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Stress responses and disease in three wintering house finch (*Carpodacus mexicanus*) populations along a latitudinal gradient

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Abstract

In laboratory studies, stress hormones have been shown to impair immune functions, and increase susceptibility to diseases. However, the interactions between stress hormones and disease have rarely been studied in free-ranging populations. In this study, we measured concentrations of the avian stress hormone corticosterone across four winter months (December–March) over two years in three eastern North American house finch populations (*Carpodacus mexicanus*) along a latitudinal gradient. Because *Mycoplasma gallisepticum* infections appear in these populations in late winter, we hypothesized that the timing of the disease outbreaks could be mediated by changes in corticosterone concentrations. We found a significant increase in baseline and stress-induced plasma corticosterone concentrations in house finches without *Mycoplasma* symptoms in late winter; when the prevalence of *Mycoplasma* infection peaks. We also found that house finches with *Mycoplasma* symptoms had elevated stress-induced corticosterone concentrations. High baseline concentrations were associated with a low body condition and a high fat load. We found that the relationship between corticosterone concentrations and the latitude of the study population changed between years. The first year, corticosterone concentrations were lowest in the southern latitude, but became higher in the second year when average winter temperatures were low. A causal understanding of the implications for this variation in corticosterone concentrations for *Mycoplasma* disease dynamics awaits further studies.

Keywords: *Carpodacus mexicanus*; House finch; Stress; Corticosterone; *Mycoplasma gallisepticum*; Latitude; Infection

1. Introduction

Although the majority of vertebrate species that have been studied have been found to modulate their stress responses seasonally (Romero, 2002), it is not clear why these seasonal modulations occur. Several ideas have been put forward that could explain how modulations of stress responses could be adaptive (reviewed in Romero, 2002). First, glucocorticosteroid rhythms have been suggested to reflect variation in the energy balance of an organism. Thus, when energetic needs exceed the available amounts of energetic resources, glucocorticosteroids will become elevated (Goymann and Wingfield, 2004). Second, the frequency of exposure to stressors could vary with the seasons. If, for example, competition for mates is more intense in the breeding season, this could lead to increased stress responses at this time of the year (Sapolsky et al., 2000). And third, the need to respond to a stressor can vary over the season. While it may be adaptive to respond strongly to a stressor and abandon the territory when there are many alternative options, it may be more beneficial to stay when the alternative options are few (Wingfield, 1994).

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Glucocorticoid concentrations can also vary between populations living at different latitudes. A study by Silverin et al. (1997) found that willow warblers (*Phylloscopus trochilus*) in northern Sweden had lower magnitudes of stress responses than those from a population further south. This observation led to the suggestion that stress responses in birds were down-regulated at higher latitudes to prevent nest abandonment when breeding seasons were short, and the alternative options were few. However, other studies have found the opposite pattern with high responses at high latitudes in both breeding and wintering birds (Holberton and Able, 2000; Wingfield et al., 1995). High corticosterone concentrations in the winter could be expected because birds that over-winter in colder climates have greater energetic demands and live in a less predictable environment. A stronger stress response might therefore be required to respond to changes in energy demands and food availability. Thus, it has become clear that just like seasonal differences, differences in glucocorticoid responses between populations can occur for many reasons, and several models may be required in order to explain differences between populations on different latitudes and within populations at the same latitude (Breuner et al., 2003; Romero, 2004).

Life-history theory provides an alternative approach to understand seasonal and latitudinal variation in stress responses. Because an organism's physiological resources are limited, investments of resources like energy and nutrients have to be balanced between competing demands (Ricklefs and Wikelski, 2002). In this framework, hormones like corticosterone may serve as mediators that reallocate limited resources across different functions. Because the maintenance of an immune defense is physiologically costly (Bonneaud et al., 2003; Martin et al., 2003), and stress hormones have several down-regulatory effects on immune functions (Apanius, 1998; Nelson et al., 2002), an up-regulation of the stress response can be viewed as an adaptive re-direction of resources away from the immune system to meet more urgent demands (Lochmiller and Deerenberg, 2000; Sheldon and Verhulst, 1996). Observations that immunity in passerines is compromised during energetically demanding activities, such as reproduction, have lent support to this view (Lochmiller and Deerenberg, 2000; Zuk and Stoehr, 2002).

