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Infection reduces anti-predator behaviors in house finches

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Abstract

Infectious diseases can cause host mortality through direct or indirect mechanisms, including altered behavior. Diminished anti-predator behavior is among the most-studied causes of indirect mortality during infection, particularly for systems in which a parasite's life-cycle requires transmission from prey to predator. Significantly less work has examined whether directly-transmitted parasites and pathogens also reduce anti-predator behaviors. Here we test whether the directly-transmitted bacterial pathogen, *Mycoplasma gallisepticum* (MG), reduces responses to predation-related stimuli in house finches (*Haemorrhous mexicanus*). MG causes conjunctivitis and reduces survival among free-living finches, but rarely causes mortality in captivity, suggesting a role for indirect mechanisms. Wild-caught finches were individually housed in captivity and exposed to the following treatments: 1) visual presence of a stuffed, mounted predator (a Cooper's Hawk (*Accipiter cooperii*)) or control object (a vase or a stuffed, mounted mallard duck (*Anas platyrhynchos*)), 2) vocalizations of the same predator and non-predator, 3) approach of a researcher to enclosures, and 4) simulated predator attack (capture by hand). MG infection reduced anti-predator responses during visual exposure to a mounted predator and simulated predator attack, even for birds without detectable visual obstruction from conjunctivitis. However, MG infection did not significantly alter responses during human approach or audio playback. These results are consistent with the hypothesis that predation plays a role in MG-induced mortality in the wild, with reduced locomotion, a common form of sickness behavior for many taxa, as a likely mechanism. Our results therefore suggest that additional research on the role of sickness behaviors in predation could prove illuminating.

Keywords

infectious disease; *Mycoplasma*; predation; sickness behavior

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Introduction

Many infectious diseases do not directly kill their hosts, but increase the risk of mortality through indirect interaction with other ecological factors (Yuill 1987, Scott 1988, Minchella and Scott 1991, Gulland 1992, Holmes 1995). One of the most commonly studied sources of such indirect mortality is an increased risk of predation among infected individuals (*e.g.*, Temple 1987, Hudson et al. 1992, Murray et al. 1997, Mesa et al. 1998, Johnson et al. 2006, Koprivnikar et al. 2012, Miller et al. 2014). However, most studies exploring the links between infection and anti-predation behavior focus on systems in which transmission from prey to predator is crucial for the parasite to complete its life-cycle, known as trophic transmission (*e.g.*, Dobson 1988, Poulin 1994, Moore 2002). In such systems, parasites may manipulate their hosts' anti-predation behavior, ensuring transmission to definitive hosts (Moore 2002). Less is known about how directly transmitted parasites and pathogens, including those transmitted among prey individuals, might alter these hosts' anti-predation defenses (but see *e.g.*, Lefcort and Blaustein 1995, Parris et al. 2004, 2006, Han et al. 2011). In such systems, reductions in anti-predation behaviors would not benefit the parasite or pathogen directly, but may be an unavoidable consequence of replicating within the host. Because additive or synergistic effects of predators and pathogens can regulate host populations in complex ways (Hudson et al. 1992, Koprivnikar et al. 2012, Miller et al. 2014), elucidating the relationship between infection and predation is crucial to understanding and predicting the dynamics of directly transmitted pathogens. Here we investigate potential links between infection and predation risk in house finches (*Haemorrhous mexicanus*) infected with an ecologically relevant and directly transmitted pathogen, *Mycoplasma gallisepticum* (MG).

