 587–622. https://doi.org/10.1111/j.1469-185x.1990.tb01239.x

Mangel, M., & Munch, S. B. (2005). A Life-History Perspective on Short-and Long-Term Consequences of Compensatory Growth. *The American Naturalist*, *166*(6), E155–E176.

Maule, A. G., Tripp, R. A., Kaattari, S. L., & Schreck, C. B. (1989). Stress alters immune function and disease resistance in chinook salmon (*Oncorhynchus tshawytscha*). *Journal of Endocrinology*, *120*(6), 135–142.

Metcalfe, N. B., & Monaghan, P. (2001). Compensation for a bad start: grow now, pay later? *Trends in Ecology and Evolution*, *16*(5), 254–260. https://archive.org/details/sim_trends-in-ecology-evolution_2001-05_16_5

Moe, B., Brunvoll, S., Mork, D., Brobakk, T. E., & Bech, C. (2004). Developmental plasticity of physiology and morphology in diet-restricted European shag nestlings (*Phalacrocorax aristotelis*). *Journal of Experimental Biology*, *207*(23), 4067–4076. https://doi.org/10.1242/jeb.01226

Monaghan, P. (2008). Early growth conditions, phenotypic development and environmental change. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *363*(1497), 1635–1645. https://doi.org/10.1098/rstb.2007.0011

Moyers, S. C., Adelman, J. S., Farine, D. R., Moore, I. T., & Hawley, D. M. (2018). Exploratory behavior is linked to stress physiology and social network centrality in free-living house finches (*Haemorhous mexicanus*). *Hormones and Behavior*, *102*, 105–113. https://doi.org/10.1016/j.yhbeh.2018.05.005

Murillo-Rincón, A. P., Laurila, A., & Orizaola, G. (2017). Compensating for delayed hatching reduces offspring immune response and increases life-history costs. *Oikos*, *126*(4), 565–571. https://doi.org/10.1111/oik.04014

Musgrove, A. B., Wiebe, K. L., Parker, C. E., & Romero, L. M. (2017). Brood size manipulations reveal relationships among physiological performance, body condition and plumage colour in Northern Flicker *Colaptes auratus* nestlings. *Ibis*, *159*(3), 600–610.

Naguib, M., Flörcke, C., & Van Oers, K. (2011). Effects of social conditions during early development on stress response and personality traits in great tits (*Parus major*). *Developmental Psychobiology*, *53*(6), 592–600.

Nettle, D., Andrews, C., Reichert, S., Bedford, T., Gott, A., Parker, C., Kolenda, C., Martin-Ruiz, C., Monaghan, P., & Bateson, M. (2016). Brood size moderates associations between relative size, telomere length, and immune development in European starling nestlings. *Ecology and Evolution*, *6*, 8138–8148.

Neuenschwander, S., Brinkhof, M. W. G., Kölliker, M., & Richner, H. (2003). Brood size, sibling competition, and the cost of begging in great tits (*Parus major*). *Behavioral Ecology*, *14*(4), 457–462.

Nowicki, S., Peters, S., & Podos, J. (1998). Song Learning, Early Nutrition and Sexual Selection in Songbirds. *American Zoologist*, *38*, 179–190.

Nowicki, S., Searcy, W. A., & Peters, S. (2002). Brain development, song learning and mate choice in birds: A review and experimental test of the "nutritional stress hypothesis." *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology*, *188*(11–12), 1003–1014. https://doi.org/10.1007/s00359-002-0361-3

O'Dwyer, K., Dargent, F., Forbes, M. R., & Koprivnikar, J. (2020). Parasite infection leads to widespread glucocorticoid hormone increases in vertebrate hosts: A meta-analysis. *Journal of Animal Ecology*, *89*(2), 519–529. https://doi.org/10.1111/1365-2656.13123

O'Mahony, S. M., Marchesi, J. R., Scully, P., Codling, C., Ceolho, A. M., Quigley, E. M. M., Cryan, J. F., & Dinan, T. G. (2009). Early Life Stress Alters Behavior, Immunity, and Microbiota in Rats: Implications for Irritable Bowel Syndrome and Psychiatric Illnesses. *Biological Psychiatry*, *65*(3), 263–267.

Owen, J. C., Nakamura, A., Coon, C. A., & Martin, L. B. (2012). The effect of exogenous corticosterone on West Nile virus infection in Northern Cardinals (*Cardinalis cardinalis*). *Veterinary Research*, *43*(34), 1–9.

R Core Team. (2023). *R: A Language and Enivornment for Statistical Computing*. https://www.R-project.org/

Råberg, L. (2014). How to Live with the Enemy: Understanding Tolerance to Parasites. *PLoS Biology*, *12*(11), 1–4.

Råberg, L., Sim, D., & Read, A. F. (2007). Disentangling Genetic Variation for Resistance and Tolerance to Infectious Diseases in Animals. *Science*, *318*(5851), 812–814.

Ricklefs, R. E. (1979). Adaptation, constraint, and compromise in avian postnatal development. *Biological Reviews*, *54*(3), 269–290. https://doi.org/10.1111/j.1469-185x.1979.tb01013.x

Romero, L. M., & Romero, R. C. (2002). Corticosterone Responses in Wild Birds: the Importance of Rapid Initial Sampling. *The Condor*, *104*(1), 129–135.

Sagar, R. L., Cockrem, J., Rayner, M. J., Stanley, M. C., Welch, J., & Dunphy, B. J. (2019). Regular handling reduces corticosterone stress responses to handling but not condition of semi-precocial mottled petrel (*Pterodroma inexpectata*) chicks. *General and Comparative Endocrinology*, *272*, 1–8. https://doi.org/10.1016/j.ygcen.2018.11.004

Saino, N., Calza, S., & Moller, A. P. (1997). Immunocompetence of Nestling Barn Swallows in Relation to Brood Size and Parental Effort. *Journal of Animal Ecology*, *66*(6), 827–836.

Schmidt, K. L., Kubli, S. P., Macdougall-Shackleton, E. A., & Macdougall-Shackleton, S. A. (2015). Early-life stress has sex-specific effects on immune function in adult song sparrows. *Physiological and Biochemical Zoology*, *88*(2), 183–194. https://doi.org/10.1086/680599

Schoenle, L. A., Moore, I. T., Dudek, A. M., Garcia, E. B., Mays, M., Haussmann, M. F., Cimini, D., & Bonier, F. (2019). Exogenous glucocorticoids amplify the costs of infection by reducing resistance and tolerance, but effects are mitigated by co-infection. *Proceedings of the Royal Society B: Biological Sciences*, *286*, 1–10. http://www.royalsocietypublishing.org/doi/10.1098/rspb.2018.2913

Schoenle, L. A., Schoepf, I., Weinstein, N. M., Moore, I. T., & Bonier, F. (2018). Higher plasma corticosterone is associated with reduced costs of infection in red-winged blackbirds. *General and Comparative Endocrinology*, *256*, 89–98.

Sears, B. F., Rohr, J. R., Allen, J. E., & Martin, L. B. (2011). The economy of inflammation: When is less more? *Trends in Parasitology*, *27*(9), 382–387. https://doi.org/10.1016/j.pt.2011.05.004

Shanks, N., Windle, R. J., Perks, P. A., Harbuz, M. S., Jessop, D. S., Ingram, C. D., & Lightman, S. L. (2000). Early-life exposure to endotoxin alters hypothalamic-pituitary-adrenal function and predisposition to inflammation. *Proceedings of the National Academy of Sciences*, *97*(10), 5645–5650.

Smith, K. E., & Pollak, S. D. (2020). Early life stress and development: potential mechanisms for adverse outcomes. *Journal of Neurodevelopmental Disorders*, *12*(1). https://doi.org/10.1186/s11689-020-09337-y

Soler, J. J., De Neve, L., Pérez-Contreras, T., Soler, M., & Sorci, G. (2003). Trade-off between immunocompetence and growth in magpies: An experimental study. *Proceedings of the Royal Society B: Biological Sciences*, *270*(1512), 241–248.

Song, Z., Zou, Y., Hu, C., Ye, Y., Wang, C., Qing, B., Komdeur, J., & Ding, C. (2019). Silver spoon effects of hatching order in an asynchronous hatching bird. *Behavioral Ecology*, *30*(2), 509–517. https://doi.org/10.1093/beheco/ary191

Spencer, K. A., & MacDougall-Shackleton, S. A. (2011). Indicators of development as sexually selected traits: The developmental stress hypothesis in context. *Behavioral Ecology*, *22*(1), 1–9. https://doi.org/10.1093/beheco/arq068

Tschirren, B., Rutstein, A. N., Postma, E., Mariette, M., & Griffith, S. C. (2009). Short- and long-term consequences of early developmental conditions: A case study on wild and domesticated zebra finches. *Journal of Evolutionary Biology*, *22*(2), 387–395. https://doi.org/10.1111/j.1420-9101.2008.01656.x

Vinkler, M., Leon, A. E., Kirkpatrick, L., Dalloul, R. A., & Hawley, D. M. (2018). Differing House Finch Cytokine Expression Responses to Original and Evolved Isolates of *Mycoplasma gallisepticum*. *Frontiers in Immunology*, *9*(13), 1–16.

Wada, H., & Coutts, V. (2021). Detrimental or beneficial? Untangling the literature on developmental stress studies in birds. *Journal of Experimental Biology*, *224*(19). https://doi.org/10.1242/jeb.227363

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York. https://ggplot2.tidyverse.org

Wingfield, J. C., Maney, D. L., Breuner, C. W., Jacobs, J. D., Lynn, S., Ramenofsky, M., & Richardson, R. D. (1998). Ecological Bases of Hormone-Behavior Interactions: The "Emergency Life History Stage." *American Zoologist*, *38*(1), 191–206.

Table 1. Statistical output for each parameter estimate in our three-parameter logistical growth curve model analyzing nestling mass gain. L is the maximum mass value, k is the scale parameter of how quickly growth rate changed over time, and x_0 is the time at which growth rate was maximal.

Parameter	Control (est ± SE)	Nutritional Stress	Difference	*p*-value
L (g)	18.79 ± 0.16	18.05 ± 0.21	-0.74 ± 0.27	0.0065
$k\ (1/days)$	0.41 ± 0.02	0.31 ± 0.02	0.69 ± 0.22	0.0018
x_0 (days)	6.35 ± 0.13	7.04 ± 0.17	-0.10 ± 0.03	0.0023

Table 2. Statistical output for each parameter estimate in our three-parameter logistical growth curve model analyzing tarsus growth. L is the maximum tarsus length value, k is the scale parameter of how quickly tarsus growth rate changed over time, and x_0 is the time at which tarsus growth rate was maximal.

Parameter	Control (est ± SE)	Nutritional Stress	Difference	*p*-value
L (g)	16.91 ± 0.13	16.72 ± 0.13	-0.19 ± 0.19	0.320
$k\ (1/days)$	0.38 ± 0.03	0.41 ± 0.04	0.03 ± 0.05	0.515
x_0 (days)	3.37 ± 0.22	3.73 ± 0.20	0.36 ± 0.30	0.223

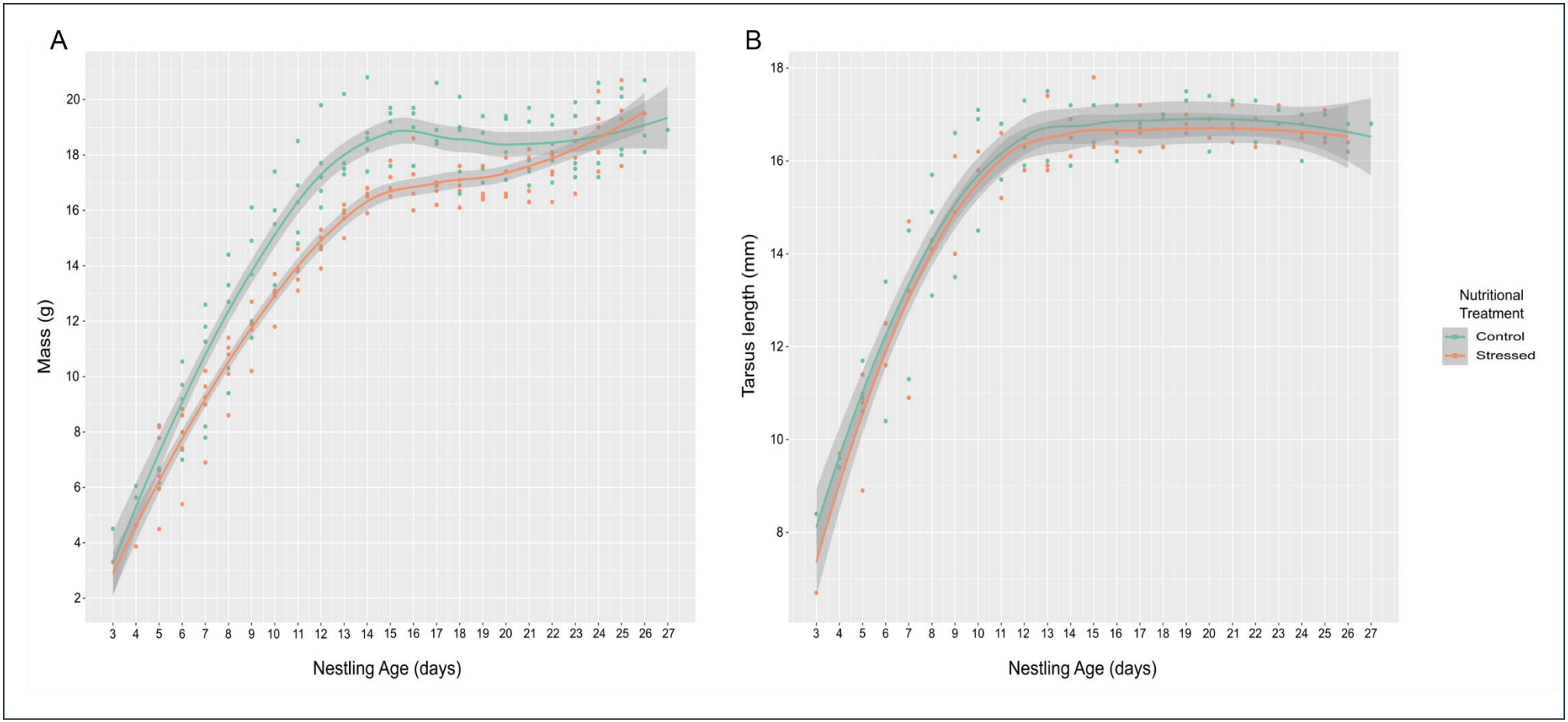

Figure 1. (A) Mass growth over hand-rearing period. All three parameters of our three-parameter logistic growth model were significantly different between captive-raised nutritional controls and nutritional stressed birds, with nutritionally stressed birds having a lower maximum mass and gaining mass more slowly overall than nutritional controls. (B) Tarsus length growth over hand-rearing period. All three parameters of our three-parameter logistic growth model showed no differences between tarsus growth in captive-raised nutritional controls and nutritional stressed birds.

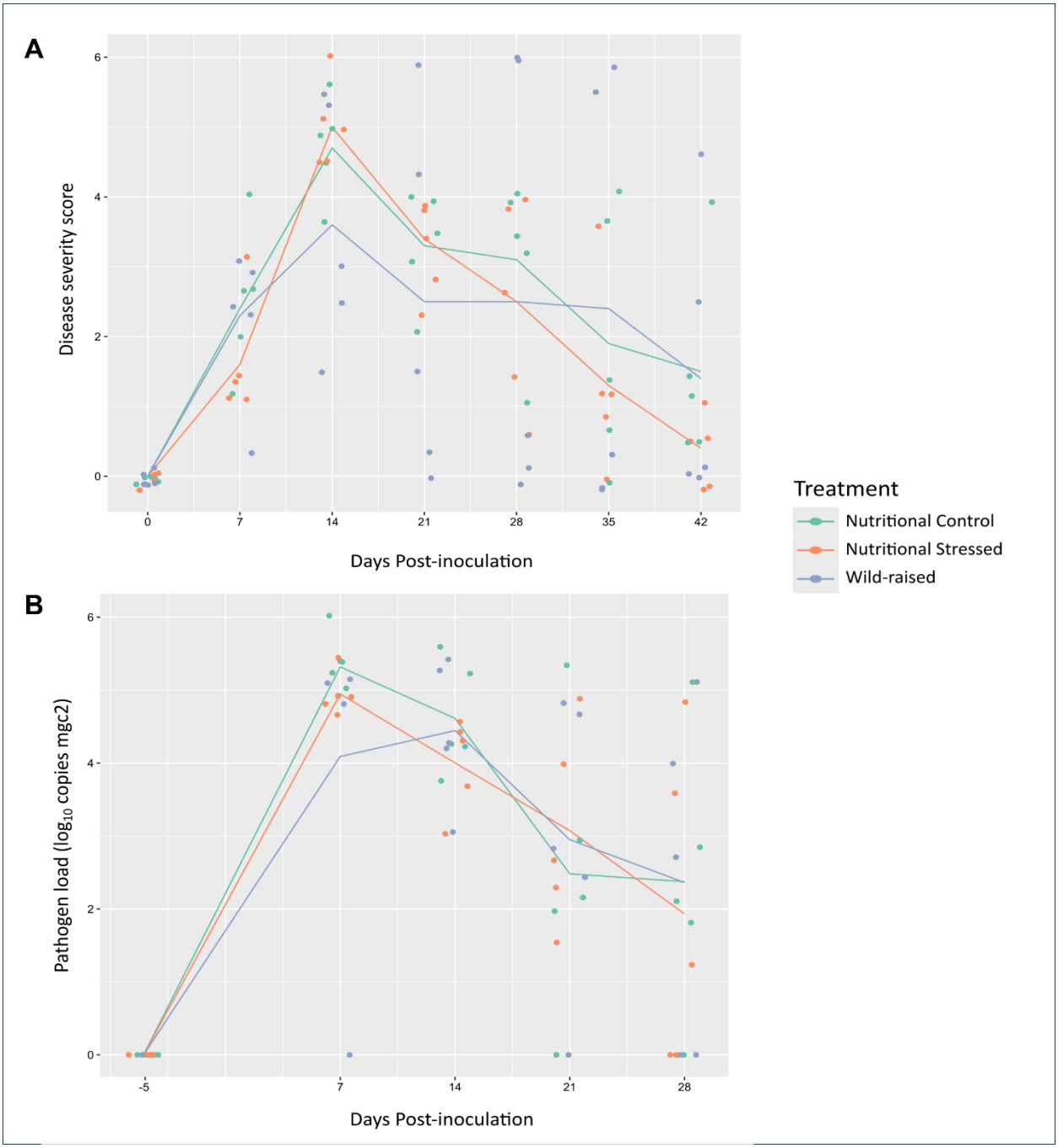

Figure 2. Within the house finches directly inoculated in each eye with MG, neither (A) disease severity (p=0.82) nor (B) pathogen load (p=0.90) differed between our three treatments. Captive-raised controls (n=5), captive-raised nutritionally-stressed (n=5), and wild-raised birds (n=5) all displayed similar levels of conjunctival swelling and amount of pathogen present in the conjunctiva throughout infection.

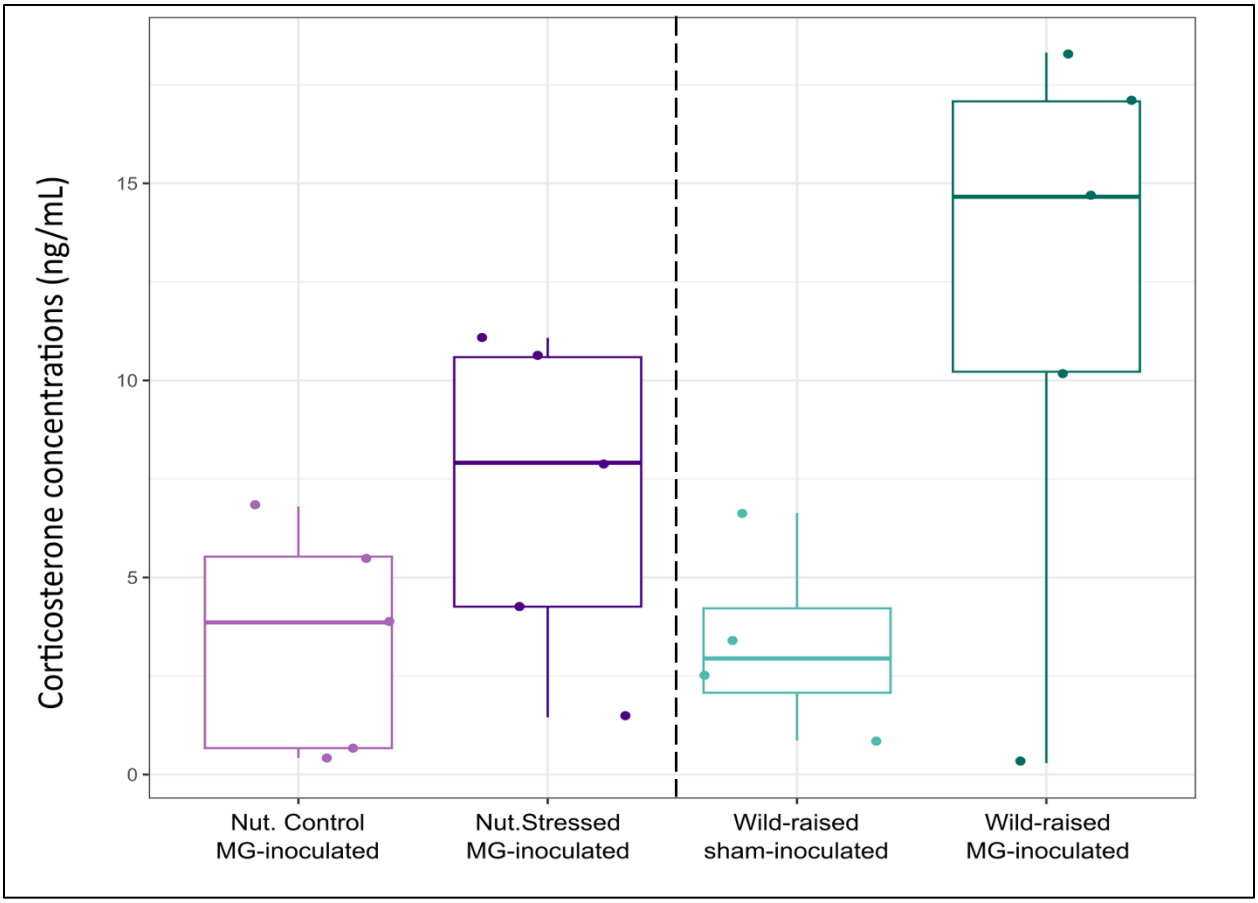

Figure 3. Wild-raised, MG-inoculated house finches had significantly higher levels of circulating plasma corticosterone on day 5 post-inoculation with *Mycoplasma gallisepticum* than captive-raised nutritional controls (p=0.01). In the wild-raised birds only, infected birds tended to have higher baseline corticosterone than sham controls (p=0.06). There were no differences between the corticosterone concentrations during infection detected in our captive-raised nutritionally-stressed birds and our captive-raised nutritional control birds (p=0.12).

Table S1. Nest identification number, nutritional stress treatment, intake age, and intake mass for each captive-raised nestling in the experiment. Nest 1 was collected on 27/04/2021, Nest 2 was collected on 28/04/2021, and Nest 3 was collected on 29/04/2021. The asterisk by nest ID for nestling 2232 denotes that this nestling hatched well before its Nest 3 siblings and was therefore taken into captivity on 27/4/2021 and fed with Nest 1 nestlings. However, its nutritional stress treatment was assigned based on Nest 3, not Nest 1.