In this study, we investigated the variation in corticosterone concentrations in free-ranging house finches (*Carpodacus mexicanus*) at three different latitudes. We studied three eastern populations of house finches that during the last decade have been subject to regular outbreaks of infections caused by the bacterium *Mycoplasma gallisepticum*. We were interested in quantifying the variation in stress responses between years, winter months and latitudes in house finch populations. This would allow us to explore the hypothesis that seasonal modulation of the stress response could impacts disease dynamics in this study system.

House finches infected with *Mycoplasma* first appeared on the North American east coast in 1994, and since then the disease has spread rapidly in house finch populations across the country and reduced the abundance of house finches in affected areas (Hochachka and Dhondt, 2000). Because the disease can be visually detected, *Mycoplasma* infection in house finches has become a model system for the study of emerging disease in wildlife (Dhondt et al., 2005). From the long-term data set that has been collected, it has become clear that the variation in *Mycoplasma* prevalence has a strong seasonal component, and the proportion of infected individuals show peaks in the late summer and late winter (Altizer et al., 2004).

Seasonal variations in disease prevalence are common and can be caused by both extrinsic factors such as climate, or intrinsic factors such as changes in host immunity (Nelson et al., 2002). Several hormones can influence immune functions, but some of the more potent immuno-modulators are the glucocorticoids that are released by the hypothalamic–pituitary–adrenal (HPA) axis during stress (Apanius, 1998). The interaction between the stress hormones of the HPA axis and the immune system have been studied extensively in the laboratory, but few studies have examined this link in wildlife populations (Nelson et al., 2002).

In this study, we measured monthly mean corticosterone concentrations in three free-ranging house finch populations in two winter seasons (December–March), at a time of the year when we expected the prevalence of *Mycoplasma* infection to increase. We examined the yearly, monthly, and latitudinal patterns of variation in stress responses between populations. We also examined if the sex, body condition or infection status could explain variation in stress responses between individuals.

2. Materials and methods

2.1. Study species and populations

The house finch is a small (20 g) North American Cardueline finch (Badyaev, 2003). In the winter, house finches aggregate in large feeding flocks, and their diet consists primarily of seeds (Hill, 1995). In the spring, the winter feeding-flocks disperse and house finches form breeding pairs. Male song and pair formation becomes evident in January in the southern latitudes and in February in northern latitudes (Hill et al., 1999). The breeding season of house finches is extended, and covers a period of six months in which pairs can fledge between 2 and 5 broods (McGraw et al., 2001). Finally, house finches are partial migrants, and some birds leave their breeding area in the autumn and over-winters in milder climates (Hill, 1993).

The house finch populations used in this study were all located in the mid-Atlantic region of the United States, and three study sites were chosen to represent three different climate regions that show different seasonal patterns of *Mycoplasma* disease dynamics (Altizer et al., 2004). In short, the infection peak in the late winter occurs earlier in the north, but with lower amplitude, and the prevalence of infection is generally higher in southern populations. For this study, the northernmost study population was located in Ithaca, NY (42°26′N, 76°30′E), the central population in Princeton, NJ (40°21′N, 74°40′W) and the southern population in Atlanta, GA (33°45′N, 84°23′W).

Mean temperatures for the study sites, in the two study years and months were obtained by the National Oceanographic and Atmospheric Administration (www.noaa.gov). The mean temperature of each state (NY, NJ, and GA) during the study period increased with decreasing latitude (mean for each latitude with years and months combined) was for the north region, −2.7 °C; central, 2.4 °C; and south, 10.2 °C. The average temperatures (mean for each year with latitudes and months combined) were for the first year 2001/2002: 5.0 °C and for the second year 2002/2003: 1.5 °C. The monthly mean temperatures (for the latitudes combined) were the following in 2001/2002: December, 6.0 °C; January, 3.5 °C; February, 3.5 °C; March, 7.0 °C; and in 2002/2003: December, 1.5 °C; January −2.2 °C; February, 0.0 °C; March, 6.7 °C. At the three latitudes, the coldest months (mean for each month, with years, and latitudes combined) were January (0.6 °C) and February (1.9 °C) while temperatures were higher in December (3.7 °C) and March (6.8 °C).