The house finch-MG system is particularly suited to exploring the links between predation risk and infection with a directly-transmitted pathogen. First, this pathogen causes severe conjunctivitis in house finches, often partially or fully obscuring the eyes, and potentially reducing the ability to visually perceive predators (Kollias et al. 2004, Fernández-Juricic and Tran 2007). Second, while MG is highly contagious among house finches and can transmit to other prey species, particularly other members of the Fringillidae family, there is no evidence of trophic transmission to predators (Farmer et al. 2005, Lierz et al. 2008, Dhondt et al. 2014). This suggests that any alterations to anti-predator behaviors in this system are unlikely to result from parasite manipulation. Third, MG induces prototypical sickness behaviors in house finches, including severe lethargy (Bouwman and Hawley 2010, Adelman et al. 2013b). Such reductions in activity and responsiveness could leave animals less able or motivated to perform anti-predator behaviors (Hart 1988, Owen-Ashley and Wingfield 2007, Tizard 2008, Adelman and Martin 2009, Lopes 2014). Finally, although finches displaying the clinical signs of MG show reduced survival in the wild (Faustino et al. 2004), experimental infection in the laboratory rarely results in mortality (*e.g.*, Kollias et al. 2004, Hawley et al. 2010, 2013). This discrepancy suggests that some form of indirect mortality, such as increased risk of predation, must underlie reduced survival in the wild.

Healthy house finches exhibit a variety of predator-avoidance behaviors, including scanning (Fernández-Juricic and Tran 2007, Valcarcel and Fernández-Juricic 2009), vigilance (Fernández-Juricic et al. 2008), and flight (Valcarcel and Fernández-Juricic 2009). Because

MG can both obscure vision and reduce locomotion, any of these critical behaviors could be reduced during an MG infection. Additionally, because MG infection alters vision, but is not known to alter audition, we would predict that behaviors performed in response to visual stimuli would be affected to a greater degree than behaviors performed in response to auditory stimuli. To test how MG infection alters anti-predator behaviors, we performed two experiments. In experiment 1, we measured scanning, vigilance, flight, and avoidance behavior (trial A only) among MG-inoculated or sham-inoculated (control) house finches exposed to three trials: A) visual exposure to either a simulated predator (a stuffed Cooper's Hawk (*Accipiter cooperii*) or non-predator (a stuffed mallard (*Anas platyrhynchos*) or a vase), B), an auditory stimulus of a vocalization from either a predator (Cooper's hawk) or a non-predator (mallard duck), C), the multi-sensory (visual and auditory) stimulus of a human entering the room and approaching the cage. In experiment 2, we tested whether MG infection alters captive finches' abilities to escape capture by researchers. We hypothesized that finches experimentally infected with MG would exhibit less pronounced anti-predator behavior than healthy birds due to the lethargy associated with infection and the greater difficulty visually distinguishing predators (Tisdale and Fernández-Juricic 2009). When MG-treated birds showed differential anti-predator responses, we then asked whether visual obstruction alone could explain these differences by conducting additional analyses that excluded any infected birds that displayed signs of conjunctivitis.

Materials and methods

Experiment 1 Overview

In experiment 1, 12 control (sham-inoculated) and 12 experimentally infected (MG-inoculated) finches were sequentially exposed to three predation-related stimuli: A) the visual presence of a stuffed, mounted Cooper's hawk and one of two control objects (a vase or a stuffed, mounted mallard duck); B), the playback of vocalizations from a Cooper's hawk and a control (mallard duck); and C) the approach of an observer directed at their cages (see below for details). We chose not to repeatedly expose birds to these stimuli to avoid any potential for habituation.

Subjects

House finches were captured using sunflower seed-baited traps in September of 2011 in Auburn, Alabama and from November 2011-January 2012 in Blacksburg, VA. Twenty-four birds that had been previously exposed to, but recovered from, MG in a separate experiment were chosen for use in Experiment 1. During February 2012, prior to inoculation and behavioral trials, birds were housed in pairs (cage dimensions: 76cm x 46cm x 46cm) in three separate rooms at constant day length (12h light : 12h dark) and temperature (22°C), and were provided *ad libitum* access to water and food (Daily Maintenance Diet, Roudybush, Woodland, CA, USA).

Whenever birds were scored for the severity of mycoplasmal conjunctivitis, we used an "eye score" on a scale from 0 to 3 per eye (Hawley et al. 2011, modified from Sydenstricker et al. 2006): 0) no visible signs of disease in or around the eye, 1) minor swelling, 2) moderate swelling and slight eversion of the conjunctiva, and 3) severe swelling, noticeable exudate,

and eversion of the conjunctiva. We quantified the total eye score for each subject as the sum of the scores from each eye, for a maximum possible total score of 6. Given the partial immunity conferred by prior exposure to MG (Sydenstricker et al. 2005), total eye scores were fairly low among birds in Experiment 1 (mean = 2.1).