Nestling ID	Nest ID	Nut. Stress Treatment	Intake Age (days)	Intake Mass (g)
2230	1	Stressed	5	7.79
2233	1	Control	5	6.69
2231	1	Stressed	4	4.62
2234	2	Control	5	8.20
2236	2	Stressed	5	8.17
2235	2	Control	3	4.51
2232	3*	Stressed	5	6.41
2239	3	Control	5	6.6
2237	3	Control	4	5.64
2238	3	Stressed	3	3.32

Ch. 3

Let's stick together: Infection enhances preferences for social grouping in a songbird species

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Published in *Ecology and Evolution,* 2023, *13*(10)

Abstract

Acute infections can alter foraging and movement behaviors relevant to sociality and pathogen spread. However, few studies have directly examined how acute infections caused by directly-transmitted pathogens influence host social preferences. While infected hosts often express sickness behaviors (e.g., lethargy) that can reduce social associations with conspecifics, enhanced sociality during infection might be favored in some systems if social grouping improves host survival of infection. Directly assaying social preferences of infected hosts is needed to elucidate potential changes in social preferences that may act as a form of behavioral tolerance (defined as using behavior to minimize fitness costs of infection). We tested how infection alters sociality in juvenile house finches (*Haemorhous mexicanus*), which are both highly gregarious and particularly susceptible to infection by the bacterial pathogen *Mycoplasma gallisepticum* (MG). We inoculated 33 wild-caught but captive-held juvenile house finches with MG or media (sham control). At peak infection, birds were given a choice assay to assess preference for associating near a flock versus an empty cage. We then repeated this assay after all birds had recovered from infection. Infected birds were significantly more likely than controls to spend time associating with, and specifically foraging near, the flock. However, after infected birds had recovered from MG infection, there were no significant differences in the amount of time birds in each treatment spent with the flock. These results indicate augmented social preferences during active infection, potentially as a form of behavioral tolerance. Notably, infected birds showed strong social preferences regardless of variation in disease severity or pathogen loads, with 14/19 harboring high loads (5-6 $\log_{10}$ copies of MG) at the time of assay. Overall, our results show that infection with a directly-transmitted pathogen can augment social preferences, with important implications for MG spread in natural populations.

Introduction

Social interactions are critical for the spread of directly-transmitted pathogens, yet infection often induces behavioral changes, such as sickness behaviors, that affect host sociality (Hawley et al., 2021; Stockmaier et al., 2021). Therefore, revealing how active infection alters host social preferences is important for understanding population-level disease dynamics. Despite extensive work on how host sociality predicts transmission risk (e.g., Rifkin et al., 2012; Sah et al., 2018) and growing evidence that healthy hosts avoid infected conspecifics in many systems (e.g., Behringer et al., 2006; Poirotte et al., 2017; Stephenson, 2019), few studies specifically examine the social preferences of hosts actively infected with directly-transmitted pathogens (Siva-Jothy & Vale, 2019; Stephenson, 2019; Wu et al., 2023). Quantifying social preferences of infected hosts is critical because they can inform our understanding of important yet understudied host strategies for mitigating the fitness costs of infection, such as enhanced sociality for group-living animals (Ezenwa et al., 2016).

Acute infections can alter host social preferences via diverse mechanisms, mediated by the pathogen or host. While some pathogens appear to manipulate infected hosts to increase sociality in ways that benefit pathogen transmission (Klein, 2003; Rode et al., 2013), the most common host-mediated behavioral changes during infection are sickness behaviors (e.g., lethargy, anorexia (Hart, 1988), which generally reduce social interactions and pathogen transmission potential (Cárdenas-Canales et al., 2022; D. G. Hamilton et al., 2020; Lopes et al., 2016; Ripperger et al., 2020). However, social interactions may also be decreased when uninfected individuals actively avoid their infected conspecifics (Zylberberg et al., 2013), obscuring the true social preferences of infected hosts. Recent work suggests that gregariousness may reduce fitness costs of infection for hosts via improved food acquisition (Almberg et al., 2015; Ezenwa & Worsley-Tonks, 2018), territory defense (Almberg et al., 2015), and increased predator vigilance by conspecifics (Ezenwa & Worsley-Tonks, 2018). Thus, sociality during infection may act as a key form of "behavioral tolerance" by improving host survival of infection (Ezenwa et al., 2016; Stockmaier et al., 2023). Direct assays of social preferences of actively infected hosts are crucial for revealing how hosts cope with infection behaviorally, and the potential consequences of these responses for pathogen spread.

We tested how experimental infection influences social preferences in a naturally-occurring host-pathogen system, house finches (*Haemorhous mexicanus*) and the bacterial

pathogen *Mycoplasma gallisepticum* (MG), which causes conjunctivitis in this species (Kollias et al., 2004; Fig. 1). House finches are gregarious songbirds that commonly experience MG outbreaks during the non-breeding season, when flocks congregate to forage at bird feeders (Hosseini et al., 2009). Feeders facilitate MG spread through shared use of fomites and augmentation of direct contacts between conspecifics (Adelman et al., 2015; Dhondt et al., 2007; Fig.1). Because MG has a short survival time on feeder surfaces (Dhondt et al., 2007) and MG prevalence is density dependent (Altizer, Hochachka, et al., 2004), social preferences of infected birds at feeders are likely critical for transmission. This may be particularly true for juvenile hatch-year birds, which join large foraging flocks and harbor high MG prevalence (Altizer, Davis, et al., 2004), suggesting they are important drivers of MG epidemics (Hosseini et al., 2009).

Behavioral studies show that MG infection causes sickness behaviors including lethargy (Kollias et al., 2004) and reduced behavioral responses to visual predator stimuli (Adelman et al., 2017). While the conjunctivitis associated with MG infection can be sufficiently severe to obscure vision (Kollias et al., 2004), infected house finches show behavioral changes such as reduced anti-predator responses even in the absence of severe eye swelling (Adelman et al., 2017). With respect to social behaviors, free-living finches with conjunctivitis are observed in smaller flocks than those of healthy birds (Hawley et al., 2007; Hotchkiss et al., 2005). Because uninfected finches do not avoid MG-infected conspecifics (Bouwman & Hawley, 2010), such patterns may reflect decreased sociality of actively infected hosts, a common component of sickness behaviors. However, these patterns could also reflect an inability of diseased birds to move readily among feeding sites (Hawley et al., 2007), rather than social preferences. In fact, infected finches may directly benefit from social behaviors because MG reduces anti-predator behaviors in house finches (Adelman et al., 2017), a source of MG-mediated mortality that may be partially offset by flock membership during infection (Cresswell, 1994). Overall, while past studies document how the behaviors of individually-housed birds change during MG infection (Kollias et al., 2004) and whether healthy house finches avoid MG-infected flockmates (Bouwman & Hawley, 2010), the social preferences of infected birds have not yet been directly examined. Understanding how social preferences toward healthy conspecifics change during acute infection, and whether such changes occur in ways that might benefit infected hosts or

influence ongoing transmission, requires assays that explicitly quantify the social preferences of infected hosts.

The house finch-MG system offers an opportunity to directly test whether infected hosts show decreased sociality due to sickness behaviors, increased sociality as a potential form of behavioral tolerance, or neither. Further, because there is individual variation in disease severity in response to MG infection in house finches (Adelman et al., 2017), this system also provides important insights into how the social preferences of birds with less severe disease and overall lethargy may influence disease dynamics in this system. To elucidate whether and how MG infection influences social preferences, we experimentally inoculated hatch-year house finches with MG or control media and used choice assays to compare social preferences of infected versus uninfected individuals. We also examined whether heterogeneity in infection severity predicts variation in sociality, which would potentially underlie individual-level covariation in infectiousness and contact rates (Stephenson, 2019). Finally, to investigate whether any detected changes in social preferences were related to active infection per se, we conducted this same choice assay after infected birds were allowed to recover.

Methods

Study Subjects, Sexing, and Housing

Thirty-three hatch-year house finches, used as *focal birds* (20 males, 13 females; 1-3 months old), were captured in Blacksburg, Virginia, USA and the City of Radford, Virginia, USA in May and June 2019. Three of these birds were collected as nestlings and hand-fed until nutritional independence (their inclusion did not alter result; see Results); the remaining 30 were nutritionally independent at capture. Age (hatch-year or after hatch-year) was determined at capture by plumage, lack of a brood patch or cloacal protuberance, and presence of a distinct yellow gape line. All birds showed no clinical signs of MG infection, and all birds were seronegative for prior MG exposure (Hawley et al., 2011) prior to experimental infection. Sex was assigned to each bird prior to the start of the experiment using DNA extracted from packed red blood cells using Qiagen 96 DNeasy Blood and Tissue Kit. The presence of sex chromosomes (ZW for females and ZZ for males) was determined using PCR (Griffiths et al., 1998).

Upon capture, all birds were housed in pairs in cages (76 x 46 x 46 cm) for up to a month depending on capture date. All birds were kept in indoor temperature-controlled rooms with a 12L:12D light cycle for the duration of the study. All birds were moved into individual cages of the same size one week before inoculation, where they were housed for the remainder of the experiment.

Stimulus birds

Eight additional hatch-year house finches served as our flock *stimulus birds* for assaying social preferences. All stimulus birds showed no clinical signs of MG infection and were all seronegative for prior MG exposure (Hawley et al., 2011) before use in the behavioral assays. Stimulus birds were housed in separate rooms from all focal birds (prior to behavioral assays) to keep focal individuals unacquainted with the stimulus flock. Further, even during behavioral assays, stimulus birds remained in separate cages from focal birds, preventing any MG transmission to stimulus birds. Four days prior to the start of behavioral assays, four of the eight stimulus birds were placed together into a new cage in the room where the sociality assay occurred. The first group of four stimulus birds were used for 40 trials (two replicate trials for 20 unique focal birds). After 40 trials, these four stimulus birds were switched out with a different flock of four birds, which were used as the stimulus birds for the remaining 26 behavioral trials (two replicate trials for 13 unique focal birds).

Inoculation and behavioral assays

Focal birds were randomly assigned to treatment using a random number generator within sex, with higher sample sizes allotted to the infection versus control treatment to account for heterogenous responses to infection (MG infection treatment: n=19; sham control treatment: n=14). Birds were split into two experimental rounds (seven days apart; each individual bird was only included in one unique round) in order to complete all behavioral assays during the infectious period (days 10-20 post infection (Dhondt et al., 2008)), when sociality is most relevant for ongoing spread. On experimental day 0, birds were inoculated bilaterally in the conjunctiva with 35 µL of MG (infection treatment) in Frey's media or with media alone (sham control treatment). We used an MG strain collected in North Carolina, USA, in 2006 (NC2006,

2006.080-5 4P 7/26/12, David H. Ley, NC State University, College of Veterinary Medicine, Raleigh, NC, USA 27606), with a viable count of 2.49 x 10^6 color-changing units (CCU).

We monitored disease severity weekly and on the day of behavioral assays by scoring conjunctivitis on a 0-3 scale per side, with scores of 3 representing severe conjunctivitis (Hawley et al., 2011). Scores for each side (left and right) were summed within sampling day for a maximum total eye score of 6 for a given focal bird. We swabbed conjunctiva weekly post-inoculation to quantify MG load, as well as immediately after behavioral trials if weekly swabs did not fall within ± 2 days of a given bird's behavioral assay. Swabs were stored in 300µL tryptose phosphate broth (TPB) and stored at -20˚C until extraction using Qiagen 96 DNeasy Blood and Tissue Kit; the amount of MG in each sample was determined via a probe-based qPCR using methods outlined in prior work (Hawley et al., 2011).

Each focal bird was tested on two consecutive days within their peak infectious period (post-infection day 10-20 (Dhondt et al., 2008)) and all behavioral assays occurred between 07:30 – 10:50 and food was withheld from focal birds for three hours before testing to standardize motivation. Focal birds were placed in a behavioral arena (Fig.2) where they could feed in proximity to a stimulus cage containing four unfamiliar, uninfected conspecifics on one side, or an empty cage on the other, and video recorded for 45 min. To account for side preferences unrelated to the presence of stimulus birds, we repeated the assay for each focal individual on consecutive mornings: once with the stimulus flock on each side of the cage (order was randomized). We quantified preference by recording time spent in one of two mutually exclusive behaviors (perching or eating) on each side of the arena during 35 min per replicate assay (allowing 10 min for acclimation). Videos were split randomly between two observers so that each observer watched videos from both infected and control individuals, while always remaining blind to treatment. However, both of an individual bird's trials were observed by the same individual.

Thirty-one days after inoculation, infected birds were given a broad-spectrum antibiotic (Tylan®, tylosin tartrate) in their drinking water (at a concentration of 1 g/L water) for five weeks until all birds showed no clinical signs of MG. After all birds were recovered from infection, we repeated the choice assay with eight new stimulus birds. The first group of four stimulus birds were used for 38 trials (two replicate trials for 19 unique focal birds). After 38 trials, the other group of four stimulus birds were used for the remaining 26 behavioral trials

(two replicate trials for 13 unique focal birds). All post-infection videos were watched and coded using BORIS (Friard & Gamba, 2016)

Statistical Analyses

All data was analyzed in R v 3.6.1 (R Core Team, 2021). For both of our assays (during infection and post-infection), we calculated two behavioral metrics: 1) the proportion of time a focal bird spent perching near the stimulus flock and 2) proportion of time spent eating near the stimulus flock (with eating defined as a bird being perched on the food dish and pecking at food at least every 20 seconds). Our definition of each behavior resulted in the time spent in each behavior as mutually exclusive (i.e., a bird perched on the food dish and actively pecking at food was designated as "eating" but not "perching"). Thus, we also calculated a summary measure of preference to associate with the flock as the proportion of time each bird spent either perching, eating, or both perching and eating near the stimulus flock. For each variable, we summed a bird's time engaged in that activity (eating, perching, or either) near the stimulus flock across replicate trials (for 70 total minutes of observation), utilizing only data from the front half of the arena (near the stimuli), which represented >98% of assay time. We then divided these sums by the total time spent engaged in the respective activity (eating, perching, or either). Thus, although each bird in our study had two replicate trials (with the stimulus flock located on each side of the arena), only one response value per behavior was analyzed for each unique focal bird in our study. Three infected birds did not eat during the infection assay, consistent with prior work documenting infection-induced anorexia in this species (Adelman, Carter, et al., 2013); thus, these three birds were only included in the perching model and the combined model of eating or perching. One bird died prior to starting our post-infection assays, so only 32 birds of the original 33 birds were tested once infected birds had recovered.

We used these proportions as response variables in separate generalized linear models (using quasibinomial error distributions) with treatment (infected or control; or recovered or control for post-recovery assays) as the main effect. Models were weighted by total time eating (eating model), total time perching (perching model), or total time engaged in either behavior (combined perching or eating model). We tested for significance using t-values generated by our GLM for each variable in R. Sex, day post-infection (which always fell between days 10-20 but varied across individuals), and experimental round were initially included in all infection models, but covariates were removed from final models if the GLM parameter estimate for that covariate

and associated t-test was $p > 0.1$. Only sex and experimental round were included as covariates in our post-infection models and were also removed from the final model using the cutoff stated above. Within the infected treatment only, we also asked whether variation in the severity of conjunctivitis or pathogen load at the time of the sociality assay predicted behavioral preference. We used ggplot2 (Wickham, 2016) for all graphing.

Results

For our behavioral trials performed during infection, there was individual variation within and between treatments in time spent eating (infected: 1.47-46.27 min; control: 0-34.01 min) and perching (infected: 15.93-59.88 min; control: 2.23-62.56 min) near the flock, out of an average total assay time of 70 minutes (2 replicates of 35 minutes each). For eating, this variation was significantly predicted by infection treatment, with infected house finches spending significantly more time eating near the stimulus flock, relative to uninfected birds (Fig.3; n=30; Intercept (Control)=0.55 ± 0.24, Beta (Infected)=1.07 ± 0.43, t=2.51, p=0.018). However, we did not find statistically significant support for effects of infection treatment on time perching near the stimulus flock (Fig.3; n=33; Intercept (Control)=-0.92 ± 0.52, Beta (Infected)=0.62 ± 0.32, t=1.94, p=0.062). When the two quantified behaviors were pooled in a combined analysis (time spent eating or perching with the flock), infected house finches were significantly more likely to spend time associating with the flock when engaged in either behavior (n=33; Intercept (Control)=-0.56 ± 0.49, Beta (Infected)=0.69 ± 0.30, t=2.30, p=0.028), relative to uninfected individuals. All covariates included in initial models (see methods) showed p>0.1 and were removed, except experimental round in the model of perching (Beta (round 2)=0.95 ± 0.32, t=2.97, p=0.01) and the combined model of time spent eating or perching (Beta (round 2)=0.72 ± 0.30, t=2.39, p=0.02) (Appendix: Fig.A1)

Birds in the infected treatment showed variable disease severity at the time of assay, from summed (left plus right conjunctiva) severity scores of 0.5 to 6 (mean: 3.76, sd: 1.91) out of a maximum of 6. However, among infected birds, severity of conjunctivitis did not predict the proportion of time eating (n=16; Intercept=1.38 ± 0.52, Beta=0.07 ± 0.14, t=-0.53, p=0.60), perching (n=19; Intercept=1.78 ± 0.58, Beta=-0.18 ± 0.13, t=-1.38, p=0.18), or generally associating (eating or perching) with the flock (n=19; Intercept=1.70 ± 0.51, Beta=-0.13 ± 0.12, t=-1.15, p=0.27). Pathogen load in the conjunctiva at the time of assay varied from 0 to 6.35

$\log_{10}$ copies of MG (mean: 4.59 $\log_{10}$ copies of MG, sd: 2.22 $\log_{10}$ copies of MG) for infected birds, with 14/19 birds harboring "high" MG loads (defined as $\geq$ 4.71 $\log_{10}$ copies of MG, the average load for this isolate (Fleming-Davies et al., 2018) and 15/19 harboring loads predicted to be infectious (defined as $\geq$ 3.13 $\log_{10}$ copies of MG as per (Adelman et al., 2015). Among infected birds, pathogen load did not predict the proportion of time spent eating (Fig.4A; n=16; Intercept=2.26 ± 0.64, Beta=-0.14 ± 0.12, t=-1.14, p=0.27), perching (Fig.4B; n=19; Intercept=1.88 ± 0.67, Beta=-0.17 ± 0.13, t=-1.30, p=0.21), or generally associating (eating or perching) with the flock (n=19; Intercept=2.0 ± 0.60, Beta=-0.17 ± 0.11, t=-1.49, p=0.16).

For our behavioral trials performed after infected birds had recovered, there was also individual variation within treatment in the amount of time spent eating (recovered: 0-49.63 min; control: 1.2-61.78 min) and perching (recovered: 3.58-38.82 min; control: 1.73-35.87 min) near the flock, out of an average total assay time of 72 minutes (2 replicates of 36 minutes each). However, in contrast to assays during active infection, a bird's prior infection treatment (recovered or uninfected control) did not significantly predict either the amount of time eating near the stimulus flock (Fig.5; n=32; Intercept (Control)=-1.35 ± 0.84, Beta (Infected)=-0.60 ± 0.54, t=-1.11, p=0.28), nor the amount of time spent perching near the flock (Fig.5; n=32; Intercept (Control)=-0.16 ± 0.25, Beta (Infected)=0.56 ± 0.35, t=1.58, p=0.12). When eating and perching behaviors were pooled, there was no significant difference between treatments in the amount of time spent associating with the flock (n=32; Intercept (Control)=-1.15 ± 0.69, Beta (Infected)=0.02 ± 0.42, t=0.04, p=0.97) In all post-recovery models, covariates were removed if they showed p>0.1, with the exception of experimental round in our eating model (Beta (round 2)=1.52 ± 0.54, t=2.82, p=0.01) and the combined model (Beta (round 2)=0.97 ± 0.42, t=2.31, p=0.03).

To ensure that inclusion of three hand-fed birds did not alter our results, we repeated the generalized linear models (using quasibinomial error distributions) with these birds excluded from the analysis. We found that there were no differences in the effects of treatment on the amount of time spent eating (n=27; Intercept (Control)=0.524 ± 0.249, Beta (Infected)=1.25 ± 0.480, t=2.60, p=0.015) or perching (n=30; Intercept (Control)=0.459 ± 0.279, Beta (Infected)=0.6684 ± 0.369, t=1.81, p=0.082) near the flock during infection compared to the models including these three hand-fed birds.

Discussion

We found that house finches actively infected with a directly-transmitted pathogen spent significantly more time than uninfected controls associating with, and specifically eating near, a flock of healthy conspecifics. Notably, birds in the infected treatment generally displayed uniformly high levels of sociality, regardless of individual variation in their disease severity or pathogen load at the time of assay. Because most (15/19) infected birds harbored pathogen loads well above prior estimates for MG's minimum infectious dose in finches (Adelman et al., 2015), such augmented sociality likely has key consequences for transmission. In this system, pathogen transmission increases with both the time that birds spend on feeders (Adelman et al., 2015) and the degree of host pathology (Bonneaud et al., 2020; Ruden & Adelman, 2021), which enhances pathogen deposition onto bird feeders (Adelman, Carter, et al., 2013). Because finches with severe pathology are often less active (Adelman et al., 2017), pathogen spread is predicted to be maximized at moderate degrees of conjunctivitis severity (Bonneaud et al., 2020). Thus, the augmented sociality seen during infection here, including in finches with high pathogen loads (Fig.4) but only moderate pathology (e.g., 25th-75th percentiles, or scores 2-5 in this study, n = 9/19 birds), is likely to facilitate MG spread in the wild.

Changes in behavior during infection can broadly be driven by host- or pathogen-mediated mechanisms, including direct manipulation of host behavior by pathogens. Directly-transmitted parasites should benefit from manipulating host sociality, and some studies show higher sociality in infected animals consistent with parasite manipulation of host behavior (Petkova et al., 2018; Rode et al., 2013). Nonetheless, examples of parasite manipulation to increase host sociality are rare, with observed behavioral changes more often manifesting as host-mediated declines in sociality (Cárdenas-Canales et al., 2022; Hawley et al., 2021). Our results represent a case of a directly-transmitted pathogen causing augmented rather than reduced host sociality, potentially due to host-mediated behavioral changes. While our experimental design does not allow us to rule out the possibility that the observed behavioral changes are pathogen-mediated, Poulin (2010) hypothesized that selection on directly-transmitted parasites to manipulate the sociality of gregarious hosts is rare because such parasites already have ample transmission opportunities. Further, in systems where augmented sociality during infection has been observed, there are clear hypothesized benefits to hosts for such behavioral changes. For example, Stephenson found increases in sociality in male guppies (*Poecelia reticulata*) that

harbored the highest loads of a directly-transmitted ectoparasite, a behavioral change that the authors hypothesized may increase mating opportunities and the ability to permanently shed worms onto other hosts, potentially benefiting infected host fitness (Stephenson, 2019). Further, Wu *et al.* (2023) found that *C. elegans* hermaphrodites will shift their mating preferences when exposed to a bacterial pathogen, increasing the rate that they associate and mate with males. Together with our results, such studies indicate that infected hosts in some systems augment sociality in ways that likely ultimately benefit host fitness. However, it is notoriously challenging to tease apart whether behavioral changes during infection represent host-mediated changes, pathogen-mediated changes, or some combination (Nadler et al., 2023).