Pre-trial housing and experimental infection

Two weeks prior to behavioral trials, twelve birds were inoculated in both conjunctiva with an isolate of MG originally collected from an infected house finch in VA in 1994 (Ley et al. 1996) (“VA1994”, stock ID 7994-1-7P 2/12/09; D. H. Ley, North Carolina State University, College of Veterinary Medicine, Raleigh). Birds received 25 μ L per eye of this inoculum, which was suspended in Frey’s medium at a viable count of 2.24×10^7 color-changing-units / mL, as determined by the most-probable-number method (Meynell and Meynell 1970). Twelve additional birds served as controls and were sham inoculated with the same volume of Frey’s medium alone. Birds from the different populations of origin (Alabama and Virginia) were distributed between both treatment groups, such that treatment groups had the same proportion of birds from each population. After inoculation, infected and control birds were housed in separate cages to prevent cross-contamination. Within treatment groups, however, birds were housed in pairs. Animal care was always performed on control cages first, and animal care workers changed their latex gloves between rooms.

Observation Room: Animal Housing and Setup

We conducted our behavioral trials in twelve rounds, one per day, exposing two birds (one control and one infected) at a time to the predation-related stimuli. On the evening prior to behavioral trials, two birds (one control and one MG-inoculated; randomly assigned to trial pairs) were transferred to separate experimental cages in an observation room with the same temperature and lighting conditions as above, but isolated from all other captive finches. Each experimental cage measured 122cm x 46cm x 46cm and was covered across the back with opaque cloth. In addition, the left and right sides were partially covered with the same material, leaving two side “windows” near the back of the cage out of which birds could observe test stimuli (Fig. 1). The inside of each cage was divided into three equal-sized partitions using opaque cloth: left, center, and right. Both the left and right partitions contained identical food dishes, while the center contained the sole water source. Birds could freely move among partitions, but could only see out of the left window while in the left partition and could only see out of the right window while in the right partition. Thus, to access food, each bird had to choose between clear views of one of the two stimuli (Fig. 1). When birds were initially placed in the cages, the internal cloth partitions were rolled up so birds could learn the placement of food and water dishes. Three hours after they were placed in experimental cages, the partitions were unrolled.

Experimental cages were placed at the back of the room oriented at an obtuse angle to each other, such that a common object placed between cages could be seen by each bird through the cages’ side “windows” (the left window for one bird and the right window for the other), though birds could not see each other (Fig. 1). A curtain was also placed between the cages so that birds could not see each other out of the front of the cages. However, the two birds in a pair were able to hear each other throughout the trial. An audio speaker, used for playback

experiments, was located in the center of the room equidistant from the two cages. A video camera was trained on the front of each cage, with a clear view into all three within-cage partitions.

One experimental cage was consistently used for the controls and one cage consistently used for infected birds to avoid any possibility of pathogen cross-contamination. Prior work on MG suggests that after 24 hours, surfaces contaminated with this pathogen are unlikely to be infectious (Dhondt et al. 2007). Still, to be conservative, we used cages that had not housed any house finches for several years prior to these experiments and cages were thoroughly washed before the start of the experiments. Between each pair of birds, the placement of these two cages within the room was switched, so that half of the trials took place with the control cage on the right side and half took place with the MG cage on the right side. After seven trial rounds (~1/2 the total number), the cages were thoroughly cleaned and the cage initially used for control birds was from that point used for infected birds, and vice versa. This design ensured that any effect of cage or cage position would be distributed evenly across experimental treatments.

Presentation of Stimuli and Behaviors Recorded

The 12 experimental rounds, each consisting of a single pair of birds exposed to the three predation-related trials, were conducted during March and April 2012. Approximately fifteen minutes before the lights turned on an experimenter entered the room and turned on the video cameras. To minimize any bias created by the sequence in which trials were presented, the order of trials was balanced across rounds and randomly assigned to experimental pairs.