Due to the energetic costs of both MG infection and social behaviors, as well as the lethargy common among house finches infected with MG (Kollias et al., 2004), increased sociality during infection may seem counterintuitive as a potential host-mediated strategy. However, maintenance of social behaviors may be one form of behavioral tolerance in this system, lowering the survival costs of infection (Ezenwa et al., 2016). One cost of MG infection in house finches is a reduction in anti-predator behaviors (Adelman et al., 2017), which likely contributes to MG-related mortality in the wild (Faustino et al., 2004). Birds that forage with flocks while infected would likely have increased protection from predation threats (Fernández-Juricic et al., 2004), and thus higher likelihood of surviving infection. However, it must be noted that, given the reduced ability of infected finches to evade capture in mock predation trials (Adelman et al., 2017), associating with flocks may also elevate predation risk for infected birds if larger flocks attract more predators and infected birds serve as easier targets than their uninfected flockmates. Interestingly, differences in sociality between infected individuals and uninfected controls were no longer present once infected birds had recovered from infection, which may further indicate that infected birds utilize increased sociality to offset the costs of sickness behavior, which becomes unnecessary after recovery.

Another mechanism that may alleviate high fitness costs of infection is improved foraging and food acquisition (Ezenwa et al., 2016; Ezenwa & Worsley-Tonks, 2018), a key benefit of flocking behavior in non-breeding birds (Fernández-Juricic et al., 2004). During infection, sickness behaviors like lethargy may decrease an individual's ability to locate or use a food source (Ezenwa et al., 2016). Group membership may offset these foraging costs of sickness behavior by assisting infected individuals in locating or acquiring a food source

(Almberg et al., 2015) or through increase predator vigilance, allowing infected animals to allocate more time towards foraging (Ezenwa & Worsley-Tonks, 2018). Given that infected birds were significantly more likely to associate with the flock while eating but not perching in our study, foraging benefits of sociality may be particularly important during infection. Notably, even control birds showed a non-random preference to feed near the flock versus the empty cage, though that preference was not as strong as that seen in infected birds. This likely reflects the benefits of group feeding in this species and their high degree of sociality (Badyaev et al., 2020). Although the hypothesized effects of MG infection on perching behavior, which included any resting or preening behaviors done while remaining perched in one location in the arena, did not have statistically significant support, the detected patterns for perching behavior in infected versus control birds were qualitatively similar to that found for time eating (Fig.3). When the two behaviors were pooled, this contributed to an overall significant preference for infected birds to associate with the flock when either eating or perching in our combined analysis. Overall, the potential anti-predation and foraging benefits of sociality are likely not mutually exclusive in house finches, with social groups providing multiple benefits to infected individuals.

The preferences for augmented sociality seen in infected birds in our study could also reflect changes in the relative cost-benefit ratio associated with sociality. For example, while increased risk of infection is considered a broader cost of sociality (Hawley et al., 2021), already-infected hosts may be less motivated to avoid this cost. In a study of avoidance of infected conspecifics in a gregarious lobster species, Caribbean spiny lobsters (*Panulirus argus*) were given a choice to den alone or with a virus-infected conspecific; while healthy lobsters strongly avoided denning with an infected conspecific, infected lobsters showed no detectable preference (Behringer et al., 2006). Enhanced social preference of infected birds could also result from more generalized, and potentially non-adaptive, changes to host sensory processing whereby infected birds are attracted to feed near a wide range of sensory stimuli; however, prior work showing that infected house finches are less responsive than healthy birds to both visual and auditory stimuli of potential predation threats (Adelman et al., 2017) suggests that generalized attraction is unlikely in this system. Further study should examine whether the social preferences seen in infected versus uninfected birds in our study result from potential benefits of sociality to infected birds (e.g., reduced predation risk, increased foraging efficiency), reduction in the potential costs of sociality for infected birds (e.g., increased infection risk), changes in generalized attraction to

sensory stimuli during infection, or some combination thereof. Interestingly, house finches from populations that have had longer time with MG endemic in their population display lower conjunctivitis severity per unit pathogen (Henschen et al., 2023), suggesting that natural populations that have co-evolved with MG show potential adaptive responses to MG infection. Performing MG inoculations of birds from populations where MG has not yet been documented may help to elucidate whether the behavioral changes detected here represent evolved strategies of behavioral tolerance to MG infection, though such differences may have evolved in response to infection and sickness behaviors more generally. Finally, we cannot eliminate the possibility that pathogen-mediated manipulation contributes to the augmented sociality in infected house finches, which could be assessed using non-infectious immune challenges.

Regardless of the mechanisms driving our results, the increased time that infected birds spend eating near conspecifics is likely to have important consequences for MG transmission. This pathogen appears to spread primarily at bird feeders (Adelman et al., 2015) from indirect contacts that occur within minutes to hours, when MG deposited onto surfaces from infected birds is still viable (Dhondt et al., 2007). Increases in the probability that infected birds feed in the presence of a flock should therefore enhance fomite-based transmission. Thus, uninfected birds in flocks might be expected to actively avoid eating near their infected conspecifics, regardless of the infected individual's social preferences. However, it has been found that uninfected house finches do not actively avoid eating near MG-infected individuals, and in some cases male house finches preferentially feed near infected versus healthy male conspecifics (Bouwman & Hawley, 2010). While no studies have specifically looked at the mechanisms driving the lack of avoidance of infected conspecifics in this system, such behaviors may arise because the benefits of flocking behavior in this system outweigh the costs, even for uninfected individuals. Overall, because uninfected birds do not actively avoid infected conspecifics (Bouwman & Hawley, 2010), our findings on the social preferences of the infected flockmates are especially interesting and suggest that augmented sociality plays a key role in determining disease dynamics within this system.

While our behavioral assays allowed us to specifically isolate social preferences of infected versus uninfected birds, these assays also have limitations when extrapolating to social behaviors and transmission implications in the wild. The captive behavioral arena may not reflect the energetic costs an infected bird incurs while moving with flocks of uninfected

conspecifics. In our small arena, even birds with the most severe pathology were able to move and eat without utilizing much energy, an unlikely situation in wild flocks. This may explain why we found no relationship between individual variation in disease severity and time spent associating near the flock in our assays. While our experiment showed that infected birds almost universally prefer to forage near a flock, only individuals with low to moderate pathology may be able to exercise their social preferences in the wild by keeping up with mobile foraging flocks (Hawley et al., 2007). Overall, future attention should be put on the implications of these preferences for transmission in the wild, focusing on whether only those animals with moderate pathology are able to carry out their social preferences and, thus, become primary drivers of pathogen transmission across a landscape.

Acknowledgements

We thank Dr. Chava Weitzman and Allison Rowley for their assistance with sampling and Cynthia Harrison for assistance watching behavioral videos. We also thank Drs. Kendra Sewall, Michael Emmerson, Ignacio Moore, and Jeff Walters for helpful input. Our experiment and animal use were conducted under approved Virginia Tech Institutional Animal Care and Use Committee (IACUC) protocol (19-055-BIOL) and state and federal permits (Virginia Department of Game and Inland Fisheries permit 061440, USFWS permit MB154804-0). All experimental methods were carried out in accordance with the guidelines and regulations set forth by the Virginia Tech IACUC and Virginia Department of Game and Inland Fisheries. This work was funded by NSF grants IOS-1754872 to D.M.H and IOS-1950307 to J.S.A.

References

Adelman, J. S., Carter, A. W., Hopkins, W. A., & Hawley, D. M. (2013). Deposition of pathogenic *Mycoplasma gallisepticum* onto bird feeders: Host pathology is more important than temperature-driven increases in food intake. *Biology Letters*, *9*(5). https://doi.org/10.1098/rsbl.2013.0594

Adelman, J. S., Mayer, C., & Hawley, D. M. (2017). Infection reduces anti-predator behaviors in house finches. *Journal of Avian Biology*, *48*, 519–528.

Adelman, J. S., Moyers, S. C., Farine, D. R., & Hawley, D. M. (2015). Feeder use predicts both acquisition and transmission of a contagious pathogen in a North American songbird. *Proceedings of the Royal Society B*, *282*(20151429).

Almberg, E. S., Cross, P. C., Dobson, A. P., Smith, D. W., Metz, M. C., Stahler, D. R., & Hudson, P. J. (2015). Social living mitigates the costs of a chronic illness in a cooperative carnivore. *Ecology Letters*, *18*(7), 660–667. https://doi.org/10.1111/ele.12444

Altizer, S., Davis, A. K., Cook, K. C., & Cherry, J. J. (2004). Age, sex, and season affect the risk of mycoplasmal conjunctivitis in a southeastern house finch population. *Canadian Journal of Zoology*, *82*, 755–763.

Altizer, S., Hochachka, W. M., & Dhondt, A. A. (2004). Seasonal dynamics of mycoplasmal conjunctivitis in eastern North American house finches. *Journal of Animal Ecology*, *73*, 309–322. www.birds.cornell.edu/hofi.

Badyaev, A. V., Belloni, V., & Hill, G. E. (2020). House Finch (*Haemorhous mexicanus).* In *Birds of the World*, A. F. Poole (Ed.). Cornell Lab of Ornithology. https://doi.org/10.2173/bow.houfin.01

Behringer, D. C., Butler, M. J., & Shields, J. D. (2006). Avoidance of disease by social lobster. *Nature*, *441*, 421.

Bonneaud, C., Tardy, L., Hill, G. E., McGraw, K. J., Wilson, A. J., & Giraudeau, M. (2020). Experimental evidence for stabilizing selection on virulence in a bacterial pathogen. *Evolution Letters*, *4*(6), 491–501. https://doi.org/10.1002/evl3.203

Bouwman, K. M., & Hawley, D. M. (2010). Sickness behaviour acting as an evolutionary trap? Male house finches preferentially feed near diseased conspecifics. *Biology Letters*, *6*, 462–465.

Cárdenas-Canales, E. M., Stockmaier, S., Cronin, E., Rocke, T. E., Osorio, J. E., & Carter, G. G. (2022). Social effects of rabies infection in male vampire bats (*Desmodus rotundus*). *Biology Letters*, *18*(9). https://doi.org/10.1098/rsbl.2022.0298

Cresswell, W. (1994). Flocking is an effective anti-predation strategy in redshanks, *Tringa totanus*. *Animal Behaviour*, *47*(2), 433–442. https://doi.org/10.1006/anbe.1994.1057

Dhondt, A. A., Dhondt, K. V., Hawley, D. M., & Jennelle, C. S. (2007). Experimental evidence for transmission of *Mycoplasma gallisepticum* in house finches by fomites. *Avian Pathology*, *36*(3), 205–208.

Dhondt, A. A., Dhondt, K. V., & McCleery, B. V. (2008). Comparative infectiousness of three passerine bird species after experimental inoculation with *Mycoplasma gallisepticum*. *Avian Pathology*, *37*(6), 635–640. https://doi.org/10.1080/03079450802499100

Ezenwa, V. O., Ghai, R. R., McKay, A. F., & Williams, A. E. (2016). Group living and pathogen infection revisited. *Current Opinion in Behavioral Sciences*, *12*, 66–72. https://doi.org/10.1016/j.cobeha.2016.09.006

Ezenwa, V. O., & Worsley-Tonks, K. E. L. (2018). Social living simultaneously increases infection risk and decreases the cost of infection. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1892). https://doi.org/10.1098/rspb.2018.2142

Faustino, C. R., Jennelle, C. S., Connolly, V., Davis, A. K., Swarthout, E. C., Dhondt, A. A., & Cooch, E. G. (2004). *Mycoplasma gallisepticum* infection dynamics in a house finch population: seasonal variation in survival, encounter and transmission rate. *Journal of Animal Ecology*, *73*(4), 651–669.

Fernández-Juricic, E., Siller, S., & Kacelnik, A. (2004). Flock density, social foraging, and scanning: An experiment with starlings. *Behavioral Ecology*, *15*(3), 371–379. https://doi.org/10.1093/beheco/arh017

Fleming-Davies, A. E., Williams, P. D., Dhondt, A. A., Dobson, A. P., Hochachka, W. M., Leon, A. E., Ley, D. H., Osnas, E. E., & Hawley, D. M. (2018). Incomplete host immunity favors the evolution of virulence in an emergent pathogen. *Science*, *359*, 1030–1033. https://www.science.org

Friard, O., & Gamba, M. (2016). BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. *Methods in Ecology and Evolution*, *7*(11), 1325–1330. https://doi.org/10.1111/2041-210X.12584

Griffiths, R., Double, M. C., Orr, K., & Dawson, R. J. G. (1998). A DNA test to sex most birds. *Molecular Ecology*, *7*(8), 1071–1075. https://doi.org/10.1046/j.1365-294x.1998.00389.x

Hamilton, D. G., Jones, M. E., Cameron, E. Z., Kerlin, D. H., McCallum, H., Storfer, A., Hohenlohe, P. A., & Hamede, R. K. (2020). Infectious disease and sickness behaviour: tumour progression affects interaction patterns and social network structure in wild Tasmanian devils. *Proceedings of the Royal Society B: Biological Sciences*, *287*(1940), 20202454. https://doi.org/10.1098/rspb.2020.2454

Hart, B. L. (1988). Biological basis of the behavior of sick animals. *Neuroscience and Biobehavioral Reviews*, *12*(2), 123–137. https://doi.org/10.1016/S0149-7634(88)80004-6

Hawley, D. M., Davis, A. K., & Dhondt, A. A. (2007). Transmission-relevant behaviours shift with pathogen infection in wild house finches (*Carpodacus mexicanus*). *Canadian Journal of Zoology*, *85*(6), 752–757.

Hawley, D. M., Gibson, A. K., Townsend, A. K., Craft, M. E., & Stephenson, J. F. (2021). Bidirectional interactions between host social behaviour and parasites arise through ecological and evolutionary processes. *Parasitology*, *148*(3), 274–288. https://doi.org/10.1017/S0031182020002048

Hawley, D. M., Grodio, J., Frasca, S., Kirkpatrick, L., & Ley, D. H. (2011). Experimental infection of domestic canaries (*Serinus canaria domestica*) with *Mycoplasma gallisepticum*: A new model system for a wildlife disease. *Avian Pathology*, *40*(3), 321–327. https://doi.org/10.1080/03079457.2011.571660

Henschen, A. E., Vinkler, M., Langager, M. M., Rowley, A. A., Dalloul, R. A., Hawley, D. M., & Adelman, J. S. (2023). Rapid adaptation to a novel pathogen through disease tolerance in a wild songbird. *PLoS Pathogens*, *19*(6 June). https://doi.org/10.1371/journal.ppat.1011408

Hosseini, P. R., Dhondt, A. A., Dobson, A. P., & Dobson, P. (2009). Spatial spread of an emerging infectious disease: Conjunctivitis in house finches. *Ecology*, *87*(12), 3037–3046.

Hotchkiss, E. R., Davis, A. K., Cherry, J. J., & Altizer, S. (2005). Mycoplasmal conjunctivitis and the behavior of wild house finches (*Carpodacus mexicanus*) at bird feeders. *Bird Behavior*, *17*, 1–8.

Klein, S. L. (2003). Parasite manipulation of the proximate mechanisms that mediate social behavior in vertebrates. *Physiology and Behavior*, *79*(3), 441–449. https://doi.org/10.1016/S0031-9384(03)00163-X

Kollias, G. V., Sydenstricker, K. V., Kollias, H. W., Ley, D. H., Hosseini, P. R., Connolly, V., & Dhondt, A. A. (2004). Experimental infection of house finches with *Mycoplasma gallisepticum. Journal of Wildlife Diseases*, *40*(1), 79–86. https://doi.org/10.7589/0090-3558-40.1.79

Lopes, P. C., Block, P., & König, B. (2016). Infection-induced behavioural changes reduce connectivity and the potential for disease spread in wild mice contact networks. *Scientific Reports*, *6*(31790), 1–10. https://doi.org/10.1038/srep31790

Nadler, L. E., Adamo, S. A., Hawley, D. M., & Binning, S. A. (2023). Mechanisms and consequences of infection-induced phenotypes. *Functional Ecology*, *37*(4), 796–800. https://doi.org/10.1111/1365-2435.14309

Petkova, I., Abbey-Lee, R. N., & Løvlie, H. (2018). Parasite infection and host personality: *Glugea*-infected three-spined sticklebacks are more social. *Behavioral Ecology and Sociobiology*, *72*(11). https://doi.org/10.1007/s00265-018-2586-3

Poirotte, C., Massol, F., Herbert, A., Willaume, E., Bomo, P. M., Kappeler, P. M., & Charpentier, M. J. E. (2017). Mandrills use olfaction to socially avoid parasitized conspecifics. *Science Advances*, *3*(e160172), 1–8.

Poulin, R. (2010). Parasite Manipulation of Host Behavior: An Update and Frequently Asked Questions. In *Advances in the Study of Behavior* (Vol. 41, Issue C, pp. 151–186). https://doi.org/10.1016/S0065-3454(10)41005-0

R Core Team. (2021). *R: A language and environment for statistical computing.* R Foundation for Statistical Computing.

Rifkin, J. L., Nunn, C. L., & Garamszegi, L. Z. (2012). Do animals living in larger groups experience greater parasitism? A meta-analysis. *The American Naturalist*, *180*, 70–82. https://doi.org/10.5061/dryad.v5007p45

Ripperger, S. P., Stockmaier, S., & Carter, G. G. (2020). Tracking sickness effects on social encounters via continuous proximity sensing in wild vampire bats. *Behavioral Ecology*, *31*(6), 1296–1302. https://doi.org/10.1093/beheco/araa111

Rode, N. O., Lievens, E. J. P., Flaven, E., Segard, A., Jabbour-Zahab, R., Sanchez, M. I., & Lenormand, T. (2013). Why join groups? Lessons from parasite-manipulated *Artemia*. *Ecology Letters*, *16*(4), 493–501. https://doi.org/10.1111/ele.12074

Ruden, R. M., & Adelman, J. S. (2021). Disease tolerance alters host competence in a wild songbird. *Biology Letters*, *17*(10). https://doi.org/10.1098/rsbl.2021.0362

Sah, P., Mann, J., & Bansal, S. (2018). Disease implications of animal social network structure: A synthesis across social systems. *Journal of Animal Ecology*, *87*(3), 546–558. https://doi.org/10.1111/1365-2656.12786

Siva-Jothy, J. A., & Vale, P. F. (2019). Viral infection causes sex-specific changes in fruit fly social aggregation behaviour. *Biology Letters*, *15*(9). https://doi.org/10.1098/rsbl.2019.0344

Stephenson, J. F. (2019). Parasite-induced plasticity in host social behaviour depends on sex and susceptibility. *Biology Letters*, *15*, 20190557. https://doi.org/10.1098/rsbl.2019.0557

Stockmaier, S., Stroeymeyt, N., Shattuck, E. C., Hawley, D. M., Meyers, L. A., & Bolnick, D. I. (2021). Infectious diseases and social distancing in nature. *Science*, *371*(6533). https://doi.org/10.1126/science.abc8881

Stockmaier, S., Ulrich, Y., Albery, G. F., Cremer, S., & Lopes, P. C. (2023). Behavioural defences against parasites across host social structures. *Functional Ecology*, *37*(4), 809–820. https://doi.org/10.1111/1365-2435.14310

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York. https://ggplot2.tidyverse.org

Wu, T., Ge, M., Wu, M., Duan, F., Liang, J., Chen, M., Gracida, X., Liu, H., Yang, W., Dar, A. Li, C., Butcher, R. A., Saltzman, A. L., & Zhang, Y. (2023). Pathogenic bacteria modulate pheromone response to promote mating. *Nature*, *613*(7943), 324–331. https://doi.org/10.1038/s41586-022-05561-9

Zylberberg, M., Klasing, K. C., & Hahn, T. P. (2013). House finches (*Carpodacus mexicanus*) balance investment in behavioural and immunological defences against pathogens. *Biology Letters*, *9*(1). https://doi.org/10.1098/rsbl.2012.0856

Figure 1. Two juvenile house finches eating together at a bird feeder. The bird on the left has noticeable clinical signs of MG infection (redness and swelling of the conjunctiva). In contrast, the bird on the right shows no signs of MG infection. Photo taken by Ivey Fennell, access for use courtesy of the Cornell Lab of Ornithology Project FeederWatch.

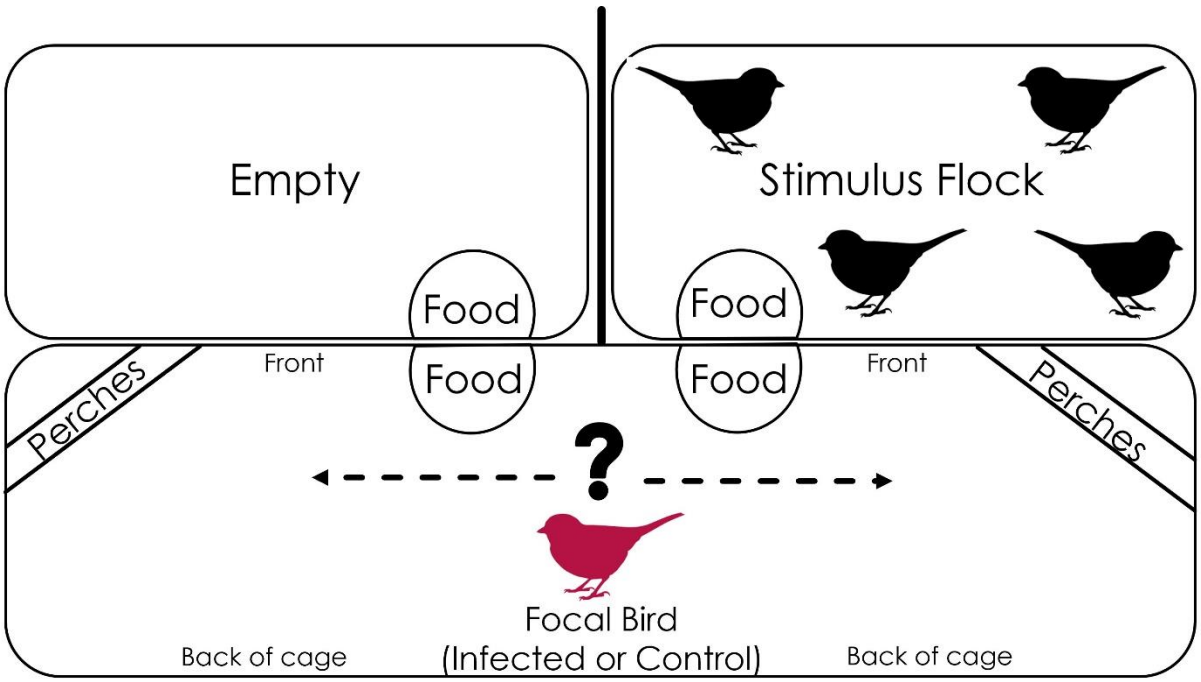

Figure 2. Top-down view of social preference behavioral arena, with food dishes at the front of the focal cage (dimensions: 105 x 46 x 40 cm). This large focal cage was placed directly in front of two smaller stimulus cages (dimensions: 76 x 46 x 46) containing a flock of four juvenile stimulus birds. The side of the stimulus flock was switched between replicates for a given focal bird such that every focal bird was assayed with the stimulus flock on each side.

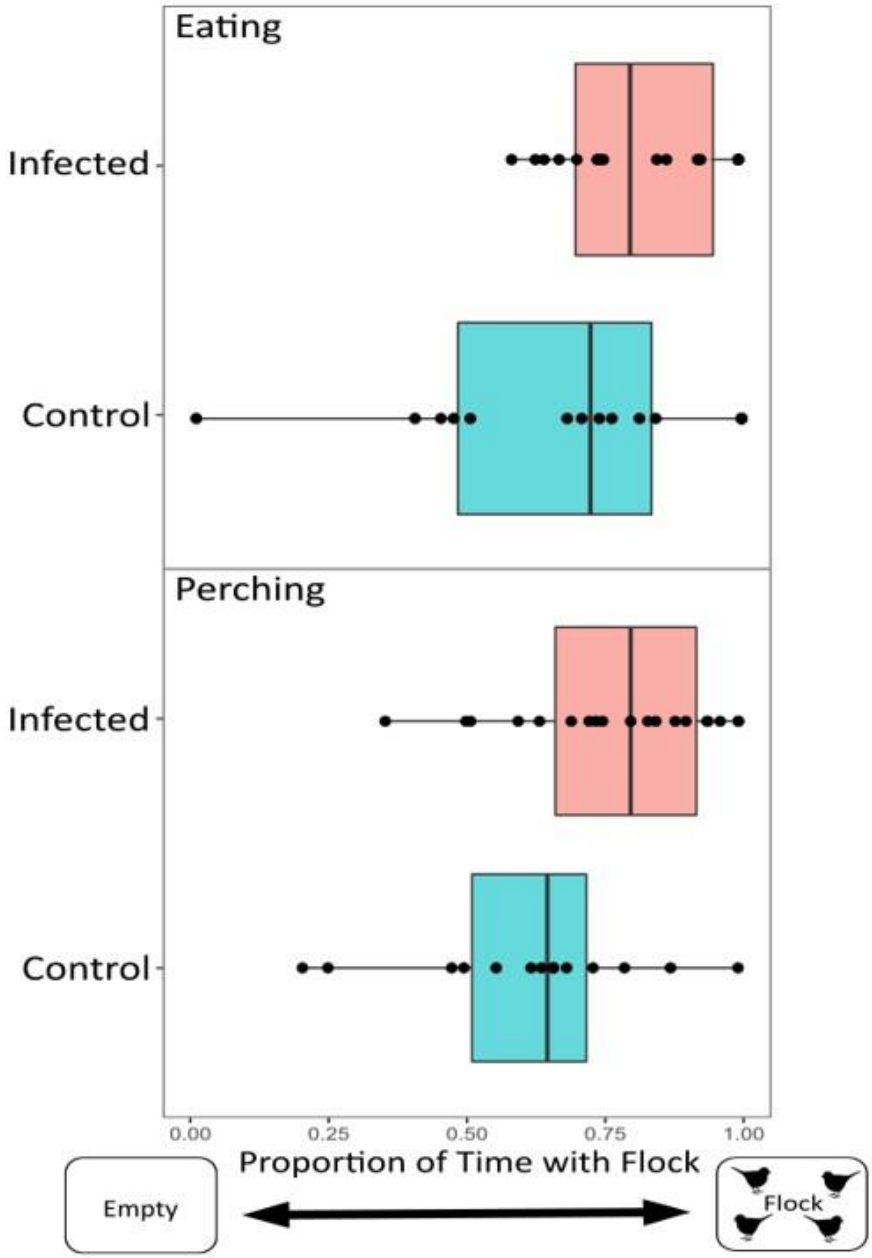

Figure 3. House finches infected with *Mycoplasma gallisepticum* spent significantly more time eating (p=0.018; n=16 individuals) though not significantly more time perching (p=0.062; n=19 individuals), near a flock of novel conspecifics than did uninfected controls (n=14 individuals). Note that the sample sizes are lower for time eating versus perching because three infected individuals did not eat during the assay (see Methods).

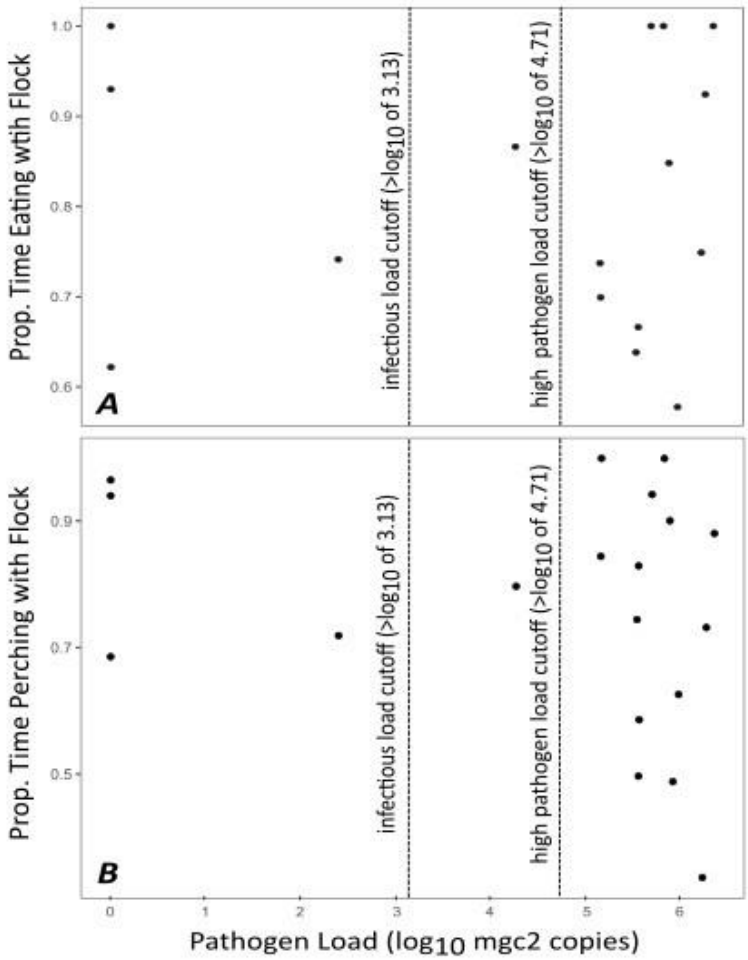

Figure 4. Among infected birds, there was no significant relationship between individual variation in pathogen load at the time of assay (x-axis) and the proportion of time eating (panel A; n=16) or perching (panel B; n=19) near the stimulus flock (y-axis). At the time of assay, infected house finches largely had conjunctival pathogen loads that were above the infectious load for MG (Adelman et al., 2015) (loads ≥ 3.13 $\log_{10}$ copies of MG; 15/19 birds; left vertical dashed line). We further defined pathogen loads as "high" if they fell above the average pathogen load for the NC2006 isolate detected in a past study (Fleming-Davies et al., 2018) (loads ≥ 4.71 $\log_{10}$ copies of MG; right vertical dashed line), which was the case for 14/19 infected birds at the time of assay.

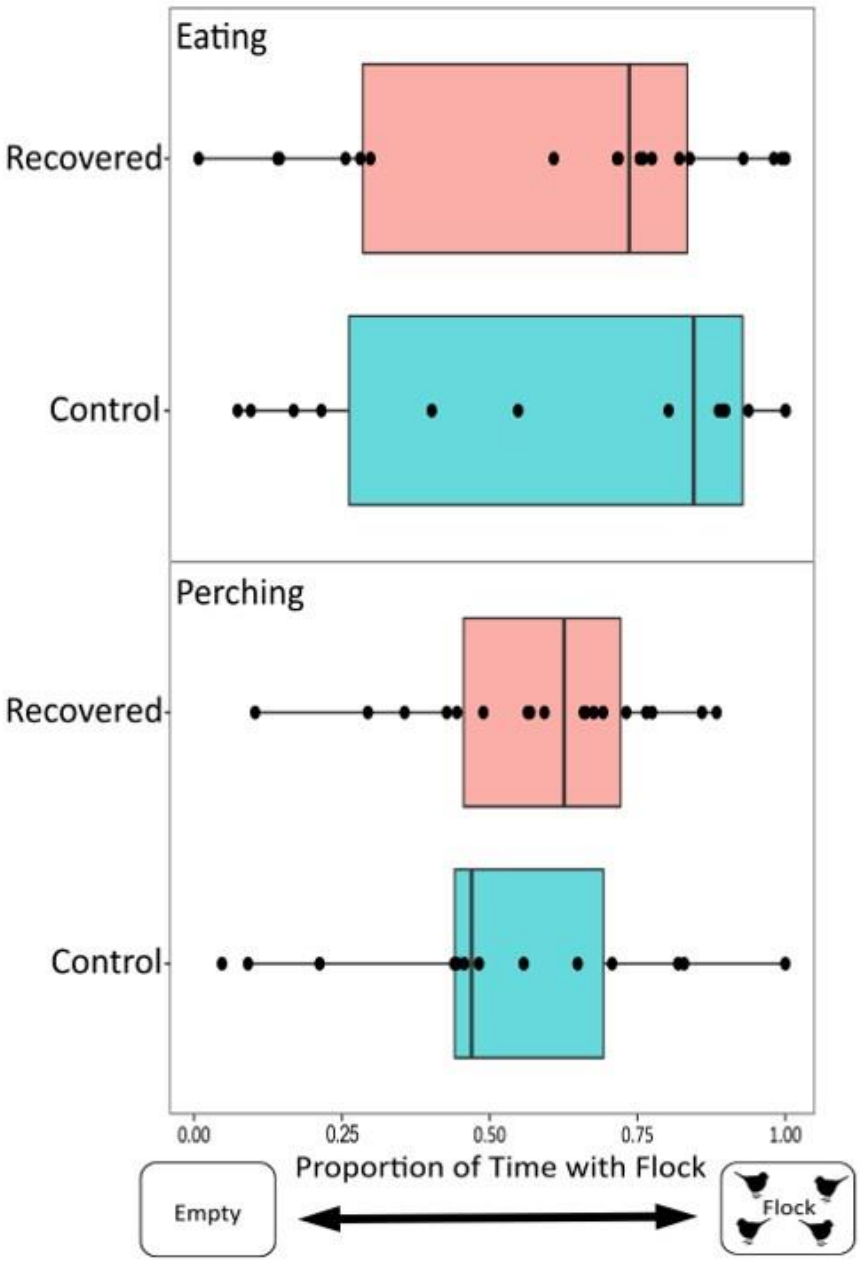

Figure 5. House finches that had recovered from *Mycoplasma gallisepticum* did not spend a significant amount of time eating (p=0.28, n=18 individuals) or perching (p=0.12, n=18 individuals) near a flock of novel conspecifics than did uninfected controls (n=14 individuals).

Appendix

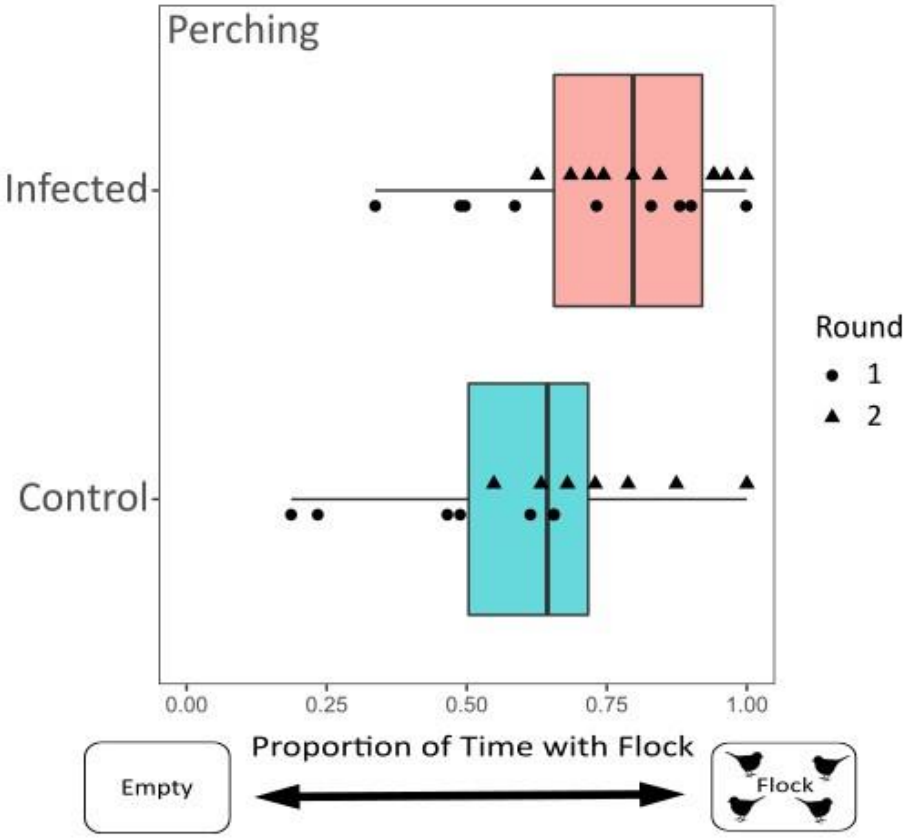

Figure A1. For all birds experimental round (round 1, circles; round 2, triangles) was a significant covariate in the generalized linear models for perching only. Any effect of round was accounted for in our analysis and, thus, did not influence our interpretation of treatment effects.

Ch. 4

In sickness and in health: Group-living augments behavioral responses to food and predation risk for sick house finches (*Haemorhous mexicanus*)

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Prepared for submission in Proceedings of the Royal Academy B: Biological Sciences

Abstract

Group-living provides many fitness benefits for individual members, including improved foraging and predator vigilance. If such benefits are especially pronounced for sick members, group-living can act as a form of behavioral tolerance by offsetting mortality costs of infection. We experimentally tested this possibility by examining whether group-living impacts foraging and anti-predator behaviors in house finches (*Haemorhous mexicanus*) infected with the pathogen *Mycoplasma gallisepticum* or sterile media (control). We varied both group-living (single-housed or group-housed) and infection (MG-inoculated or sham-inoculated) and performed four behavioral assays at peak infection: two assessing how birds respond to foraging opportunities and two assessing responses to predation threats. Both social treatment and disease status influenced most measured behaviors, with single-housed, diseased birds consistently the least responsive to foraging opportunities and predation threats. While benefits of group-living were also apparent for healthy individuals in most assays, our results suggest that diseased birds particularly benefit from group-living. Further, detected behavioral differences with group-living were not explained by effects of sociality on disease severity or pathogen load, which did not differ with group-living. By augmenting behaviors key to survival during infection, group-living may act as a form of behavioral tolerance for social species, with important implications for transmission dynamics.

Introduction

While benefiting all animals generally, for gregarious species, maintaining social groups is vitally important for individual fitness and overall health. Despite potential costs of group-living such as competition (e.g., Forero et al., 2002; Markham & Gesquiere, 2017) and increased pathogen risk (e.g., Rifkin et al., 2012), sociality has evolved multiple times across a variety of taxa (Hofmann et al., 2014), suggesting that the benefits of sociality outweigh its costs for many

animals. The key benefits of group-living, which include increased food acquisition and decreased time spent on predator vigilance by individual group members (e.g., Beauchamp, 2008; Kenward, 1978; Olson et al., 2015), may be especially important for individuals actively experiencing acute infections (Ezenwa et al., 2016). Such infected individuals might rely heavily on healthy group members for fitness benefits such as food acquisition and predator detection (Ezenwa & Worsley-Tonks, 2018). Nonetheless, few studies have explicitly examined the potential benefits of group-living for actively infected individuals (but see Almberg et al., 2015). Determining whether group-living particularly benefits infected individuals is key for understanding social preferences during infection as well as its possible impacts on transmission dynamics within a population.

Infection with a pathogen often results in sickness behaviors characterized by overall reductions in activity level and social interactions, which may limit the ability of infected animals to behave normally within their environment (Dantzer & Kelley, 2007; Hart, 1988) . Animals displaying sickness behaviors are often more susceptible to predation (Adelman et al., 2017) and can have difficulty locating or partaking in food resources (Almberg et al., 2015). Results from several studies suggest that group-living can reduce the fitness costs of infection for hosts through several different possible mechanisms, including increased predator vigilance by conspecifics, territory defense, and improved foraging success and food acquisition (Almberg et al., 2015; Ezenwa & Worsley-Tonks, 2018). Thus, for gregarious species, infected individuals may utilize group-living as a form of behavioral tolerance, defined here as using behavior to offset the fitness costs incurred during infection (Ezenwa et al., 2016).

Both foraging and anti-predator benefits of group-living require the transmission of information among group members (Markham & Gesquiere, 2017). Social groups could provide infected individuals with information they would not be able to acquire otherwise, such as the location of a foraging site or the presence of a predator. One mechanism that utilizes this information transfer to benefit individuals within a group is the “many-eyes effect”, where groups provide more total individuals to scan for predators, thereby allowing group members to both allocate more of their daily time budget towards feeding and to respond more quickly to predators (Lima & Dill, 1990). For example, flock size influences the latency to detect and respond to a predator in several species of gregarious birds, with birds in larger flocks responding more quickly to a perceived predator threat than birds within smaller flocks (Morelli

et al., 2019). The importance of group-living has also been demonstrated in individuals harboring infectious parasites. Herding gazelle infected with gastrointestinal nematodes had increased foraging efficiency due to the dilution of individual anti-predator vigilance (Ezenwa & Worsley-Tonks, 2018). Information transfer and the presence of "many eyes" that are associated with group-living could play a role in offsetting the fitness costs incurred by sickness behaviors such as lethargy (Hetem et al., 2008), which are hypothesized to help acutely infected animals divert energy toward immunity (Dantzer & Kelley, 2007), but can also lower an individual's ability to maintain other aspects of fitness (Lopes et al., 2021).

Host-pathogen systems where acute infections occur largely during periods of group-living provide key opportunities for directly testing whether grouping behavior can offset fitness costs of infection, thereby acting as a form of behavioral tolerance. One such example is the naturally-occurring host-pathogen system, house finches (*Haemorhous mexicanus*) and their bacterial pathogen *Mycoplasma gallisepticum* (MG), which leads to severe conjunctivitis in infected birds (Kollias et al., 2004). House finches are a gregarious North American songbird species that form large foraging flocks, particularly during the fall and winter, congregating around bird feeders which facilitate MG spread (Dhondt et al., 2007) and seasonal outbreaks of disease (Hosseini et al., 2009).

Group-living can potentially offset the costs of MG infection in several ways. First, flocking may offset the higher predation risk experienced by infected individuals, which likely constitutes the primary source of MG-mediated mortality in wild birds (Faustino et al., 2004). MG-infected house finches show extreme lethargy (Kollias et al., 2004) and reduced anti-predator responses relative to healthy birds (Adelman et al., 2017), both of which may increase their likelihood of predation. Notably, even though conjunctivitis in MG-infected house finches can lead to obscured vision, reductions in anti-predator behaviors also occur in infected birds who do not yet exhibit conjunctival swelling (Adelman et al., 2017), likely due to sickness-induced lethargy. Thus, even infected birds able to visualize a predator may still experience predation mortality during infection if not alerted by conspecifics about a predation threat. Group-living may help offset the cost of decreased anti-predator vigilance during infection by alerting infected individuals to and, consequently, increasing foraging time, through the "many-eyes effect" (Fernández-Juricic et al., 2004; Morelli et al., 2019), allowing birds in a flock a higher likelihood of surviving infection. Finally, group-living itself may result in suppression of

sickness behaviors for sick individuals surrounded by uninfected conspecifics, leading sick individuals to be more active when in a group than if isolated, an effect seen in zebra finches and hypothesized to decrease behavioral costs associated with infection (Lopes et al., 2012). Because infection with MG is associated with an overall reduction in activity levels (Kollias et al., 2004), suppression of sickness behaviors may allow infected group members to take better advantage of the benefits associated with group-living, increasing their chances of surviving infection.

Infection with MG also impacts the social preferences of house finches in ways consistent with group-living benefitting infected finches. When given the choice to associate with a flock or alone, house finches experimentally inoculated with MG preferred to eat in association with a flock significantly more so than uninfected birds (Langager et al., 2023). This social preference may indicate that benefits of group membership are especially important for infected birds in this system. Here we assessed the two primary benefits of group-living in songbirds (foraging and anti-predator behaviors) to test whether infected house finches benefit directly from group-living. To elucidate the role of group-living on foraging behavior and predator responses of MG-infected house finches, we conducted a 2x2 factorial design in which wild-caught, but captive-held, house finches were assigned to one of two treatments – a group-living treatment (group-housed or isolated) and an infection treatment (inoculated with MG or sham-inoculated with sterile media) and then tested via four behavioral assays to measure responses to both foraging and predation challenges. We also examined whether social treatment altered the severity of infection (pathogen load) or disease (conjunctivitis severity) that experimentally-infected birds experienced, because such effects could contribute to detected behavioral differences in response to group-living (Adelman et al., 2017). In line with the "many eyes" hypothesis, we predicted that infected birds housed in groups would respond more rapidly to foraging opportunities and predation threats than single-housed infected birds, despite birds in both social treatments harboring equivalent pathogen loads and conjunctival swelling.

Methods

Experimental design

Our experiment varied both group-living treatment (single-housed or group-housed in a flock of 5 total birds) and infection status (sham- or MG-inoculated) for 32 "focal" birds in a fully factorial design (n=8 per social housing / infection status combination). Each group-housed

focal bird was co-housed with four flockmates, one of which (per flock) was used as a sham control to better account for potential variability among flocks. To control for potential effects of cage size on behavioral responses in the four assays, all birds, regardless of social group treatment, were housed in identically-sized large flight cages. As noted below, because MG transmission from focal infected birds to healthy flockmates occurred rapidly in group-housed treatments, in some cases prior to our behavioral assays, we also collected behavioral data from birds that became MG-infected via transmission for certain assays (see *Dominance Trials and Infection Treatment*), resulting in the use of data from all birds (focal birds plus additional flockmates from group-housed treatments; Table 1) for some assays.

We assayed behavioral responses (foraging and anti-predator behaviors) using four distinct assays conducted on different days during the window of peak infection (days 10 to 20; Dhondt et al., 2008). To complete the required behavioral tests during this window, we split the experiment into two temporal batches (one month apart, with each individual bird only included in one unique batch). Experimental batch 1 (n=28 total birds; n=16 focal birds) ran from the end of June 2022 to mid-July 2022. Experimental batch 2 (n=28 total birds; n=16 focal birds) ran from the end of July 2022 to mid-August 2022. Both batches contained equal proportions of all treatment combinations.

Study subjects, capture, and initial housing

Fifty-six hatch year house finches were captured from eight sites in Radford or Blacksburg, Virginia, USA in May – July of 2022 using baited wire traps placed over a bird feeder. Age (hatch-year) for each bird was determined at capture by plumage, lack of a brood patch or cloacal protuberance, and presence of a distinct yellow gape line. Because the study examined responses to experimental MG infection, we ensured that all birds used in the study did not have prior exposure to MG in two ways. First, all captive-held house finches were examined for clinical signs of MG at least three times over a quarantine period of 14 days post-capture. Second, the fifty-six birds used in this experiment were also seronegative for MG prior to experimental infection, which was determined by testing blood plasma with an IgY ELISA kit specifically for MG detection (Hawley et al., 2011). Based on prior work demonstrating that hatch year juvenile house finches show no sex differences in social affiliative behavior

(Langager et al., 2023) and because sex differences in plumage were not yet present in juvenile birds at the time of the study, we did not sex birds for this experiment.

After capture but prior to being moved into experimental housing, all birds were housed in pairs in indoor cages (76 x 46 x 46 cm) for up to 50 days of quarantine and acclimation, depending on capture date. All pairs were kept in temperature-controlled rooms with a 12L:12D light cycle and fed a mixture of 80% Roudybush Maintenance Nibbles and 20% hulled sunflower seeds in a single dish. Water was provided in a single dish *ad libitum*.