Trial A: Visual Presence of a Mounted Predator—A stuffed Cooper's hawk was placed between the cages so that birds in each cage were able to view the mount through the cages' side "windows" near the food dishes (Fig. 1). Two control objects, a stuffed mallard duck and ceramic vase, were placed at the other end of the two cages (Fig. 1), visible through "windows" on the sides of cages opposite the hawk. When the birds were placed in the cages the hawk was initially covered; when the mount was uncovered by an experimenter, the trial began and lasted for one hour (if this was the first trial for a given pair, the experimenter uncovered the hawk before the lights came on and the trial was noted as beginning at lights-on). Using video from this section of the trial, we performed a 15min focal sample, starting 30min after the hawk was uncovered. We recorded the amount of time each bird spent in each section of the cage (the partition closest to the hawk, the center partition, or the partition furthest from the hawk).

Trial B: Predator vs. Non-Predator Vocalizations—To assay responses to auditory predation-related stimuli, we played a vocalization from a predator (Cooper's hawk) and a non-predator (mallard) to each pair of birds. Both are common species, present in the locations where house finches were captured for this study (Drilling et al. 2002, Curtis et al. 2006), and we have seen both species near house finches at our study sites, suggesting that finches have some experience with each. Each playback consisted of 5s of a naturally repeated call, *i.e.*, the animal repeating the same vocalization, without experimental

alteration. One playback of each species was presented during each trial and these were separated by 10 minutes of silence. The order of playbacks (hawk first or mallard first) was balanced across pairs. Four exemplars of hawk calls and four exemplars of mallard calls were obtained from the Macaulay Library (Cornell Laboratory of Ornithology, Ithaca, NY) and used as stimuli, with the occurrence of each exemplar balanced across pairs. We calculated the maximum amplitude of each exemplar at 2m from the speaker (the approximate distance to the cages) using a sound level meter (model no. 33-2055, Radio Shack, Fort Worth, TX, USA). Means $\pm$ 1 standard error were 63 ± 1.08 dBA for the hawk exemplars and 62.5 ± 0.29 for the mallard exemplars ($t_6 = 0.45$, $p = 0.67$). The playback device was placed outside of the experimental arena and connected to the speaker inside via cable, allowing experimenters to play the calls without entering the room. After the final playback, ten minutes elapsed before the next stimulus was presented or the experimental round was terminated (depending upon the order of trials for that pair). Using video, we performed a focal observation of each bird for 2min before and 2min after each playback, counting the total number of head-turns and the total amount of locomotion (a summation of hops and flights). Locomotion (a hop or flight) was defined as any change in position that left the bird more than one body width from its original location.

Trial C: Approach of Observer—This trial consisted of an observer entering the experimental room and walking briskly directly toward the midpoint of the two cages. The observer then waited approximately five seconds before leaving the room. We allowed ten minutes to elapse before presenting the next stimulus (if trial C was not the end of the experimental round). From the video, we extracted the birds' latency to move in response to the observer, with the observation period beginning with entry into the room and terminating 5min later.

Analysis, Experiment 1

Behaviors were quantified from videos using JWatcher (Blumstein et al. 2006). All statistical analyses were performed in R (R Development Core Team 2015).

For the mounted predator trials (Trial A), we calculated the time a bird spent in each partition of the cage to determine if it avoided the mounted predator. We counted a bird as having a preference if it spent 50% or more of the focal observation period (15min) in one out of the three partitions. All birds spent 50% or more of their time in either the partition nearest the predator mount or the partition farthest from the mount, both of which contained food dishes. We therefore analyzed preference differences between control and MG-treated birds using a 2 x 2 contingency table and a χ^2 test. To determine if any preference differences could be explained by visual obstruction alone, this analysis was repeated excluding any MG-treated birds that showed eye score > 0 . Because multiple tests were performed on these data, reported p-values reflect Bonferroni correction.

In the audio playback trials (Trial B), the number of head turns correlated strongly with the number of hops or flights (Spearman's $\rho = 0.72$), and thus these measurements likely reflect similar behavioral responses and are not statistically independent from one another. As such, we combined these into a single metric of response to playback: head turns + hops + flights.