Social Group Treatment and Experimental Housing

Ten days before inoculation, the 56 birds in the experiment were assigned to a social group treatment, either group-housed (a flock of 5 birds housed together) or single-housed (Fig.1). Each group-housed treatment contained one MG-inoculated and one sham-inoculated individual (see *Dominance Trials and Infection Treatment).* All five flock members were housed together in a large flight cage (100 x 34 x 34 cm) and provided food *ad libitum* across three food dishes to account for the increased number of birds present within the cage. Single-housed birds were provided food *ad libitum* in a single food dish. House finches were given a unique set of color bands according to their social group treatment (i.e., no flock member had the same color bands), before being moved into their assigned group housing. Each identically-sized flight cage was given a unique cage ID number and equipped with three perches as well as a cardboard box in the upper right corner to provide a refuge area. While this refuge area was specifically installed for use in the predator assay, cages were outfitted with the boxes prior to housing birds, in order to minimize disturbance as well as acclimatize birds to the presence of the refuge area.

A total of seven housing rooms were used for each temporal batch, and all housing rooms were temperature-controlled and kept on a 12L:12D light schedule throughout the duration of the experiment. Due to space constraints, two cages of flock-housed individuals were kept within the same room during each batch of the experiment and were separated by a barrier so that each flock was visually, but not auditorily, isolated from one another throughout the experiment. Single-housed, infected birds were housed alone in a room to simulate true social isolation, and single-housed sham-inoculated individuals were placed together into a room containing four cages, which were separated by barriers so that each cage was visually, but not auditorily, isolated from each other.

Dominance Trials and Infection Treatment

Because an individual's social status within a group can impact how behaviors are expressed during infection (Cohn & de Sá-Rocha, 2006), we assessed the social status of each of the flock members prior to experimental infection to determine which bird in each flock would be directly inoculated with MG (group-housed infected treatment) (Fig. 1). To assess social status without human interference, we video-recorded each flock for 60 minutes and observed dyadic interactions around a single food dish. To motivate birds to interact at the food dish, all three of their normal food dishes were removed 30 minutes before lights off the night before. All assays began 30 minutes after lights turned on for the day (for a total of 60 minutes food restriction while lights were turned on, but 13 hours in total), when a single food dish was placed on the floor of each flock cage and video recording began. The total wins of each dyadic interaction were used to quantify relative social status within each flock. A bird was considered as "winning" the interaction if they maintained their place at the food dish or displaced a flockmate from the dish; losses were recorded for the bird in a pair who was displaced or kept away from the food dish. We assigned each bird a social rank from 1-5, with the bird at rank 1 having won the most dyadic interactions and the rank 5 bird having won the fewest interactions. To avoid directly inoculating a bird at either extreme within the group (most dominant or most submissive), we assigned birds at rank 3 (the most central social status within each flock, neither highly dominant nor highly submissive) to the MG-inoculated, group-housed treatment (n=8). Sham-inoculated treatments within the flock were randomly assigned to either the 2nd or 4th ranked flock member using a coin toss (n=8). The remaining 3 birds in a flock were not assigned to a specific infection treatment, serving as "flockmates".

Though only 2 birds in each flock served as focal individuals (1 MG-inoculated and 1 sham-inoculated per flock) and were directly inoculated on Experiment Day 0, there was rapid MG transmission among our group-housed birds such that by experiment day 11, 4/8 group-housed sham-inoculated birds and 12/24 of the birds designated as flockmates were displaying visible signs of MG infection; on experiment day 15, the number of flockmates displaying visible signs of MG had increased to 14/24 flockmates. Because disease status of an individual's group members can impact their behavior (Lopes, 2023), we recorded the disease status (diseased or healthy) for each bird (including MG-inoculated, sham-inoculated, and flockmates)

at the time of each behavioral assay and included all birds, with their current disease status, in some analyses (Table 1). For the purposes of our analyses, "healthy" birds are those without clinical signs of MG and "diseased" birds are those displaying visible signs of MG infection. We use disease status rather than infection status (determined via qPCR) because 1) disease status is a highly reliable indicator of infection for birds of unknown MG status (capturing 97% of positive cases in past work; (Adelman et al., 2015), and 2) we had higher temporal sampling resolution for disease status versus infection status in our study (see "Inoculation and Disease Monitoring"), allowing us to more robustly determine disease status versus infection status at the time of the assay for a given individual.

Inoculation and Disease Monitoring

To ensure that birds developed sufficiently high disease severity to influence foraging and anti-predator behaviors, we used an MG strain known to be virulent in this finch population (Fleming-Davies et al., 2018; Langager et al., 2023), that had been collected from North Carolina, USA, in 2006 (NC2006, 2006.080-5 4P 7/26/12, David H. Ley, NC State University, College of Veterinary Medicine, Raleigh, NC, USA 27606). On experimental day 0, birds assigned to a given treatment were inoculated bilaterally in the conjunctiva with 70 µL of MG in Frey's media (MG-inoculated treatment: single-housed n=8, group-housed n=8) or with the same volume of sterile media alone (sham-inoculated treatment: single-housed: n=8, group-housed n=8, but see Table 1). To ensure that handling stress was uniform across all birds in the experiment, the remaining 24 flock-housed birds that were not assigned to either the infection or sham-inoculated treatment were handled in the same manner on experiment day 0 as the birds in an assigned focal treatment, but the clean pipette was only held above the eye briefly.

Disease severity was monitored weekly for all birds until experiment day 16 by scoring conjunctivitis on a 0-3 scale per eye, with scores of 3 displaying the most severe conjunctivitis (Hawley et al., 2011). If weekly conjunctival scoring did not occur within ±2 days of a behavioral assay, we reassessed eye score after the behavioral assay was completed for the day. Within each sampling day, disease severity scores were summed across eyes to calculate a combined severity score of 0 to 6 for birds. In addition to scoring disease severity, we also swabbed the conjunctiva twice, on experiment days 7/8 and 15/16, to quantify MG load. Swabs were placed into 300 µL tryptose phosphate broth (TPB) and stored at -20˚ C until extraction

using Qiagen 96 DNeasy Blood and Tissue Kit; a probe-based qPCR was used to determine the amount of MG in each sample (methods outlined in Hawley et al., 2011).

Behavioral Assays

Feeder Assays

All birds were video recorded in their home cage on two consecutive days for two different feeder behavioral assays. First, on experiment day 11, we assessed latency to approach and use the normal feeder dish in its usual location within the home cage (the front left corner) following removal of the dish to simulate food disturbance. Second, on experiment day 12, we assessed latency to approach and use a novel, brightly colored feeder type that was placed in a different location than their normal food dish, hanging near the back right side of the home cage. To standardize motivation to feed, we removed all normal food dishes from the cages 30 minutes before lights out the night before the assay and only returned food at the start of the assay, 30 minutes after the lights had turned on for the day (totaling 60 minutes of food restriction, as house finches fast during the night). Video was recorded for 45 minutes starting at 07:00, when food (either in the normal or novel feeder) was returned to the cage. The novel food dishes were open, such that birds could visually identify their usual food source within the dish. Each dish also had a small perching area, so birds had somewhere to perch while eating.

All videos were scored by a single observer using BORIS (Friard & Gamba, 2016), with perching and eating behaviors coded continuously (Table 2) throughout the entirety of the video. While it was impossible for the observer to be blind to social group treatment, observers were blind to infection treatment during video scoring.

Capture Evasion Assay

To determine how long a given bird could evade capture by a potential threat to survival, we quantified the time it took to capture each bird from its home cage, with all captures throughout the study done by a single person unfamiliar with the goals of the study. During weekly disease sampling periods (experiment days 7/8 and 15/16), all focal birds in both the MG-inoculated (single-housed: n=8; flock-housed: n=8) and sham-inoculated treatments (single-housed: n=8; flock-housed: n=8) were captured as rapidly as possible. The total time that each bird was able to evade capture by the observer was recorded as the time elapsed between opening

the cage door to the time the bird was securely caught. Because all group-housed treatment cages contained a single bird in the assigned MG-inoculated treatment as well as a single bird in the assigned sham-inoculated treatment, we spread our capture trials across two consecutive days, with one focal bird (either MG- or sham-inoculated) in each flock being captured per day. This ensured that we were able to accurately assess the fastest capture time for each of the two focal birds within the flock. Birds from both the single- and group-housed social group treatments were randomly assigned to be sampled on one of the two consecutive days, so that each focal bird was only timed for capture evasion and sampled once in a weekly period. Time to evade capture was not recorded for any of the birds within the group-housed treatment that were assigned as "flockmates".

Simulated Aerial Predator Assay

We used a simulated aerial predator attack (a hawk silhouette kite mounted on a zipline) to assess response time of birds to a mock predator flyover. To standardize the mounting of the predator and zipline, all aerial predator assays were conducted in a single assay room within the same facility. Each home cage was transferred from its captive room to the predator assay room, approximately 3-6 m from each housing room. Tests were carried out only once for each treatment cage during the study, and for only one cage at a time, such that a given cage was the only one present in the assay room when the aerial predator was released. To ensure that all cages were tested during peak foraging times (07:00-10:30), tests were carried out over three days (experiment days 13, 14, or 15). All birds were food restricted for 60 minutes prior to their assay start (birds assayed at 07:00 were food restricted as outlined in the *Feeder Assay* section).

To prevent prolonged visual access to the simulated predator during the trial, a temporary black plastic sheet was clipped to the back of the cage prior to that cage being moved into the predator assay room. Further, to prevent birds from seeing the simulated predator before the assay began, all cages were moved into the predator assay room before lights were turned on. After placing each cage in the room, video recording began and then the lights were turned on. Overall, each cage was recorded for 35 minutes, with four cages being tested each morning (the first test started at 7:00 and the final test cage of the day started at 9:30).

Approximately 30 seconds after the tester left the room, a 5 second playback from a common aerial predator of finches, a Cooper's Hawk (*Accipiter cooperii*), was played every 15

seconds over the course of 1 minute. During the last 5 second playback section, a kite representing a bottom-up view of a hawk was released, causing it to fly down a zipline system over the birds being tested. Each bird's response during the simulated predator flyover (from the time the hawk was released to the time it landed against the far wall, out of sight) was recorded (outlined in Table 3). In addition to their response during the simulated hawk flyover, we also recorded the time each individual spent immobile for at least 5 seconds. All videos were scored by a single observer who was blind to disease status, but not social treatment, using BORIS (Friard & Gamba, 2016).

Statistical analysis

All analyses were completed in R v. 4.3.2 (R Core Team, 2023) and all plots were made in ggplot2 (Wickham, 2016). Because transmission within the group-housed treatment cages resulted in some sham-inoculated focal birds showing clinical signs of conjunctivitis by the time of a given assay, we used the actual disease status (diseased or healthy; Table 1) of a bird at the time of each behavioral assay in all analyses. Because such rapid transmission limited the sample sizes of healthy control birds (reduced from the 8 original sham controls), we included untreated flockmates (with their actual disease status at the time of the assay) in analyses of both feeder assays and the aerial predator assay, with the unique cage ID of each flock included as a random effect to account for non-independence of birds housed in the same group. However, only focal birds (n=32) and their disease status at the time of capture were included in the analysis of capture evasion because capture times were not recorded for flockmates. Cage ID was still included as a random effect here to account for the non-independence of the two focal birds in each group-housed treatment cage (one MG-inoculated and one sham-inoculated bird per group).

For all behavioral analyses, we included disease status (diseased or heathy) at time of assay, social treatment (group-housed or single-housed), and the interaction of social treatment and individual disease status as predictor variables. Interactions were removed from final models if $p > 0.1$. The significance of the predictor variables in our models were tested using the Anova function (for GLMMs; car package; Fox & Weisberg, 2019) or Anova.clmm function (RVAideMemoire package; Herve, 2023) to generate either Type II likelihood-ratio tests if interactions were not significant or Type III tests in models with significant pairwise interactions.

Foraging assay analyses

Using data from our feeder assays, we addressed two questions about the effect of group living and disease status on foraging behaviors: how likely an individual bird in each treatment category was to approach and eat from their normal feeder following disturbance, and how likely they were to eat from the brightly colored, novel feeder. We quantified willingness to approach each feeder type (normal feeder or novel feeder) by assigning a score from 0-5 to denote how closely an individual bird came to eating from the feeder (Fig. 2A; Table 2), with a score of 0 indicating birds that stayed more than 30 cm from the feeder (considered in our assay to be "no approach") and a score of 5 assigned to birds who ate from the feeder during the assay. The approach scores recorded during the novel feeder assay were treated as ordinal factors and used as the response variable in cumulative link mixed models (CLMM) in the ordinal package (Christensen, 2023). For our normal feeder assays, because the distribution of response scores (with only single-housed birds showing any variation in score) did not allow a CLMM to converge, we used a Fisher's Exact Test comparing approach scores across the four treatment combinations.

To complement the above analyses, we used mixed effect Cox models (coxme package; Therneau, 2024) to analyze latency to approach and eat from the feeder for each assay. For both the normal feeder and novel feeder assays, a binary measure denoting whether or not a bird had eaten from the feeder (0 – did not eat; 1 – did eat) as well as the latency to feed were used as response variables in our analysis.

Anti-predator behavior analyses

Next, we examined how group living and disease status impact anti-predator behaviors in the face of a perceived predation threat (either capture by a human or a simulated aerial predator flyover). Latency to capture a bird was used as the response variable in separate generalized linear mixed models (GLMMs, using Gamma error distribution linked with the log function) for each time capture evasion was sampled (i.e., one model for time to capture on experiment day 7/8 and another for time to capture on experiment day 15/16).

The latency to respond to the aerial predator flyover (in seconds) was determined from subtracting the time the hawk started to "fly" from the time of the bird's first recorded response during the flyover (for behaviors that we considered a predator response, see Table 3). The

latency time recorded for birds who did not exhibit a response during the hawk flyover was the time that the hawk hit the back wall (and was no longer visible to birds in the cage), indicating that these birds did not react during the entirety of the hawk flyover. This time to first response was divided into quartiles, with the birds that were slowest to respond (or did not respond at all) to the predator flyover being sorted into the lowest quartile (quartile 4) and the birds who responded most quickly into the highest (quartile 1). These quartiles were used as ordinal factors which were the response variable in a cumulative link mixed model (CLMM; Christensen 2023).

In addition to the cumulative link mixed models, we used mixed effect Cox models (coxme package; Therneau, 2024) to analyze the latency to respond to the predator flyover. Similar to the Cox models analyzing latency to feed, a binary measure of whether or not a bird responded to the predator as well as the latency to their response were used as response variables in our models.

To analyze a potential proxy for sickness behaviors, as well as the degree to which individuals resumed activity after the aerial predator flyover, we measured time spent immobile in the 30 minutes directly after the aerial predator flyover, as a proxy for sickness-induced lethargy. The proportion of time spent immobile was used as the response variable in an LMM, which were weighted by the total time of the assay.

Disease and pathogen severity

Within the MG-inoculated treatment only (n=16 focal birds; half single-housed and half group-housed), we tested whether group-living treatment influenced either disease severity or pathogen load using separate GLMMs. Because both disease severity and pathogen load were taken over multiple sampling periods (see Fig. 1), each GLMM incorporated bird ID as a random effect. For this analysis alone, cage ID was not included as a random effect because the 16 infected focal birds were all in independent cages.

Results

Feeder Assays

The combination of social treatment and individual disease status influenced the likelihood that house finches would approach and eat from their normal feeder following disturbance (Fisher's Exact test $p<0.0001$). Specifically, only 37% (3/8) of single-housed,

diseased birds ate from their normal feeder during the assay, whereas 100% of birds in all other treatment groups ate during the assay (group-housed diseased: 24/24; group-housed healthy: 16/16; single-housed healthy: 8/8; Fig. 2B). In terms of latency to feed at the normal dish, social treatment and disease status both had independent effects in our model. Group-housed birds had a lower latency to eat from the feeder than single-housed birds, and healthy birds had a lower latency to start feeding than diseased birds (Fig. 2D; n=56; social treatment likelihood ratio Chi-squared=20.16, d.f.=1, $p<0.0001$; disease status likelihood ratio Chi-squared=11.22, d.f.=1, $p<0.001$; mean time to eat (s): group-housed healthy, 220.72s; group-housed diseased, 529.02s; single-housed healthy, 912.77s; single-housed diseased, 2083.95s).

Only group-living treatment significantly predicted how likely a bird was to approach and eat from the novel feeder, with group-housed birds more likely to eat from the novel feeder than single-housed birds, independent of disease status (Fig. 2C; n=56; social treatment likelihood ratio Chi-squared=9.90, d.f.=1, $p<0.002$; disease status likelihood ratio Chi-squared=2.07, d.f.=1, $p=0.15$). Notably, however, none of the birds in the single-housed diseased group ate from the novel feeder, though one bird did perch on it, but never ate (group-housed diseased: 12/24; group-housed healthy: 9/16; single-housed diseased: 0/8; single-housed healthy: 1/8). The patterns for latency to feed from the novel feeder largely mirrored results from the normal feeder assay, with group-housed birds generally (though not significantly at $\alpha=0.05$) displaying a lower latency to locate and eat from the feeder than single-housed birds (Fig. 2E; n=56; social group treatment likelihood ratio Chi-squared=3.14, d.f.=1, $p=0.076$), and diseased birds having a significantly longer latency to eat from the novel feeder than healthy birds (disease status likelihood ratio Chi-squared=4.31, d.f.=1, $p=0.038$). Interestingly, the significant effect of disease status appears driven by the universal lack of eating from the novel feeder in single-housed, diseased birds (mean time to eat (s): group-housed diseased, 2388.98s; group-housed healthy, 2312.026s; single-housed diseased, N/A; single-housed healthy, 2922.008s).

Capture Evasion

The interaction between an individual's social group treatment and their disease status influenced the speed at which they were captured on day 7/8, such that single-housed diseased birds were captured significantly more quickly (1.82-7.44s) by a human than group-housed healthy birds (3.13-23.52s) (Fig. 3A; n=32; social treatment*disease status Chi-squared=14.27,

d.f.=1, p<0.001). As a main effect, social treatment did not predict time to capture (Chi-squared=0.76, d.f.=1, p=0.38). However, disease status as a main effect did significantly predict capture time (Chi-squared=15.1, d.f.=1, p<0.001). The interaction between social treatment and disease status was no longer significant by experiment day 15/16 (Fig. 3B; n=32, social treatment*disease status Chi-squared=0.011, d.f.=1, p=0.92), and was thus removed from our model. Our additive model found that on day 15/16, social group treatment did not predict capture evasion times (social treatment Chi-squared=1.42, d.f.=1, p=0.23). However, we did find an effect of disease status on capture evasion at this timepoint (disease status Chi-squared=4.51, d.f.=1, p=0.03).

Aerial Predator Assay

Both social treatment and a bird's disease status interacted to predict how quickly an individual bird responded to the mock predator flyover, as measured by quartile scores (Fig. 4A; n=56; CLMM social treatment*disease status likelihood ratio Chi-square=3.94, d.f.=1, p=0.047), with single-housed diseased birds responding most slowly (mean=1.51 sec) relative to all other treatments (Fig. 4B). Neither social treatment as a main effect (likelihood ratio Chi-square=0.00, d.f.=1, p=1.00) nor disease status (likelihood ratio Chi-squared=0.00, d.f.=1, p=0.99) predicted quartile scores of latency to respond to the predator flyover. In our Cox models analyzing raw individual latency times, we found that, while not quite significant, our single-housed birds were moderately slower to respond to the predator (likelihood ratio Chi-squared= 3.58, d.f.=1, p=0.059), though individual disease status had no effect on latency to respond (likelihood ratio Chi-squared=0.39, d.f.=1, p=0.53).

The interaction of social group treatment and disease status significantly predicted the time a bird spent immobile during the 30-minute recovery period after aerial predator flyover (Wald Chi-squared=3.69, d.f.=1, p=0.05), with single-housed diseased birds spending more time immobile than birds in any other treatment category (Fig. 5). Social group treatment as a main effect did not predict the time spent immobile (Wald Chi-squared=0.35, d.f.=1, p=0.55). However, we did find a significant main effect of disease status on time spent immobile (Wald Chi-squared=10.70, d.f.=1, p=0.001).

Disease Severity and Pathogen Load

Within MG-inoculated focal birds, there was no significant effect of social treatment on either disease severity (Fig. 6A; n=16; social treatment Chi-squared=0.173, d.f.=1, p=0.68) or pathogen load (Fig. 6B; n=16; social treatment Chi-squared=0.067, d.f.=1, p=0.80).

Discussion

Here we experimentally demonstrate that infected individuals benefit directly from group living by increasing responsiveness to both foraging opportunities and potential predation threats, relative to single-housed infected individuals. While healthy and diseased birds both showed detectable behavioral benefits of group-living in most of our assays, diseased birds housed alone consistently showed the lowest and slowest rates of feeding relative to both healthy birds and diseased birds housed in groups. Similarly, when evading capture from a human (as a proxy for a predation threat) or responding to a mock aerial predator, single-housed diseased birds showed slower responses to both predation threats relative to all other treatment groups. Notably, these differences in behavior were not due to effects of social housing versus isolation on disease severity or pathogen load, which were identical for MG-inoculated birds across social treatments. Together, our results indicate that group living particularly benefits infected house finches by improving behavioral responsiveness to foraging opportunities and predator threats, both of which likely have important consequences for individual survival during infection.

In our foraging assays, group-housed birds were significantly more likely than single-housed diseased birds to approach and eat from their normal feeder after a disturbance, as well as to discover and eat from a novel feeder type. This behavioral difference may have important consequences for the overall survival of house finches in the wild, which rely heavily on bird feeders to meet their energetic needs (Badyaev et al., 2020), particularly during winter (Bonter et al., 2013). Our findings indicate that isolated diseased birds, which occur commonly in the wild (Hawley et al., 2007), are less able to respond quickly to disturbance at a feeder that they regularly use. For group-living house finches, flockmates may allow them to respond more effectively to regular disturbances (refilling of feeders, which can occur multiple times per week; Dayer et al., 2019) as well as novel or atypical situations, such as implementation of an unfamiliar feeder type or the establishment of a feeder in a backyard for the first time. In a study of another gregarious songbird, house sparrows (*Passer domesticus*), it was found that birds in

larger groups were able to innovate and problem solve more quickly in response to a novel environment than those in smaller groups (Liker & Bókony, 2009). Having different group members with varying levels of experiences may help house finches in particular, which do not appear as adept as some species at problem solving, as demonstrated in a study in male house finches in which, on average, only 27% of single-housed individuals were able to open a lid placed on top of their normal food dish (Arnold et al., 2021). Our assays suggest that group living helps individual house finches to overcome novel foraging situations, and this benefit may be particularly important for diseased individuals.

Our anti-predator assays also indicated that the benefits of group living, here in terms of predator avoidance, are particularly important for diseased birds. Single-housed diseased birds were significantly slower than all other treatment combinations in their responses to two different perceived threats at two different points during infection – capture by a human during early infection and an aerial predator flyover during peak infection. While these findings align with previous work showing that diseased house finches display reduced anti-predator responses (Adelman et al., 2017), our results demonstrate that social context plays a key role in modifying effects of disease on an individual's response to predators. In fact, diseased birds housed in groups largely had response times that aligned with their healthy flockmates, suggesting that group-living can largely ameliorate the effects of disease on anti-predator behaviors. Because predation accounts for the majority of MG-related mortality (Faustino et al., 2004), augmented anti-predator responses by infected birds living in groups could have important impacts on the ability of birds to survive infection. MG infection typically leads to swelling of the conjunctiva, which can sometimes progress to reduced visual acuity or even temporary blindness (Sydenstricker et al., 2006). Notably, even though there were no differences in disease severity between MG-inoculated birds in each social group treatment, group-housed diseased birds responded twice as fast to an aerial predator than single-housed diseased house finches. The equivalent pathogen loads harbored by infected birds, regardless of group-living treatment, further support the possibility that infected house finches are showing behavioral tolerance by behaviorally offsetting the per-pathogen fitness costs of infection. Overall, our results suggest that diseased birds in a flock are receiving key cues from their groupmates that are not available to isolated, diseased finches.

Another possible mechanism for the observed behavioral differences across social treatment and disease status is that group-housed birds might be masking the sickness behaviors commonly associated with MG infection. Consistent with this possibility, single-housed diseased birds spent significantly more time immobile after the predator flyover, displaying higher levels of lethargy than group-housed diseased birds. These findings align with the suppression of sickness behavior in social settings by another highly gregarious songbird species, zebra finches (*Taeniopygia guttata*), when exposed to a simulated infection (Lopes et al., 2012). Though we only quantified sickness behaviors *per se* during our aerial predator assay, both foraging behaviors and predator responses can be impacted by the presence or absence of sickness behaviors. Thus, by suppressing behaviors such as lethargy during infection, house finches in flocks may be increasing their likelihood of survival through increased anti-predator responses and foraging opportunities, compared to their more isolated conspecifics. Interestingly, the one single-housed, diseased bird in our study that had very low disease severity and recovered from infection quickly still displayed similar foraging and anti-predator behaviors compared to other single-housed diseased birds that had much more severe disease. While anecdotal, this, along with our other results, supports the influence of social environment on behavioral phenotypes.

Overall, our results suggest that group-living may act as a key form of behavioral tolerance for diseased house finches in the wild. These results may help explain previously demonstrated preferences for associating with a flock during experimental MG infection in house finches (Langager et al., 2023). Nonetheless, while our assays found that birds housed in flocks of five were significantly more likely to eat from a feeder, in the wild infected house finches may not be able to consistently keep up with flocks of healthy conspecifics. In fact, relative to healthy birds, house finches with severe disease are observed more often in smaller flocks while at feeding stations (Hawley et al., 2007; Hotchkiss et al., 2005). Because diseased house finches are not actively avoided by healthy conspecifics, and in the case of diseased males, are preferred over healthy birds as feeding partners (Bouwman & Hawley, 2010), the smaller observed flock sizes of diseased birds in the wild is unlikely to result from avoidance by healthy conspecifics, and instead likely represents an inability of diseased birds to adequately move across the landscape (Aberle et al., 2020). Although severely diseased wild birds may not be able to successfully associate with flocks of the size used in this study, even having one other house finch close by while feeding may be beneficial from a foraging behavior perspective. In a study

of multiple species of common North American bird feeder birds, Berberi and colleagues (2023) found that having even one conspecific with them at a feeder can help individuals combat lost foraging opportunities due to interspecific competition. However, our study was limited to observing foraging and anti-predator behaviors in the presence or absence of any group members, with birds being either single-housed or group-housed with a stable flock of four conspecifics. Considering the large body of evidence that differing group sizes impact behavioral phenotypes (e.g., Fernández-Juricic et al., 2004; Liker & Bókony, 2009; Rooke et al., 2020) and work that has shown that infected house finches in the wild are seen more often with smaller flocks (Hawley et al., 2007; Hotchkiss et al., 2005), future work should explore optimal flock sizes, as well as the benefits and costs associated with smaller or larger flocks, for diseased individuals.

Our results show that group living extends both foraging and anti-predator benefits to individual members, and these benefits are particularly strong for infected individuals. These results align with recent work showing that personality traits of individuals can mediate the benefits of social cues, with slow-exploring red knots benefiting more strongly from group foraging than fast-exploring individuals (Roncoroni et al., 2024) Together, this underscores the importance of understanding how traits of individuals (disease status, personality, etc.) drive the relative behavioral benefits of group-living. Further, although not quantified here, the foraging and anti-predator benefits of group-living are likely synergistic to some extent. Increased foraging due to dilution of time spent on predator vigilance has been documented in multiple systems (Ezenwa & Worsley-Tonks, 2018; Fernández-Juricic et al., 2004), so it is likely that any fitness benefits provided by groups through increased predator defense and increased foraging success are not mutually exclusive. Thus, maintaining social groups during infection may provide multiple and overlapping benefits to individuals. While our study was conducted on a specific songbird species, the benefits detected here for infected house finches may be similar for other gregarious taxa, such as herbivorous (Ezenwa & Worsley-Tonks, 2018) and cooperatively hunting mammals (Almberg et al., 2015). In other social systems, however, group-living during infection may come with added costs rather than benefits (McFarland et al., 2021). Such costs of sociality for some hosts during infection may help explain why many social animals actively or passively reduce their degree of social interaction when infected or expressing sickness

behaviors (e.g. Bos et al., 2012; Geffre et al., 2020; Ripperger et al., 2020; Stockmaier et al., 2020).

In summary, our results show that infected house finches benefit directly from group-living, often to a stronger extent than healthy individuals. While such benefits may be key for individuals to survive infection in the wild, group-living of infected hosts will also directly augment transmission within the group (as seen for the initially healthy 32 flockmates, which showed 50% disease prevalence by the end of the study), representing a key cost for healthy group members. Thus, group-living during infection as a form of behavioral tolerance may, in turn, favor the evolution of counterstrategies in healthy individuals, such as the ability to detect and avoid (or show aggression toward) infected group members (Stockmaier et al., 2021). Overall, understanding the ways in which pathogen infection alter the key costs and benefits of group-living is critical for ultimately predicting both the ecological and evolutionary dynamics of pathogens in gregarious taxa, and the evolution of social behavior in the face of rapidly changing natural enemies.

Acknowledgements

We thank Caro Vela for capturing birds for our capture evasion assay. We also thank Dr. Anna Perez-Umphrey, Sara Teemer Richards, and Jesse Garrett-Larsen for their assistance throughout the experiment. We thank Drs. Ignacio Moore, Kendra Sewall, and Jeff Walters for their helpful input. Our experiments were conducted under approved Virginia Tech Institutional Animal Care and Use Committee protocol. All experimental methods were carried out in accordance with the guidelines and regulations set forth by the Virginia Tech IACUC and Virginia Department of Game and Inland Fisheries (Virginia Department of Game and Inland Fisheries permit 061440, USFWS permit MB154804-0). This work was funded by NSF grant IOS-1754872 (to D.M.H).

References

Aberle, M. A., Langwig, K. E., Adelman, J. S., & Hawley, D. M. (2020). Effects of bird feeder density on the foraging behaviors of a backyard songbird (The house finch, *Haemorhous mexicanus*) subject to seasonal disease outbreaks. *Canadian Journal of Zoology*, *98*(9), 611–621. https://doi.org/10.1139/cjz-2019-0282

Adelman, J. S., Mayer, C., & Hawley, D. M. (2017). Infection reduces anti-predator behaviors in house finches. *Journal of Avian Biology*, *48*, 519–528.

Adelman, J. S., Moyers, S. C., Farine, D. R., & Hawley, D. M. (2015). Feeder use predicts both acquisition and transmission of a contagious pathogen in a North American songbird [Supplemental material]. *Proceedings of the Royal Society B*, *282*(20151429).

Almberg, E. S., Cross, P. C., Dobson, A. P., Smith, D. W., Metz, M. C., Stahler, D. R., & Hudson, P. J. (2015). Social living mitigates the costs of a chronic illness in a cooperative carnivore. *Ecology Letters*, *18*(7), 660–667. https://doi.org/10.1111/ele.12444

Arnold, S., Weaver, M. J., & McGraw, K. J. (2021). Ornamental plumage coloration interacts with habitat urbanization to predict problem-solving in the House Finch *Haemorhous mexicanus*. *Acta Ornithologica*, *56*(1), 29–37. https://doi.org/10.3161/00016454AO2021.56.1.003

Badyaev, A. V., Belloni, V., & Hill, G. E. (2020). House Finch (Haemorhous mexicanus). In *Birds of the World*, A. F. Poole (Ed.). Cornell Lab of Ornithology. https://doi.org/10.2173/bow.houfin.01

Beauchamp, G. (2008). What is the magnitude of the group-size effect on vigilance? *Behavioral Ecology*, *19*(6), 1361–1368. https://doi.org/10.1093/beheco/arn096

Berberi, I., Miller, E. T., & Dakin, R. (2023). The effect of sociality on competitive interactions among birds. *Proceedings of the Royal Society B: Biological Sciences*, *290*(1994). https://doi.org/10.1098/rspb.2022.1894

Bonter, D. N., Zuckerberg, B., Sedgwick, C. W., & Hochachka, W. M. (2013). Daily foraging patterns in free-living birds: Exploring the predation-starvation trade-off. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1760). https://doi.org/10.1098/rspb.2012.3087

Bos, N., Lefèvre, T., Jensen, A. B., & D'Ettorre, P. (2012). Sick ants become unsociable. *Journal of Evolutionary Biology*, *25*(2), 342–351. https://doi.org/10.1111/j.1420-9101.2011.02425.x

Bouwman, K. M., & Hawley, D. M. (2010). Sickness behaviour acting as an evolutionary trap? Male house finches preferentially feed near diseased conspecifics. *Biology Letters*, *6*, 462–465.

Christensen, R. H. B. (2023). *ordinal - Regression Models for Ordinal Data*. https://CRAN.R-project.org/package=ordinal

Cohn, D. W. H., & de Sá-Rocha, L. C. (2006). Differential effects of lipopolysaccharide in the social behavior of dominant and submissive mice. *Physiology and Behavior*, *87*(5), 932–937. https://doi.org/10.1016/j.physbeh.2006.02.011

Dantzer, R., & Kelley, K. W. (2007). Twenty years of research on cytokine-induced sickness behavior. *Brain, Behavior, and Immunity*, *21*(2), 153–160.

Dayer, A. A., Rosenblatt, C., Bonter, D. N., Faulkner, H., Hall, R. J., Hochachka, W. M., Phillips, T. B., & Hawley, D. M. (2019). Observations at backyard bird feeders influence the emotions and actions of people that feed birds. *People and Nature*, *1*(2), 138–151. https://doi.org/10.1002/pan3.17

Dhondt, A. A., Dhondt, K. V., Hawley, D. M., & Jennelle, C. S. (2007). Experimental evidence for transmission of Mycoplasma *gallisepticum* in house finches by fomites. *Avian Pathology*, *36*(3), 205–208.

Dhondt, A. A., Dhondt, K. V., & McCleery, B. V. (2008). Comparative infectiousness of three passerine bird species after experimental inoculation with Mycoplasma *gallisepticum*. *Avian Pathology*, *37*(6), 635–640. https://doi.org/10.1080/03079450802499100

Ezenwa, V. O., Ghai, R. R., McKay, A. F., & Williams, A. E. (2016). Group living and pathogen infection revisited. *Current Opinion in Behavioral Sciences*, *12*, 66–72. https://doi.org/10.1016/j.cobeha.2016.09.006

Ezenwa, V. O., & Worsley-Tonks, K. E. L. (2018). Social living simultaneously increases infection risk and decreases the cost of infection. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1892). https://doi.org/10.1098/rspb.2018.2142

Faustino, C. R., Jennelle, C. S., Connolly, V., Davis, A. K., Swarthout, E. C., Dhondt, A. A., & Cooch, E. G. (2004). *Mycoplasma gallisepticum* infection dynamics in a house finch population: seasonal variation in survival, encounter and transmission rate. *Journal of Animal Ecology*, *73*(4), 651–669.

Fernández-Juricic, E., Siller, S., & Kacelnik, A. (2004). Flock density, social foraging, and scanning: An experiment with starlings. *Behavioral Ecology*, *15*(3), 371–379. https://doi.org/10.1093/beheco/arh017

Fleming-Davies, A. E., Williams, P. D., Dhondt, A. A., Dobson, A. P., Hochachka, W. M., Leon, A. E., Ley, D. H., Osnas, E. E., & Hawley, D. M. (2018). Incomplete host immunity favors the evolution of virulence in an emergent pathogen. *Science*, *359*, 1030–1033. https://www.science.org

Forero, M. G., Tella, J. L., Hobson, K. A., Bertellotti, M., & Blanco, G. (2002). Conspecific food competition explains variability in colony size: A test in Magellanic Penguins. *Ecology*, *83*(12), 3466–3475. https://doi.org/10.1890/0012-9658(2002)083[3466:CFCEVI]2.0.CO;2

Fox, J., & Weisberg, S. (2019). *An R Companion to Applied Regression*. Sage. https://socialsciences.mcmaster.ca/jfox/Books/Companion/

Friard, O., & Gamba, M. (2016). BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. *Methods in Ecology and Evolution*, *7*(11), 1325–1330. https://doi.org/10.1111/2041-210X.12584

Geffre, A. C., Gernat, T., Harwood, G. P., Jones, B. M., Morselli Gysi, D., Hamilton, A. R., Bonning, B. C., Toth, A. L., Robinson, G. E., Dolezal, A. G., & Strassmann, J. E. (2020). Honey bee virus causes context-dependent changes in host social behavior. *PNAS*, *117*(19). https://doi.org/10.5061/dryad.m63xsj3z8

Hart, B. L. (1988). Biological basis of the behavior of sick animals. *Neuroscience and Biobehavioral Reviews*, *12*(2), 123–137. https://doi.org/10.1016/S0149-7634(88)80004-6

Hawley, D. M., Davis, A. K., & Dhondt, A. A. (2007). Transmission-relevant behaviours shift with pathogen infection in wild house finches (*Carpodacus mexicanus*). *Canadian Journal of Zoology*, *85*(6), 752–757.

Hawley, D. M., Grodio, J., Frasca, S., Kirkpatrick, L., & Ley, D. H. (2011). Experimental infection of domestic canaries (*Serinus canaria domestica*) with *Mycoplasma gallisepticum*: A new model system for a wildlife disease. *Avian Pathology*, *40*(3), 321–327. https://doi.org/10.1080/03079457.2011.571660

Herve, M. (2023). *RVAideMemoire: Testing and Plotting Procedures for Biostatistics*. https://CRAN.R-project.org/package=RVAideMemoire

Hetem, R. S., Mitchell, D., Maloney, S. K., Meyer, L. C. R., Fick, L. G., Kerley, G. I. H., & Fuller, A. (2008). Fever and sickness behavior during an opportunistic infection in a free-living antelope, the greater kudu (*Tragelaphus strepsiceros*). *Am J Physiol Regul Integr Comp Physiol*, *294*, 246–254. https://doi.org/10.1152/ajpregu.00570.2007.-To

Hofmann, H. A., Beery, A. K., Blumstein, D. T., Couzin, I. D., Earley, R. L., Hayes, L. D., Hurd, P. L., Lacey, E. A., Phelps, S. M., Solomon, N. G., Taborsky, M., Young, L. J., & Rubenstein, D. R. (2014). An evolutionary framework for studying mechanisms of social behavior. *Trends in Ecology and Evolution*, *29*(10), 581–589. https://doi.org/10.1016/j.tree.2014.07.008

Hosseini, P. R., Dhondt, A. A., Dobson, A. P., & Dobson, P. (2009). Spatial spread of an emerging infectious disease: Conjunctivitis in house finches. *Ecology*, *87*(12), 3037–3046.

Hotchkiss, E. R., Davis, A. K., Cherry, J. J., & Altizer, S. (2005). Mycoplasmal conjunctivitis and the behavior of wild house finches (*Carpodacus mexicanus*) at bird feeders. *Bird Behavior*, *17*, 1–8.

Kenward, R. E. (1978). Hawks and Doves: Factors Affecting Success and Selection in Goshawk Attacks on Woodpigeons. *Journal of Animal Ecology*, *47*(2), 449–460.

Kollias, G. V., Sydenstricker, K. V., Kollias, H. W., Ley, D. H., Hosseini, P. R., Connolly, V., & Dhondt, A. A. (2004). Experimental infection of house finches with *Mycoplasma gallisepticum*. *Journal of Wildlife Diseases*, *40*(1), 79–86. https://doi.org/10.7589/0090-3558-40.1.79

Langager, M. M., Adelman, J. S., & Hawley, D. M. (2023). Let's stick together: Infection enhances preferences for social grouping in a songbird species. *Ecology and Evolution*, *13*(10). https://doi.org/10.1002/ece3.10627

Liker, A. S., & Bókony, V. (2009). Larger groups are more successful in innovative problem solving in house sparrows. *PNAS*, *109*(19), 7893–7898. https://www.pnas.org

Lima, S. L., & Dill, L. M. (1990). Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology*, *68*, 619–640.

Lopes, P. C. (2023). Anticipating infection: How parasitism risk changes animal physiology. *Functional Ecology*, *37*(4), 821–830. https://doi.org/10.1111/1365-2435.14155

Lopes, P. C., Adelman, J., Wingfield, J. C., & Bentley, G. E. (2012). Social context modulates sickness behavior. *Behavioral Ecology and Sociobiology*, *66*(10), 1421–1428. https://doi.org/10.1007/s00265-012-1397-1

Lopes, P. C., French, S. S., Woodhams, D. C., & Binning, S. A. (2021). Sickness behaviors across vertebrate taxa: Proximate and ultimate mechanisms. *Journal of Experimental Biology*, *224*(9). https://doi.org/10.1242/jeb.225847

Markham, A. C., & Gesquiere, L. R. (2017). Costs and benefits of group living in primates: An energetic perspective. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*(1727). https://doi.org/10.1098/rstb.2016.0239

McFarland, R., Henzi, S. P., Barrett, L., Bonnell, T., Fuller, A., Young, C., & Hetem, R. S. (2021). Fevers and the social costs of acute infection in wild vervet monkeys. *PNAS*, *118*(44). https://doi.org/10.1073/pnas.2107881118/-/DCSupplemental

Morelli, F., Benedetti, Y., Díaz, M., Grim, T., Ibáñez-Álamo, J. D., Jokimäki, J., Kaisanlahti-Jokimäki, M. L., Tätte, K., Markó, G., Jiang, Y., Tryjanowski, P., & Møller, A. P. (2019). Contagious fear: Escape behavior increases with flock size in European gregarious birds. *Ecology and Evolution*, *9*(10), 6096–6104. https://doi.org/10.1002/ece3.5193

Olson, R. S., Haley, P. B., Dyer, F. C., & Adami, C. (2015). Exploring the evolution of a trade-off between vigilance and foraging in group-living organisms. *Royal Society Open Science*, *2*(9). https://doi.org/10.1098/rsos.150135

R Core Team. (2023). *R: A Language and Enivornment for Statistical Computing*. https://www.R-project.org/

Rifkin, J. L., Nunn, C. L., & Garamszegi, L. Z. (2012). Do animals living in larger groups experience greater parasitism? A meta-analysis. *The American Naturalist*, *180*, 70–82. https://doi.org/10.5061/dryad.v5007p45

Ripperger, S. P., Stockmaier, S., & Carter, G. G. (2020). Tracking sickness effects on social encounters via continuous proximity sensing in wild vampire bats. *Behavioral Ecology*, *31*(6), 1296–1302. https://doi.org/10.1093/beheco/araa111

Roncoroni, A., Ersoy, S., & Bijleveld, A. I. (2024). Slow-exploring captive red knots were quicker to find food in a social setting than fast explorers. *Biology Letters*, *20*(20210139). https://doi.org/10.1098/rsbl.2024.0139

Rooke, R., Rasool, A., Schneider, J., & Levine, J. D. (2020). *Drosophila melanogaster* behaviour changes in different social environments based on group size and density. *Communications Biology*, *3*(1). https://doi.org/10.1038/s42003-020-1024-z

Stephens, P. A., & Sutherland, W. J. (1999). Consequences of the Allee effect for behaviour, ecology and conservation. *Trends in Ecology and Evolution*, *14*(10), 401–405.

Stockmaier, S., Bolnick, D. I., Page, R. A., & Carter, G. G. (2020). Sickness effects on social interactions depend on the type of behaviour and relationship. *Journal of Animal Ecology*, *89*(6), 1387–1394. https://doi.org/10.1111/1365-2656.13193

Stockmaier, S., Stroeymeyt, N., Shattuck, E. C., Hawley, D. M., Meyers, L. A., & Bolnick, D. I. (2021). Infectious diseases and social distancing in nature. *Science*, *371*(6533). https://doi.org/10.1126/science.abc8881

Sydenstricker, K. V., Dhondt, A. A., Hawley, D. M., Jennelle, C. S., Kollias, H. W., & Kollias, G. V. (2006). Characterization of experimental *Mycoplasma gallisepticum* infection in captive house finch flocks. *Avian Diseases*, *50*(1), 39–44. https://doi.org/10.1637/7403-062805R.1

Therneau, T. M. (2024). *coxme: Mixed Effects Cox Models*. https://CRAN.R-project.org/package=coxme

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York. https://ggplot2.tidyverse.org

Table 1. Final sample sizes for each treatment category (group-housed diseased, group-housed healthy, single-housed diseased, and single-housed healthy) in each behavioral assay conducted. While we experimentally inoculated 16 focal birds each with MG or sterile media (sham control), our group-housed birds experienced rapid transmission, in some cases prior by day 11. Thus, disease status was assessed for each bird within ± 2 days of each assay listed. For the feeder assays and the simulated aerial predator assay, the behaviors of all birds were included in analyses. Two birds developed clinical signs of disease between their feeder assay and simulated predator assay, leading to a reduction in the sample size for healthy group-housed birds and an increase in sample size for diseased group-housed birds for the simulated aerial predator assay.

	Normal Feeder (Day 11)	Novel Feeder (Day 12)	Simulated Aerial Predator (Day 13-15)	Capture Evasion Day 7/8*	Capture Evasion Day 15/16*
Healthy Group-housed	n=16	n=16	n=14	n=8	n=4
Diseased Group-housed	n=24	n=24	n=26	n=8	n=12
Healthy Single-housed	n=8	n=8	n=8	n=8	n=8
Diseased Single-housed	n=8	n=8	n=8	n=8	n=8

**only n=8 starting focal birds per treatmetn group were assayed for capture evasion*

Table 2. Ethogram for feeder assays. Behaviors were only recorded for birds when they were on the half of the cage containing the food dish.

Behavior	**Feeder Approach Score**	**Description**
eating	5	Actively taking food from food dish and eating, trial "success"
perching	4	Perching on food dish, but not actively eating
close approach	3	Perched within 3 inches of food dish
bottom perch	2	Perched on bottom perch, 10-20 cm away from food dish
top perch	1	Perched on top perch, 20-30 cm away from food dish
no approach	0	Did not come within 30 cm of food dish

Table 3. Ethogram for behaviors recorded during simulated aerial predator assay. Latency to the first of the listed behaviors during the time of the hawk flyover (considered their first "response") was utilized in analyses.

Behavior	**Description**
fly	Flies to any part of the cage
hop	Relocates to new place in cage without using wings
ruffle	Puffs feathers and shivers
bill wipe	Wipes bill on any part of cage
flinch	Visibly flinches/ducks down
scanning	Head turning 180˚, looking at surroundings

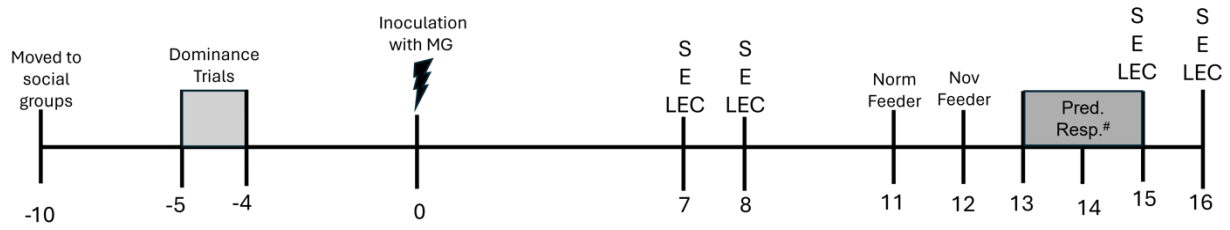

Figure 1. Timeline of experiment. Although our experiment occurred in two experimental "batches", the birds in each batch underwent the same timeline. Conjunctival swabs (S) and disease severity scores (E) were taken for every bird in the study (n=56), regardless of treatment, on experiment day 7/8 and experiment day 15/16. Latency to evade capture (LEC) was recorded for focal birds only (n=32) on both sampling periods, with a single individual being captured only once over the two days within a sampling period (e.g., either day 7 or 8).