Because hops and flights were summed during the initial data collection, using the alternative method of principal components analysis (PCA) to combine these three variables was not possible. However, if PCA is performed on the two collected variables (number of head turns; number of hops + flights), our results are qualitatively identical to those presented when using summation of the variables.

We analyzed the combined metric of movements as the dependent variable in a linear mixed effects model, R package nlme, (Pinheiro and Bates 2000, Pinheiro et al. 2016) with initial fixed effects of MG Treatment (control or infected), Playback Type (predator or duck), Before or After Playback, and all their interactions. We included random effects of bird ID nested within experimental pair ID. Interactions with $p > 0.10$ were removed by backwards elimination. Residuals were checked visually for confirmation of normality.

Latency to move upon human approach (Trial C) was analyzed similarly, using linear mixed effects models with experimental pair ID as a random effect and MG-inoculation as a main effect. Latency was log-transformed to meet the assumptions of normality.

Experiment 2 Overview

In experiment 2, birds were housed in individual cages for a separate experiment that required inoculation with different isolates of MG. Because capture by hand was frequently required to track infection dynamics in that experiment, all captures were timed (see details below) to assess how each animal's ability to evade hand-capture, a simulated predation attempt, varied with the severity of MG infection.

Subjects

During July-September, 2013, 37 birds were captured in Blacksburg, VA using seed-baited traps and housed in individual cages (76 x 46 x 46 cm) under the same environmental conditions as experiment 1. After a minimum of one month acclimation, all birds were inoculated with either Frey's medium alone (controls, n=10) or with one of the following MG isolates that were predicted to vary in virulence from low to high as follows (Hawley et al. 2013): CA2009 (n=9), NC1995 (n=9), or VA2013 (n=9). As stocks of VA2013 (2013.089-15(2P) 9/13/13) and NC1995 (13295-2 (5P) 1/13/10) were of higher concentration than CA2009 (CA2009 (2009.061-1 (3P) 10/25/10), the former stocks were diluted with Frey's medium to approximate concentration (1.87×10^7 CCU/mL) of CA2009. For all treatments, birds were inoculated with 40 μ L (7.5×10^5 CCU) per eye. All stocks were provided courtesy of David Ley, NC State University.

Capture evasion trials

All birds were captured by hand by the same observer (JSA) seven days prior to inoculation and on the following days after inoculation: 3, 7, 14, 21, and 28. The same observer also recorded the time elapsed between opening the cage door and securing the animal using a stopwatch. As the approximate severity of disease is readily apparent when approaching a cage, it was not feasible to be completely blind to treatment (MG vs. control) during captures. As such, we utilized the same observer at each capture to minimize any variation

due to observer ability. Exact eye score was recorded once the bird was in hand, on the same scale as Experiment 1, by a separate observer (DMH) who was blind to capture times.

Analysis, Experiment 2

Results of capture evasion were analyzed using linear mixed effects models in R (Pinheiro and Bates 2000, R Development Core Team 2015, Pinheiro et al. 2016). Time elapsed before capture was natural log-transformed prior to analysis to ensure normality of errors. The model included eye score as the independent variable. Because multiple time points were used for each bird, the model incorporated a random effects structure of bird ID nested within treatment (control or specific MG isolate). To elucidate whether reduced locomotion (aka, sickness behavior) or visual obstruction drove the differences in capture time, we analyzed a subset of MG-inoculated birds that showed no eye score on day 7 post-inoculation, because sickness behaviors should be readily apparent at that time point (Adelman et al. 2013b). The time-to-capture of these birds was compared to sham-inoculated controls using a Wilcoxon rank-sum test. Because multiple tests were performed on these data, p-values reported from both tests reflect Bonferroni correction.

Results

Experiment 1

Trial A: Visual Presence of a Mounted Predator—All sham-inoculated (control) birds spent over 50% of their time in the cage partition furthest from the mounted predator, suggesting strong avoidance of this visual stimulus in healthy house finches. In contrast, five out of the 12 MG-inoculated birds spent over 50% of their time in the partition closest to the predator mount (Fig. 2A, $\chi^2_1=6.32$, Bonferroni-corrected $p = 0.02$). To assess whether visual obstruction was solely responsible for this pattern, we repeated the analysis including only birds with a total eye score of 0. In this case, the pattern remains the same (Fig. 2B, $\chi^2_1=9.23$, Bonferroni-corrected $p < 0.01$), although the sample size of MG-treated birds was greatly reduced ($n = 3$).

Trial B: Predator vs. Non-Predator Vocalizations—After playback of vocalizations from either a predator (Cooper's hawk) or a non-predator (mallard duck), captive finches showed reduced scanning and locomotion, as measured by the sum of head turns, hops, and flights (Fig. 3, Before vs. After: estimate = -42.96 , SE = 10.60 , $F_{1,69} = 14.60$, $p < 0.001$). MG-treated birds showed a marginally smaller reduction in these behaviors than did control animals (Fig. 3, interaction effect: MG-treated x After: estimate = 28.63 , SE = 15.00 , $F_{1,69} = 3.64$, $p = 0.06$). However, there was no significant main-effect of MG-treatment (estimate = -24.79 , SE = 19.52 , $F_{1,11} = 0.34$, $p = 0.57$). Moreover, vocalization type (predatory or non-predatory) showed no influence on finch activity, either as a main effect (estimate = -5.27 , SE = 7.50 , $F_{1,69} = 0.49$, $p = 0.48$), or in interaction with other variables (all $p > 0.10$, removed from model).

Trial C: Approach of Observer—Upon approach by a human, MG-inoculated birds did not differ from sham-inoculated control birds in their latency to first movement (data not

shown; means $\pm$ 1SE (log scale): sham-treated = 7.88 ± 0.53 , MG-treated = 8.24 ± 0.65 ; parameter estimate(MG-treated): 0.36, SE = 0.78, $F_{1,11} = 0.22$, $p = 0.65$).

Experiment 2: Capture Evasion

Birds showing more severe eye scores (conjunctivitis) were more susceptible to a simulated predation attempt, as evidenced by reduced time to capture (Fig. 4A, estimated slope (log-scale) = - 0.11, SE = 0.020, $F_{1,180} = 29.65$, Bonferroni-corrected $p < 0.001$). To determine if visual obstruction was the sole cause of this pattern, we compared a subset of MG-inoculated birds without any visible eye score (8/27 MG-inoculated birds) to sham-inoculated birds on day 7 post-inoculation, because sickness behaviors should be readily apparent at this time point. In this analysis, MG-inoculated birds were still captured more quickly, despite no detectable visual obstruction (Wilcoxon rank-sum test: $W=67$, Bonferroni-corrected $p = 0.04$).

Discussion

Here we show that house finches experimentally infected with the directly-transmitted bacterial pathogen *Mycoplasma gallisepticum* (MG) exhibited significant reductions in anti-predator behaviors. While uninfected finches spent the majority of their time as far as possible from a stuffed, mounted predator, infected finches showed no such preference. Moreover, as the severity of conjunctivitis increased, finches were hand-captured more rapidly by an observer, suggesting impaired escape behaviors and/or threat detection. By contrast, infected finches did not significantly differ from controls in their latency to move upon approach of a human or in their response to auditory stimuli (either predator or non-predator vocalizations). Taken together, our results suggest that MG infection can affect some important anti-predator behaviors in house finches. This finding helps explain why the vast majority of house finches survive MG infection in captivity, despite reduced survival in the wild (Faustino et al. 2004, Kollias et al. 2004, Hawley et al. 2010, 2013). Additionally, because MG is not an obligately trophically transmitted pathogen (Lierz et al. 2008), our results suggest that such reductions in anti-predator behaviors are most likely unavoidable consequences of infection, rather than pathogen manipulation. Although other authors have suggested such interactions between infection and anti-predator behavior are important among wild birds (e.g., Yorinks and Atkinson 2000, Møller and Nielsen 2007, Descamps et al. 2011, Møller et al. 2012), this study provides rare empirical evidence of these links at the individual level (but see also Lindström et al. 2003).