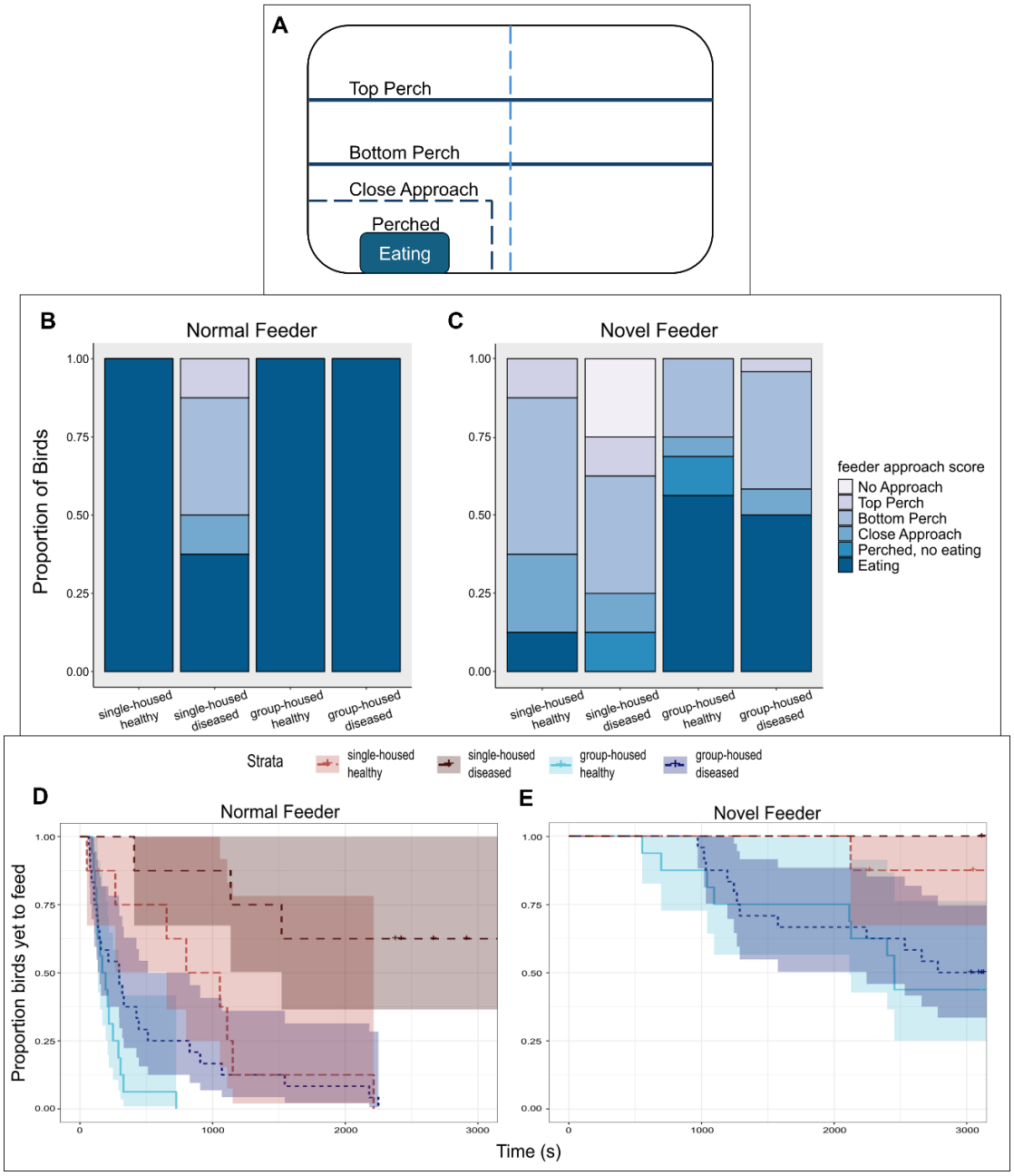

Figure 2. (A) A top-down view of the normal feeder assay. A feeder approach score from 0-5 was assigned to each bird, representing how close they came to eating from the food dish. Approach scores were only assigned when the bird was in the half of the cage containing the food dish. (B) When offered their normal feeder type after 60 min of food restriction, only birds in the single-housed diseased category showed any variation in response, with only 38% of birds eating from the normal feeder, compared to 100% of all group-housed birds and single-housed healthy. (C) Following 60 min of food restriction, single-housed house finches were significantly less likely to eat from a novel feeder than group-housed birds, regardless of disease status ($p<0.002$) (D) Single-housed birds were slower to feed from their normal food dish than group-housed birds, and (E) had single-housed birds had a higher latency to approach and eat than birds in flocks.

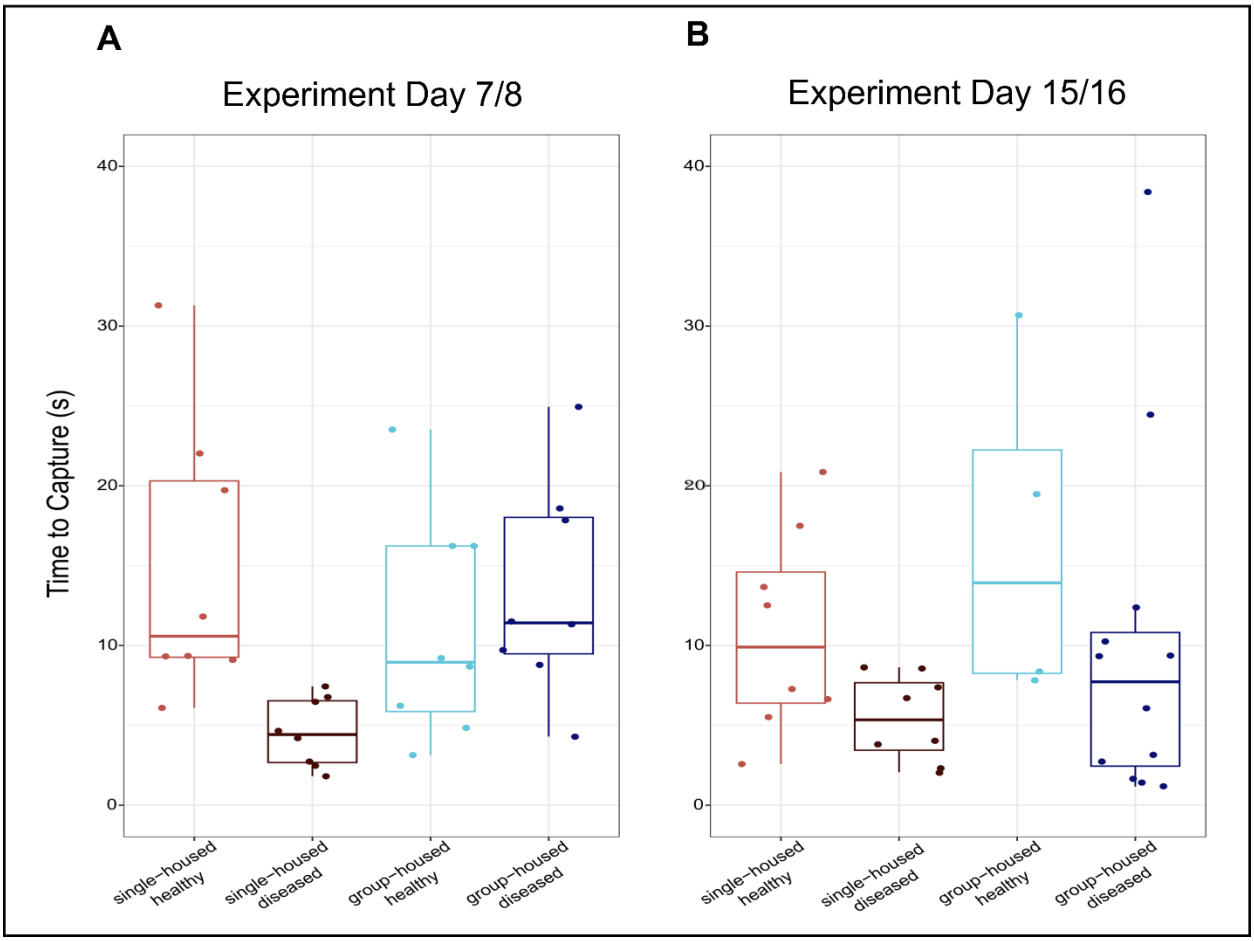

Figure 3. (A) On experiment day 7/8, single-housed house finches with mycoplasmal conjunctivitis were hand-caught significantly faster than group-housed or single-housed healthy finches ($p<0.001$). (B) On experiment day 15/16, diseased birds in both group-housed (n=12) and single-housed (n=8) social treatments were captured significantly slower than healthy birds ($p=0.03$).

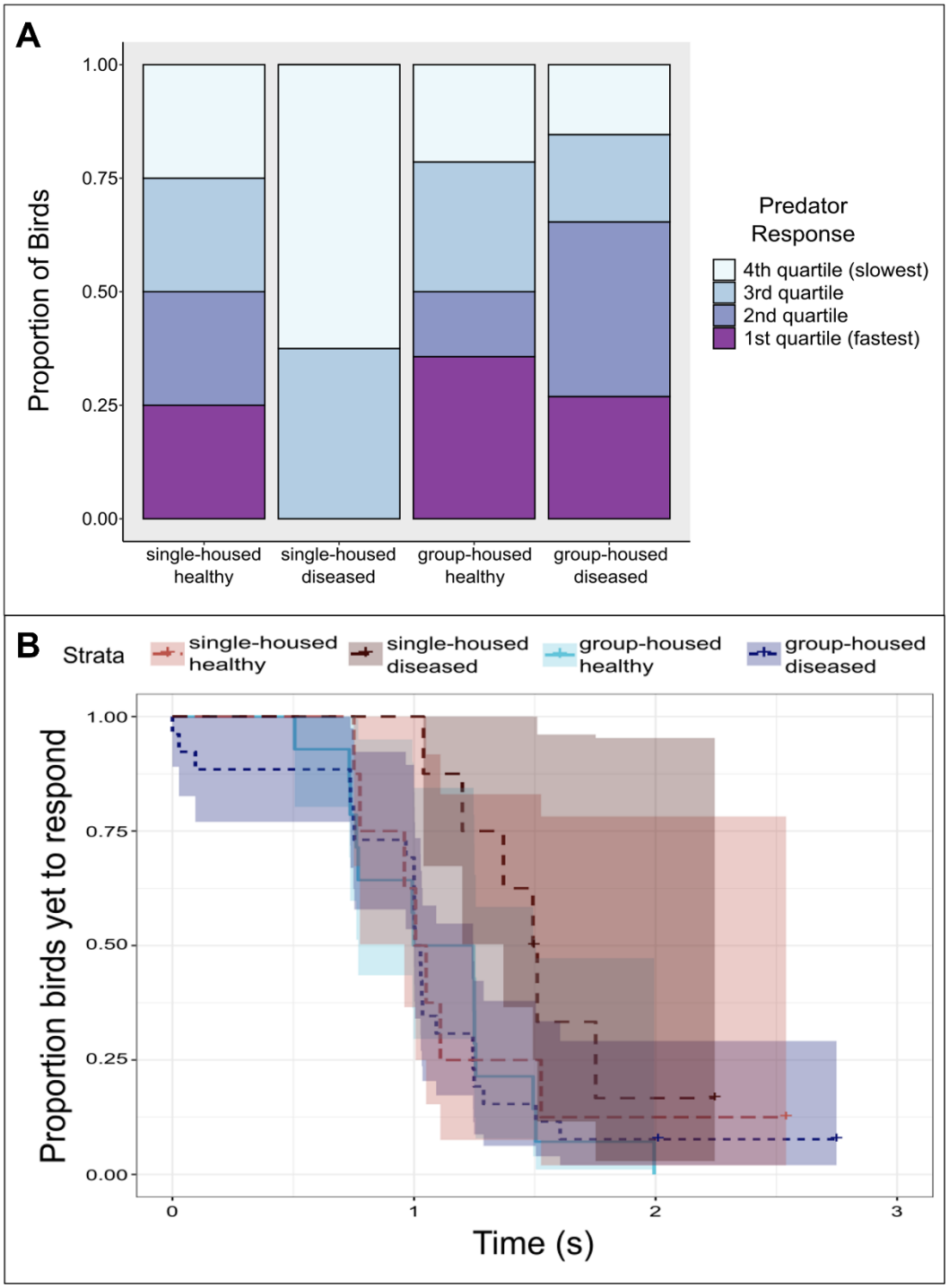

Figure 4. (A) The interaction between social group treatment and disease status significantly predicted how quickly a house finch responded to a simulated predator flyover, with group-housed birds more likely to respond quickly to the mock predator compared to single-housed, diseased birds. Here reaction time is analyzed as quartile scores (see methods). (B) Single-housed, diseased birds were slower to respond to the predator than group-housed birds or single-housed healthy birds.

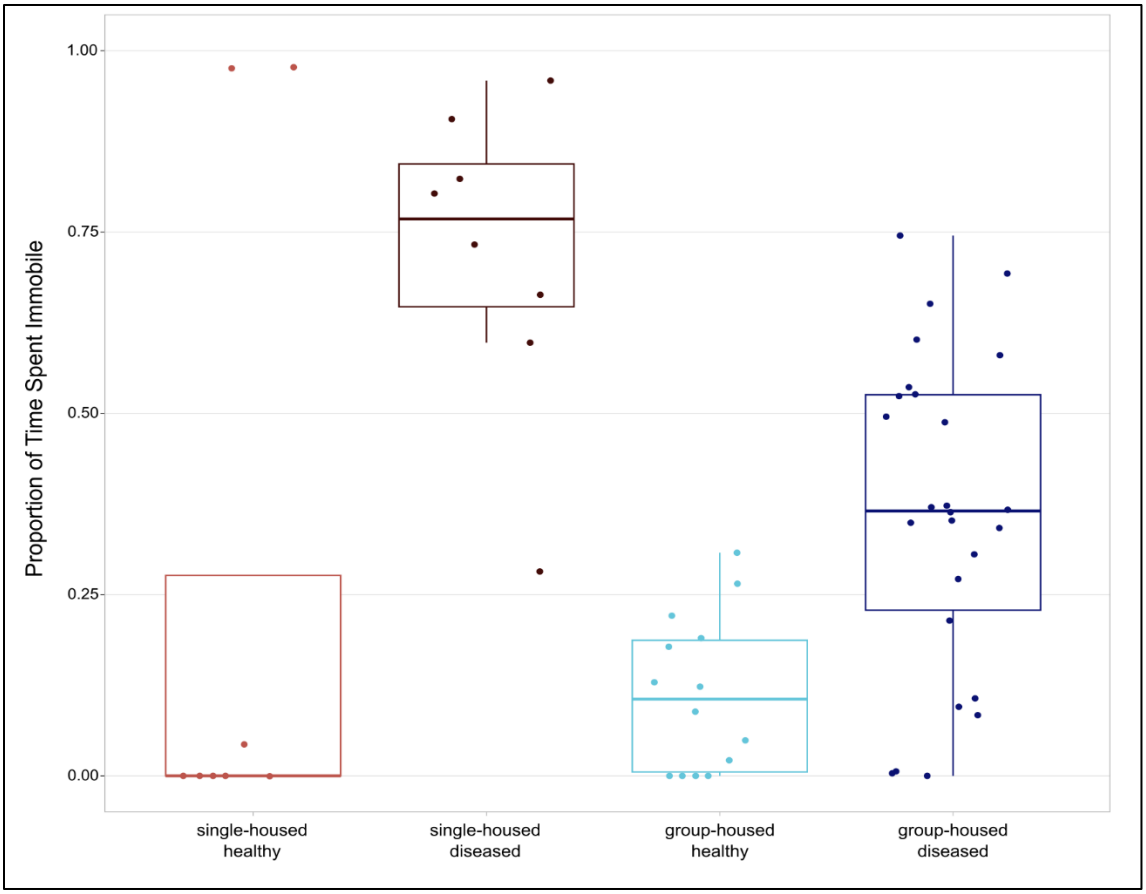

Figure 5. During the 30 minutes following the release of a mock aerial predator, single-housed diseased birds spent more time immobile than all group-housed birds and single-housed healthy birds.

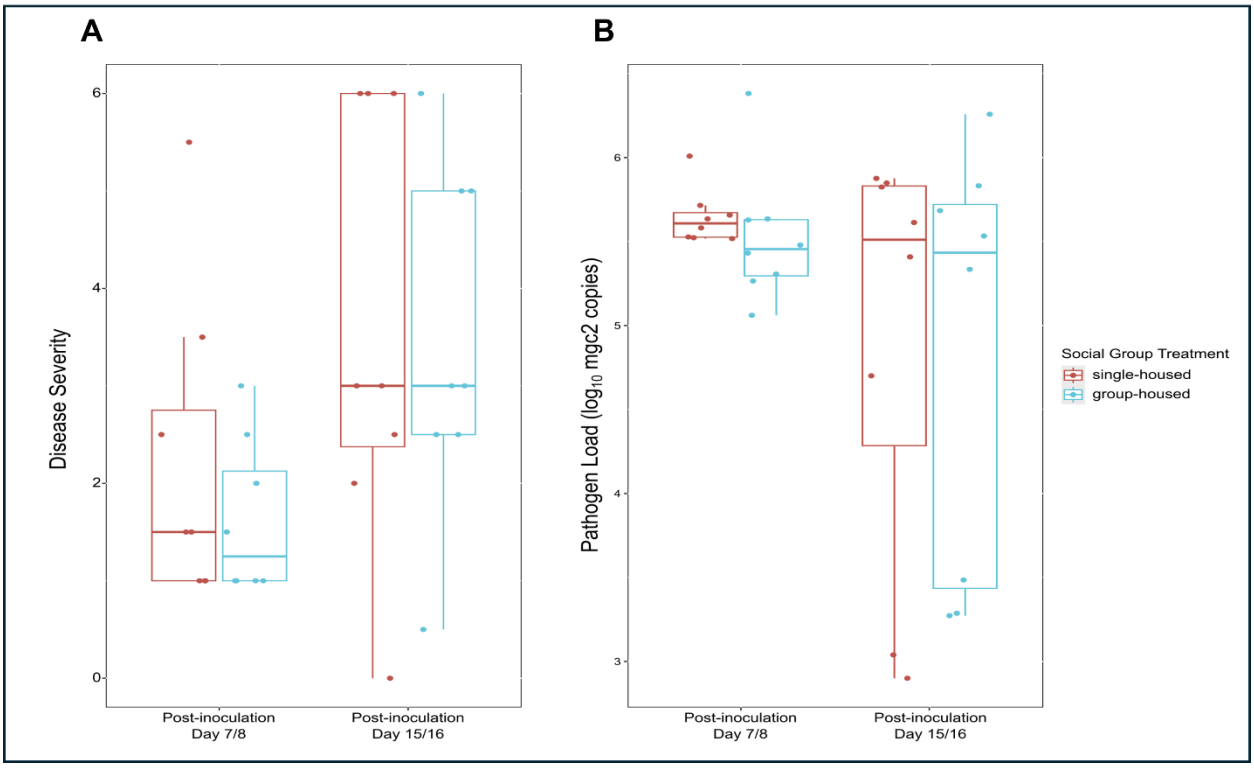

Figure 6. For house finches that were directly inoculated in each eye with MG, neither disease severity (left, p=0.68) nor pathogen load (right, p=0.80) significantly differed with social group treatment. Birds in the group-housed, infected treatment (n=8; blue) and single-housed infected treatment (n=8; red) displayed similar levels of conjunctival swelling (disease severity) as well as similar levels of pathogen present in the conjunctiva.

Ch. 5: Synthesis

Disease-causing pathogens are nearly ubiquitous components within most environments, making them one of the most common causes and consequences of phenotypic differences among hosts (Nadler et al., 2023). Emerging wildlife diseases can have disastrous effects on animal populations across the world, as vulnerable populations are exposed to novel pathogens (Smith et al., 2009; Tompkins et al., 2011). Further, human-mediated changes to environments have led to an increase in novel ecological challenges for many species. Because of these changes, it is especially important to understand how animals respond to challenges within their shifting environments. The ways in which animals respond to common ecological challenges, such as nutritional stress or infectious disease, can have long-term implications for individual host fitness as well as population dynamics. Causally elucidating such implications requires experimental studies that isolate effects of key ecological challenges faced by organisms. My dissertation aims to elucidate the ways that an individual host phenotype might shift due to challenges faced in their environment, focusing on shifting host responses to disease as both a consequence of other ecological challenges, as well infectious disease as a homeostatic challenge in itself. By studying disease through a dual lens of cause and consequence of ecological challenge, my dissertation adds to our knowledge of host fitness in the face of common ecological stressors and how behavioral responses can help contribute to individual survival, with cascading effects on population-level trajectories.

Here, I used a naturally-occurring songbird disease system, house finches and the pathogenic bacteria *Mycoplasma gallispeticum* (MG), to study how animal responses shift in the face of ecological challenge. First, I explored how disease serves as a consequence of exposure to ecological challenges in Chapter 2. Specifically, I investigated how individual responses to disease shift after a period of nutritional stress during early life, which is a vital developmental period for all animals (Burley & Johnson, 2002). Although I demonstrated that nutritionally stressed nestlings and fledglings gained significantly lower mass during development, I did not find differences in the bird's tarsus growth during the nutritional stress period, nor in their responses to pathogen infection (disease severity, pathogen load, or corticosterone levels). These results indicate that although young animals can face short-term consequences related to nutritional stress, such as reduced weight gain during development, that there may not always be notable longer-term consequences for skeletal growth or host responses to pathogens. With this

study, my work has furthered our understanding of the potential resilience of songbird responses to disease even in the face of developmental stress, and the consequences associated with decreased resource availability (which is often driven by anthropogenic change).

The results in Chapter 2 were somewhat surprising because previous work on nutritional stress during development has demonstrated negative, long-term phenotypic change across multiple taxonomic groups, which could have significant downstream impacts on individual fitness as adults (Eyck et al., 2019; Wada & Coutts, 2021). In songbirds specifically, nutritional stress can impact developing brain structures, leading to changes in song learning and quality (Buchanan et al., 2003, 2004; Nowicki et al., 2002), which may honestly indicate reduced male quality to potential mates, potentially impacting reproduction (reviewed in Spencer & MacDougall-Shackleton, 2011). Similarly, nutritional stress has been shown to induce phenotypic changes in responses to a simulated immune challenge (Hõrak et al., 1999; Nettle et al., 2016; Saino et al., 1997, 2003). Thus, while our nutritional stress treatment did not cause detectable differences in the measured host responses of house finches infected with *Mycoplasma gallisepticum* (MG), other areas of the immune system or other body systems, such as song learning or adult behavior, may be impacted by nutritional stress during development. On the other hand, our results suggest that host responses to a debilitating natural pathogen can, at least in some cases, be resilient to effects of developmental nutritional stress.