Because MG induces severe conjunctivitis, which can obscure vision, we predicted that responses to visual predator stimuli would be altered more severely than responses to auditory stimuli. Our results lend some support to this hypothesis, as MG-infected and control finches showed no significant differences in response to audio playback of heterospecific vocalizations (Fig. 3). However, it should be noted that in the audio trials, all birds, including uninfected animals, responded to both predator vs. non-predator vocalizations by reducing head-turns and locomotion, suggesting an immobility or freezing response to unexpected auditory stimuli. This result suggests that either the vocalizations played in our experiments are not among those that house finches utilize to avoid predation

or that house finches do not rely on auditory cues to detect predators. In other avian systems, vocalizations from different predator species elicit different levels of response, with some eliciting similar responses to control (non-predator) sounds (Rainey et al. 2004, MacLean and Bonte 2013). Furthermore, it is possible that captive house finches do not respond to auditory calls in the same way that free-living finches would. Regardless, further study is needed before any definitive conclusions can be made as to whether MG infection influences anti-predation behaviors in response to auditory stimuli in house finches.

In contrast, to the auditory stimulus, in two of three tests that included visual stimuli, MG-infected house finches responded to predation-related stimuli significantly differently than control birds. First, while control finches spent the majority of their time in the cage partition furthest from a mounted predator, infected finches did not show this preference (Fig. 2). This trial, however, was subject to pseudo-replication because we only had access to one hawk mount as a predatory stimulus, rather than a diversity of mounted or live predator species. While prior work showed that healthy house finches responded to a different mounted hawk species (Fernández-Juricic and Tran 2007), that study also used only a single mount. In both studies, it is possible that healthy finches avoided these mounts for reasons wholly unrelated to predation. However, the repetition of this behavior in two separate labs, with finches from separate populations and mounted predators of different species suggests that healthy finches may avoid mounted predators more generally.

Additionally, infected finches in our study were captured more quickly by hand. In both this case and during the mounted predator trial, the birds' visual fields may have been sufficiently occluded by conjunctivitis to reduce their ability to detect predation-related stimuli (Fernández-Juricic et al. 2008). In support of this possibility, we found that capture time was negatively related to the degree of conjunctivitis (Fig. 4A), suggesting a potential role for visual occlusion in the increased likelihood of capture among infected birds. However, if visual impairment was the sole explanation for the detected differences, we would expect the differences in behavior between infected and control birds in both experiments to disappear when we limited our analysis to birds without conjunctivitis. In contrast, even MG-treated birds with no conjunctivitis showed no preference for the non-predator side of the cage in trial A (Fig. 2B), although the sample sizes in that analysis were greatly reduced. Additionally, in Experiment 2, even MG-inoculated birds with no conjunctivitis on day 7 post-inoculation were captured more rapidly than control birds (Fig. 4B). Together, these results suggest that although visual impairment may play a role in the diminished anti-predator behaviors seen in infected house finches, it is likely not the sole mechanism underlying changes in behavior during infection.

The reduction in anti-predator behaviors even among MG-infected finches without detectable visual impairment strongly suggests a role for sickness behaviors. Many authors have suggested that sickness behaviors, specifically lethargy and diminished motivation, should reduce an animal's likelihood of escaping predators (Hart 1988, Owen-Ashley and Wingfield 2007, Tizard 2008, Adelman and Martin 2009, Lopes 2014), though few studies have directly evaluated this possibility (Lindström et al. 2003, Otti et al. 2012, Männiste et al. 2013). In experiment 1, MG-infected birds showed a non-significant trend toward reduced movements (Fig. 3), indicative of the sickness behaviors documented in other

experiments in this species (Bouwman and Hawley 2010, Adelman et al. 2013a, b). Such lethargy or reduced motivation could help explain reduced aversion to the mounted predator: because infected birds moved less in general, if they happened to enter the cage partition nearest the predator, they would be less likely to leave it. Similarly, a role for sickness behaviors may also help explain the lack of detectable difference between infected and control groups in the latency to move in response to an approaching observer (trial C). If the initial response to an approaching threat involved initial immobility, as has been observed in some urbanized house finch populations (Valcarcel and Fernández-Juricic 2009), sickness behaviors among infected birds may have precluded us from detecting any differences between groups in the latency to move upon human approach. More broadly, these results suggest that sickness behaviors, which occur in response to infection and immune activation across a diversity of organisms (Hart 1988), may influence indirect mortality during infection by reducing anti-predation responses.

Finally, the overall differences we observed in responsiveness to our stimuli were likely influenced by the level of perceived predation threat. Endler (Endler 1991) described six stages of successful predation, from encounter and detection to attack and consumption, escalating in their immediacy of threat to prey. In the early stages, such as during detection by predators, effective anti-predator defenses include immobility, which occurred during our audio trials and approach of a human observer. As suggested above, because sickness behaviors (*i.e.* reduced locomotion) are also characterized by immobility, we likely had limited ability to detect differences between control and infected finches in this early-stage anti-predator behavior. In contrast, during the later “attack” stage of predation, which we simulated using capture by hand, Endler’s described anti-predator behaviors are universally more active, including flight and unpredictable movements (Endler 1991). At this stage, sickness behaviors would make differences between control and infected animals in response to the threat of predation easier to detect, as effective anti-predator behaviors (flight) are highly dissimilar from sickness behaviors (immobility). Therefore, although we only detected differences in some anti-predator behaviors, it is possible that other differences are present, but increased sample sizes are required to detect differences in those behaviors, such as immobility, that naturally appear similar to sickness behaviors.

Taken together, our results show that MG infection alters house finches’ responses to predation-related stimuli, a factor that likely contributed to recent declines in wild house finch populations (Hochachka and Dhondt 2000). In doing so, these data provide rare empirical evidence of a directly-transmitted pathogen reducing anti-predation behaviors. Additionally, our results point toward a potentially important role for sickness behaviors in altering anti-predator responses in this and other systems. As others have suggested (Otti et al. 2012), increased risk of predation may be a broadly important yet underappreciated cost to immune activation during infection, with implications for host-pathogen dynamics and the maintenance of immune variation in natural populations.

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Figure 1.

Experiment 1 was set up such that each house finch (one infected, one sham-inoculated; housed individually in each cage) was forced to feed in clear view of either a control object (duck mount or vase) or a simulated predator (Cooper's hawk mount). Birds were tested in pairs but could not see one another (dark lines indicate curtains of opaque cloth). Cages used for *Mycoplasma gallisepticum*-treated and control birds alternated between left and right sides of the room between trials.

Figure 2.

Control house finches (sham-inoculated) preferred to spend time on the side of their cage furthest from a mounted predator (Cooper's hawk), whereas house finches infected with *M. gallisepticum* (MG-inoculated) showed no evidence of avoiding the mounted predator. This pattern holds true when all birds are included (A) and when only birds that showed no visual signs of infection (eye score, B) are included. Sample sizes are shown inside bars. *P*-values reflect Bonferroni correction from χ^2 tests.

Figure 3.

House finches showed reductions in locomotion and scanning behaviors after playback of vocalizations from a non-predatory species (A; mallard) and a predator species (B, Cooper's hawk). Birds inoculated with *M. gallisepticum* showed marginally smaller reductions in these behaviors than did sham-inoculated birds ($p = 0.06$, tested using linear mixed effects models). Points show group means, error bars show ± 1 standard error.

Figure 4.

House finches displaying higher eye scores (more severe conjunctivitis) after inoculation with *M. gallisepticum* (MG) were captured by hand more rapidly than those showing less severe eye scores (A). The solid line in (A) shows predictions from a linear mixed effects model, dashed lines show ± 1 standard error around these, and points have been jittered to illustrate sample sizes. Additionally, when comparing only birds that showed no conjunctivitis on day seven post-inoculation (eye score = 0, B), MG-inoculated birds were still captured more rapidly than sham-inoculated birds. In (B), whiskers show the data range, boxes show the inter-quartile range, thick bars represent medians, and the p -value is from a Wilcoxon rank-sum test. In both panels, p -values reflect Bonferroni correction.