Given the previous work on brain development, behavior, and immune responses outlined above, one area of potential future research should focus on how developmental stress impacts the development of pro-inflammatory cytokine production in wild animals, which could affect an individual's behavioral phenotype during infection (Dantzer, 2004; Devlin et al., 2022) in the long term. There is a strong body of work on this topic in rodents (Lumertz et al., 2022) that has found a positive relationship between early-life stress and increased levels of pro-inflammatory cytokines. However, it is important to study these phenomena in wild animals to understand how individuals may differ in their cytokine and behavioral responses and, in turn, how any differences might impact disease transmission among populations. Future work should also seek to discover how early-life stressors impact aspects of the adaptive immune response, such as antibody production. These adaptive antibody responses are especially important in systems where re-infection with a pathogen occurs naturally, as antibody production can have a protective

effect against future infection (Leon & Hawley, 2017; Weitzman et al., 2022). Overall, the effects of developmental nutritional stress on the phenotypes of individuals are dynamic and complex (Hoffman et al., 2018; Wada & Coutts, 2021), so it is likely that the nutritionally stressed birds in Chapter 2 did display phenotypic differences that were simply outside the scope of this experiment. Taken in the light of increasing global changes, my work has provided further understanding of how the availability of food resources during development may impact an array of individual phenotypes that could impact fitness. However, further work must be done to build a complete picture of the overall effects of developmental nutritional stress.

While responses to disease can shift as a result of ecological challenges, disease is also a frequent driver of phenotypic changes, such as shifts in behavior (Nadler et al., 2023). In gregarious species, social behaviors in particular provide an interesting viewpoint from which to study disease, as maintaining them is often key to the spread of disease throughout populations, yet they are often altered in the face of active infection (Hawley et al., 2021; Stockmaier et al., 2021). In Chapter 3, I sought to determine how infection with a directly-transmitted pathogen (MG) impacted the social behaviors of hatch-year house finches. Because the birds were infected within several months of hatching, I also examined whether any changes in social behavior during infection persisted following recovery, indicating potential long-term organizational effects of acute infection on the bird's social behavior. This study demonstrated that infected house finches augment their social preferences and behaviors during MG infection, such that they spend more time eating near a group of conspecifics versus an empty cage, relative to healthy birds. Though previous studies have demonstrated a depression in social behaviors in severely diseased individuals due to the presence of sickness behaviors (reviewed in Stockmaier et al., 2021), similar to those stimulated by MG infection (Hawley et al., 2007; Hotchkiss et al., 2005), we found that, regardless of the severity of infection or disease, house finches that were acutely infected with MG showed a strong preference to associate with healthy flockmates. It is possible that, in this system, disease leads to shifts in social behavior because group-living has positive impacts on infected hosts, by increasing behavioral tolerance, which is defined as offsetting fitness costs of infection through shifts in behavior (Ezenwa et al., 2016). Interestingly, when the social preferences of these birds were retested after infected birds had recovered from disease, we saw no differences in their preferences compared to uninfected controls. This further indicates that infected house finches may be utilizing social grouping to alleviate the costs of

infection, especially sickness behaviors. As these behaviors are not present once infection has been cleared, it would be unnecessary to maintain a heightened level of sociality permanently.

In Chapter 4, I tested whether the social preferences of infected birds observed in Chapter 3 have detectable behavioral benefits for infected individuals. Group-living can be energetically costly due to the potential costs such as competition (Ezenwa et al., 2016; Markham & Gesquiere, 2017). However, for gregarious taxa, social living also provides important benefits to individual group members, such as increased predator vigilance and foraging success (e.g., Guindre-Parker & Rubenstein, 2020; Olson et al., 2015). In Chapter 4, I investigated how group-living impacts foraging and anti-predator behaviors in house finches infected with MG. Although our experiment demonstrated behavioral benefits of group-living for both healthy and infected house finches, we found that group-living particularly benefits infected hosts, improving their ability to respond to foraging opportunities and predation threats. These behavioral responses to food opportunities and predation challenges likely have important consequences for host survival during infection, as sickness behaviors, such as lethargy and anorexia, often decrease host foraging success (Almberg et al., 2015; Ezenwa & Worsley-Tonks, 2018) and predator detection (Adelman et al., 2017). Further, maintaining energetically-costly social behaviors ultimately benefits infected hosts by allowing them to allocate more of their daily time budget towards foraging for resources if they rely on group members to provide predator vigilance (e.g., Beauchamp, 2008; Ezenwa & Worsley-Tonks, 2018; Kenward, 1978; Olson et al., 2015), freeing up time to gain more energetic resources that can be used to offset infection costs. Overall, our study suggests that infected house finches likely benefit from group-living in ways that reduce costs of infection, acting as a form of behavioral tolerance. The use of group-living as a form of behavioral tolerance is a phenomenon that has been recently documented in other gregarious species (Almberg et al., 2015; Ezenwa & Worsley-Tonks, 2018), suggesting that the patterns observed in house finches may be relevant to other social taxa.

This study found that there were benefits to infected house finches, but it is likely that these grouping behaviors come with associated costs to individual group members, such as increased competition, particularly around food sources, or even an increased likelihood of attracting the notice of a predator (Ezenwa et al., 2016). While I found that the benefits of social grouping are particularly important for infected individuals, it may also be that these individuals

feel the costs of social grouping most heavily. Though the work in Ch.4 did not directly analyze the rank order of responses to the predator within groups, this will be an important area for future study to understand the possible costs associated with group living, particularly for infected individuals within groups. For example, while I found that diseased birds in a flock had a faster response to an aerial predator flyover than single-housed diseased birds, there was still a wide variation in time to respond. It is possible that infected birds in the wild that simply show a slightly slower response compared to their flockmates, even if their response time is faster than a solitary infected bird, would be easily targeted prey during a real predation event, with slower-responding birds in a group likely to incur costs through selfish herd dynamics (W. D. Hamilton, 1971). These costs may be especially felt by less gregarious species, who may not experience the strong benefits of grouping demonstrated by the highly social birds in this study. Understanding both the costs and benefits of social grouping will further aid in the understanding of how and why species across a spectrum of sociality may experience social interactions.

Notably, although we detected individual variation in disease severity and pathogen load, neither metric was associated with the increased level of sociality displayed by infected birds in Chapter 3, with 14 of the 19 MG-infected finches displaying high pathogen loads but maintaining their augmented social preferences. Further, neither disease severity nor pathogen load contributed to any observed differences in behavior in Chapter 4, as birds in the single-housed, socially isolated treatment displayed similar disease severity and pathogen loads to diseased birds in groups but displayed a vastly different behavioral phenotype. Thus, these observed behavioral shifts in foraging and predation responses cannot be attributed to differences in the amount of pathogen present within an individual, or that individual's disease severity. The absence of a relationship between pathogen load and behavioral responses to food and predation risk in Chapter 4 further support the possibility that infected house finches are showing behavioral tolerance by using behaviors to offset the per-pathogen fitness costs of infection. As a consequence, the infected house finches in these studies were highly social while harboring infectious levels of MG (Langager et al., 2023), likely facilitating transmission among interacting groups and populations (Adelman et al., 2015). Thus, while behavioral tolerance benefits infected individuals by facilitating survival of infection, there are likely negative consequences of behavioral tolerance for healthy conspecifics. Interestingly, this is in contrast to behaviors that minimize pathogen load (often referred to as behavioral immunity or resistance),

which are generally predicted to benefit both the infected individual and its healthy groupmates (De Roode & Lefèvre, 2012).

Studying social preferences with a focus on infected hosts is a relatively new approach (but see Siva-Jothy & Vale, 2019; Stephenson, 2019; Wu et al., 2023), with most previous work focusing on uninfected group members and the increased risk of pathogen infection that is associated with of social grouping (Rifkin et al., 2012; Sah et al., 2018). While my work documents the importance of accounting for the preferences of infected individuals, social dynamics in the wild are complex and vary between species. Behavioral interactions in the wild represent an outcome of the social preferences of both infected and healthy hosts, many of which have evolved to avoid infected conspecifics as an adaption to reduce infection risk (Stockmaier et al., 2021). In house finches, healthy conspecifics do not actively avoid finches with clinical signs of MG infection, and, in fact, adult males prefer to feed in the vicinity of sick versus healthy males, likely because sick males are less aggressive to conspecifics (Bouwman & Hawley, 2010). However, there are many systems in which sick conspecifics are actively avoided or are isolated from the group (Stockmaier et al., 2021). Thus, future studies should incorporate both the preferences and behaviors of infected individuals alongside those of uninfected groupmates to further our understanding of how these behaviors are manifesting in the wild. Studying social interactions from both possible perspectives, those of infected and uninfected members, will help broaden our knowledge of the true social dynamics taking place within a group, as well as the ways diseases may spread within and among populations.

Because I used captive laboratory experiments on wild-caught birds, care must be used in extrapolating these results to birds in the wild. For example, previous work looking at social behaviors in free-living house finches found that birds with visible clinical signs of MG were observed at feeders in smaller groups than healthy conspecifics (Hawley et al., 2007; Hotchkiss et al., 2005), and were less likely to move within feeding sites (Hawley et al., 2007). This suggests that some diseased birds may be unable to travel with social groups between feeding sites, even if they inherently prefer to associate with social groups as I showed in Chapter 3. However, even if some diseased birds cannot fully express their social preferences in the wild, individual responses to disease are heterogeneous (e.g., Adelman et al., 2013; Henschen et al., 2023), with birds with less severe disease likely remaining undetected in observational studies

such as those examining flock sizes. Because we demonstrated that these birds may still harbor infectious pathogen loads, documenting the behaviors of birds with aclinical MG infection is critical to fully understand how they are driving pathogen transmission within this system. Thus, studying the behaviors of wild birds in laboratory settings may be especially important for understanding the dynamics of diseases that do not lead to observable signs within infected hosts. However, in order to fully understand how MG transmission is occurring in the wild, future work should aim to detect and observe the social preferences of all individuals, particularly those with less easily detected disease

Additionally, captive studies like those presented in my dissertation are vital to deducing causality while controlling for confounding variables, which is difficult in a wild setting. In all my chapters, I was able to isolate the variables of interest, as well as present a single focal ecological challenge (Chapter 2 – the effect of nutritional stress on host responses to disease; Chapter 3 – the effect of MG infection on individual social preferences; Chapter 4 – the effect of group-living and MG infection on fitness-relevant behaviors). It must be noted that wild animals experience the environment as a whole and that both biotic and abiotic factors may influence their response to ecological challenges (Wingfield, 2013; Wingfield et al., 2011). Further, wild animals rarely encounter only one ecological challenge at a time, especially in a rapidly changing world (Wingfield et al., 2011), which may lead to further phenotypic shifts. Because of this, captive experimental studies should be used in conjunction with wild and observational studies to more completely understand how animals are meeting the simultaneous environmental challenges they are faced with. Further, while my work was able to study aspects of physiology and behavior that have the possibility to impact host fitness, this dissertation is limited in its ability to actual deduce fitness impacts *per se*. Future work should aim to understand the ways in which the shifting phenotypes outlined in this work directly affect an individual's likelihood to survive and reproduce.

Infectious diseases can have devastating effects on populations of wild animals, causing declines in local and global population abundance (Smith et al., 2009; Tompkins et al., 2011). It is important to study host-pathogen interactions in the face of a changing environment in order to understand changes in individual host fitness as well as population-level dynamics (Tompkins et al., 2011; Warne et al., 2019). A rapidly changing world can augment the ecological challenges

faced at multiple biological levels of organization, from individuals to ecosystems (Warne et al., 2019). By studying the factors that impact individual host responses to disease, as well as how disease may shift other phenotypes important for host survival, we can better understand the overarching impacts of ecological change. My dissertation furthers our understanding of the impacts upon individuals due to the shifting and increasing ecological challenges they are likely to face, which may have further implications for populations, communities, and ecosystems.

References

Adelman, J. S., Kirkpatrick, L., Grodio, J. L., & Hawley, D. M. (2013). House Finch Populations Differ in Early Inflammatory Signaling and Pathogen Tolerance at the Peak of *Mycoplasma gallisepticum* Infection. *The American Naturalist*, *181*(5), 674–689.

Adelman, J. S., Mayer, C., & Hawley, D. M. (2017). Infection reduces anti-predator behaviors in house finches. *Journal of Avian Biology*, *48*, 519–528.

Adelman, J. S., Moyers, S. C., Farine, D. R., & Hawley, D. M. (2015). Feeder use predicts both acquisition and transmission of a contagious pathogen in a North American songbird. *Proceedings of the Royal Society B*, *282*(20151429).

Almberg, E. S., Cross, P. C., Dobson, A. P., Smith, D. W., Metz, M. C., Stahler, D. R., & Hudson, P. J. (2015). Social living mitigates the costs of a chronic illness in a cooperative carnivore. *Ecology Letters*, *18*(7), 660–667. https://doi.org/10.1111/ele.12444

Beauchamp, G. (2008). What is the magnitude of the group-size effect on vigilance? *Behavioral Ecology*, *19*(6), 1361–1368. https://doi.org/10.1093/beheco/arn096

Bouwman, K. M., & Hawley, D. M. (2010). Sickness behaviour acting as an evolutionary trap? Male house finches preferentially feed near diseased conspecifics. *Biology Letters*, *6*, 462–465.

Buchanan, K. L., Leitner, S., Spencer, K. A., Goldsmith, A. R., & Catchpole, C. K. (2004). Developmental stress selectively affects the song control nucleus HVC in the zebra finch. *Proceedings of the Royal Society B: Biological Sciences*, *271*(1555), 2381–2386. https://doi.org/10.1098/rspb.2004.2874

Buchanan, K. L., Spencer, K. A., Goldsmith, A. R., & Catchpole, C. K. (2003). Song as an honest signal of past developmental stress in the European starling (*Sturnus vulgaris*). *Proceedings of the Royal Society B: Biological Sciences, 270*(1520), 1149–1156. https://doi.org/10.1098/rspb.2003.2330

Burley, N. T., & Johnson, K. (2002). The evolution of avian parental care. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *357*(1419), 241–250. https://doi.org/10.1098/rstb.2001.0923

Dantzer, R. (2004). Cytokine-induced sickness behaviour: A neuroimmune response to activation of innate immunity. *European Journal of Pharmacology*, *500*, 399–411.

De Roode, J. C., & Lefèvre, T. (2012). Behavioral immunity in insects. *Insects*, *3*(3), 789–820. https://doi.org/10.3390/insects3030789

Devlin, B. A., Smith, C. J., & Bilbo, S. D. (2022). Sickness and the Social Brain: How the Immune System Regulates Behavior across Species. *Brain, Behavior and Evolution*, *97*(3–4), 197–210. https://doi.org/10.1159/000521476

Eyck, H. J. F., Buchanan, K. L., Crino, O. L., & Jessop, T. S. (2019). Effects of developmental stress on animal phenotype and performance: a quantitative review. *Biological Reviews*, *94*(3), 1143–1160. https://doi.org/10.1111/brv.12496

Ezenwa, V. O., Ghai, R. R., McKay, A. F., & Williams, A. E. (2016). Group living and pathogen infection revisited. *Current Opinion in Behavioral Sciences*, *12*, 66–72. https://doi.org/10.1016/j.cobeha.2016.09.006

Ezenwa, V. O., & Worsley-Tonks, K. E. L. (2018). Social living simultaneously increases infection risk and decreases the cost of infection. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1892). https://doi.org/10.1098/rspb.2018.2142

Guindre-Parker, S., & Rubenstein, D. R. (2020). Survival Benefits of Group Living in a Fluctuating Environment. *The American Naturalist*, *195*(6), 1027–1036. https://doi.org/10.5061/dryad.stqjq2c05

Hamilton, W. D. (1971). Geometry for the Selfish Herd. *Journal of Theoretical Biology*, *31*(2), 295–311.

Hawley, D. M., Davis, A. K., & Dhondt, A. A. (2007). Transmission-relevant behaviours shift with pathogen infection in wild house finches (*Carpodacus mexicanus*). *Canadian Journal of Zoology*, *85*(6), 752–757.

Hawley, D. M., Gibson, A. K., Townsend, A. K., Craft, M. E., & Stephenson, J. F. (2021). Bidirectional interactions between host social behaviour and parasites arise through ecological and evolutionary processes. *Parasitology*, *148*(3), 274–288. https://doi.org/10.1017/S0031182020002048

Henschen, A. E., Vinkler, M., Langager, M. M., Rowley, A. A., Dalloul, R. A., Hawley, D. M., & Adelman, J. S. (2023). Rapid adaptation to a novel pathogen through disease tolerance in a wild songbird. *PLoS Pathogens*, *19*(6 June). https://doi.org/10.1371/journal.ppat.1011408

Hoffman, A. J., Finger, J. W., & Wada, H. (2018). Early stress priming and the effects on fitness-related traits following an adult stress exposure. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, *329*(6–7), 323–330. https://doi.org/10.1002/jez.2190

Hõrak, P., Tegelmann, L., Ots, I., & Møller, A. P. (1999). Immune Function and Survival of Great Tit Nestlings in Relation to Growth Conditions. *Oecologia*, *121*(3), 316–322.

Hotchkiss, E. R., Davis, A. K., Cherry, J. J., & Altizer, S. (2005). Mycoplasmal conjunctivitis and the behavior of wild house finches (*Carpodacus mexicanus*) at bird feeders. *Bird Behavior*, *17*, 1–8.

Kenward, R. E. (1978). Hawks and Doves: Factors Affecting Success and Selection in Goshawk Attacks on Woodpigeons. *Journal of Animal Ecology*, *47*(2), 449–460.

Langager, M. M., Adelman, J. S., & Hawley, D. M. (2023). Let's stick together: Infection enhances preferences for social grouping in a songbird species. *Ecology and Evolution*, *13*(10). https://doi.org/10.1002/ece3.10627

Leon, A. E., & Hawley, D. M. (2017). Host Responses to Pathogen Priming in a Natural Songbird Host. *EcoHealth*, *14*(4), 793–804. https://doi.org/10.1007/s10393-017-1261-x

Lumertz, F. S., Kestering-Ferreira, E., Orso, R., Creutzberg, K. C., Tractenberg, S. G., Stocchero, B. A., Viola, T. W., & Grassi-Oliveira, R. (2022). Effects of early life stress on brain cytokines: A systematic review and meta-analysis of rodent studies. *Neuroscience and Biobehavioral Reviews*, *139*. https://doi.org/10.1016/j.neubiorev.2022.104746

Markham, A. C., & Gesquiere, L. R. (2017). Costs and benefits of group living in primates: An energetic perspective. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*(1727). https://doi.org/10.1098/rstb.2016.0239

Nadler, L. E., Adamo, S. A., Hawley, D. M., & Binning, S. A. (2023). Mechanisms and consequences of infection-induced phenotypes. *Functional Ecology*, *37*(4), 796–800. https://doi.org/10.1111/1365-2435.14309

Nettle, D., Andrews, C., Reichert, S., Bedford, T., Gott, A., Parker, C., Kolenda, C., Martin-Ruiz, C., Monaghan, P., & Bateson, M. (2016). Brood size moderates associations between relative size, telomere length, and immune development in European starling nestlings. *Ecology and Evolution*, *6*, 8138–8148.

Nowicki, S., Searcy, W. A., & Peters, S. (2002). Brain development, song learning and mate choice in birds: A review and experimental test of the "nutritional stress hypothesis." *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology*, *188*(11–12), 1003–1014. https://doi.org/10.1007/s00359-002-0361-3

Olson, R. S., Haley, P. B., Dyer, F. C., & Adami, C. (2015). Exploring the evolution of a trade-off between vigilance and foraging in group-living organisms. *Royal Society Open Science*, *2*(9). https://doi.org/10.1098/rsos.150135

Rifkin, J. L., Nunn, C. L., & Garamszegi, L. Z. (2012). Do animals living in larger groups experience greater parasitism? A meta-analysis. *The American Naturalist*, *180*, 70–82. https://doi.org/10.5061/dryad.v5007p45

Sah, P., Mann, J., & Bansal, S. (2018). Disease implications of animal social network structure: A synthesis across social systems. *Journal of Animal Ecology*, *87*(3), 546–558. https://doi.org/10.1111/1365-2656.12786

Saino, N., Calza, S., & Moller, A. P. (1997). Immunocompetence of Nestling Barn Swallows in Relation to Brood Size and Parental Effort. *Journal of Animal Ecology*, *66*(6), 827–836.

Saino, N., Suffritti, C., Martinelli, R., Rubolini, D., & Møller, A. P. (2003). Immune response covaries with corticosterone plasma levels under experimentally stressful conditions in nestling barn swallows (*Hirundo rustica*). *Behavioral Ecology*, *14*(3), 318–325. https://doi.org/10.1093/beheco/14.3.318

Siva-Jothy, J. A., & Vale, P. F. (2019). Viral infection causes sex-specific changes in fruit fly social aggregation behaviour. *Biology Letters*, *15*(9). https://doi.org/10.1098/rsbl.2019.0344

Smith, K. F., Acevedo-Whitehouse, K., & Pedersen, A. B. (2009). The role of infectious diseases in biological conservation. *Animal Conservation*, *12*(1), 1–12. https://doi.org/10.1111/j.1469-1795.2008.00228.x

Spencer, K. A., & MacDougall-Shackleton, S. A. (2011). Indicators of development as sexually selected traits: The developmental stress hypothesis in context. *Behavioral Ecology*, *22*(1), 1–9. https://doi.org/10.1093/beheco/arq068

Stephenson, J. F. (2019). Parasite-induced plasticity in host social behaviour depends on sex and susceptibility. *Biology Letters*, *15*, 20190557. https://doi.org/10.1098/rsbl.2019.0557

Stockmaier, S., Stroeymeyt, N., Shattuck, E. C., Hawley, D. M., Meyers, L. A., & Bolnick, D. I. (2021). Infectious diseases and social distancing in nature. *Science*, *371*(6533). https://doi.org/10.1126/science.abc8881

Tompkins, D. M., Dunn, A. M., Smith, M. J., & Telfer, S. (2011). Wildlife diseases: From individuals to ecosystems. *Journal of Animal Ecology*, *80*(1), 19–38. https://doi.org/10.1111/j.1365-2656.2010.01742.x

Wada, H., & Coutts, V. (2021). Detrimental or beneficial? Untangling the literature on developmental stress studies in birds. *Journal of Experimental Biology*, *224*(19). https://doi.org/10.1242/jeb.227363

Warne, R. W., Baer, S. G., & Boyles, J. G. (2019). Community Physiological Ecology. *Trends in Ecology and Evolution*, *34*(6), 510–518. https://doi.org/10.1016/j.tree.2019.02.002

Weitzman, C. L., Ceja, G., Leon, A. E., & Hawley, D. M. (2022). Protection Generated by Prior Exposure to Pathogens Depends on both Priming and Challenge Dose. *Infection and Immunity*, *90*(3). https://doi.org/10.6084/m9.figshare.16611052

Wingfield, J. C. (2013). Ecological processes and the ecology of stress: The impacts of abiotic environmental factors. *Functional Ecology*, *27*(1), 37–44. https://doi.org/10.1111/1365-2435.12039

Wingfield, J. C., Patrick Kelley, J., Angelier, F., Chastel, O., Lei, F., Lynn, S. E., Miner, B., Davis, J. E., Li, D., & Wang, G. (2011). Organism-environment interactions in a changing

world: A mechanistic approach. *Journal of Ornithology*, *152*. https://doi.org/10.1007/s10336-011-0668-3

Wu, T., Ge, M., Wu, M., Duan, F., Liang, J., Chen, M., Gracida, X., Liu, H., Yang, W., Dar, A. R., Li, C., Butcher, R. A., Saltzman, A. L., & Zhang, Y. (2023). Pathogenic bacteria modulate pheromone response to promote mating. *Nature*, *613*(7943), 324–331. https://doi.org/10.1038/s41586-022-05561-9