At each study site, feeders containing black oil–sunflower seeds were placed at 2–4 trapping sites to attract house finches. Trapping sites were located in suburban areas, with bushes surrounded by open or semi-open fields. Birds were captured with wire-mesh feeder traps and mist nets under permits from the New York State Department of Environmental Conservation (No. LCP 99-039) and the US Fish and Wildlife Service (PRT 802829). On average, birds were captured during 3 mornings each month, and the majority of samples (>90%) were taken between the 10th and the 26th in each month. After capture, all birds were sexed based on plumage characteristics (Hill, 1993). We took measurements of body mass (to the nearest 0.01 g) with a pesola scale and tarsus length (to the nearest 0.01 mm) with digital calipers. Both subcutaneous fat content in the furcular hollow (Helms and Drury, 1960) and pectoral muscle condition (Olsen et al., 1996) were estimated visually on a 0–5 scale. All birds were inspected and scored for physical signs of conjunctivitis, such as eyelid or conjunctival swelling, erythema, and discharge. This visual examination provides a reliable method to detect *Mycoplasma* infections in house finches. The presence of these symptoms is almost exclusively a result of infections with a strain of *M. gallisepticum*, and *Mycoplasma* have only rarely been isolated in cultures or detected by PCR in birds without these symptoms (Hartup et al., 2001, 2004). In aviaries, visual symptoms appear 4–5 days after house finches have been exposed to *Mycoplasma*, and they remain visible for on average 10 weeks (Kollias et al., 2004).

2.2. Blood sampling and hormone analyses

Blood samples of birds at all three latitudes were taken in December–March 2001/2002 and 2002/2003. During capture, traps and nets were monitored continuously and birds were removed from the traps or nets, and first blood samples were extracted within three minutes of entry to obtain accurate baseline stress hormone concentrations (Romero and Romero, 2002). From each bird, we collected three small (50 μl) serial blood samples at 3 min for baseline corticosterone concentrations, and at 30 and 60 min for stress-induced concentrations. Between these times, birds were kept in dark paper bags. This technique is a standardized procedure in bird studies, and referred to as the capture stress-protocol (Romero and Romero, 2002). Blood samples were obtained by puncturing a wing vein with a sterile needle and the blood was transferred into an Eppendorf tube using heparinized micro-hematocrit capillary tubes. Samples were kept cool while in the field, and were centrifuged for 5 min at 7000*g* within 3 h of sampling. The plasma fraction was removed and stored at −20 °C until assayed for total corticosterone content. In total, 515 blood samples were taken from 186 birds. The plasma corticosterone concentration of each sample was measured using the method described in Wingfield et al. (1992). In the assay, hormones are extracted from small volumes (10–20 μl) of plasma and detected with a direct radioimmunoassay. In the assays, the mean recovery of corticosterone was 76%, the inter-assay variation was 7–15% and the intra-assay variation was 15%.

2.3. Statistical analyses

Because the *Mycoplasma* infection could potentially affect corticosterone concentrations, we excluded the birds that were captured with *Mycoplasma* symptoms from the analyses when testing for seasonal and latitudinal differences. Thus, all seasonal and latitudinal differences recorded are from birds without disease symptoms. We tested for differences in corticosterone concentrations between infected and uninfected birds in separate analyses.

We tested for effects of year, latitude, and month on stress-induced corticosterone concentrations using general linear models (GLM). Because we were unable to capture house finches in all months at all three latitudes,

we only had samples from 17 of the 24 possible study months and latitudes. Variable sample sizes also exist because we trapped different numbers of birds, or were unable to get complete corticosterone series from all birds that were captured. Our statistical design was therefore incomplete. We took this into account by using a GLM that removed all effects that could not be fully estimated, such as some higher-order interaction effects. We used sums of squares type V in the model that are designed to take unbalanced designs into account (StatSoft, Inc. 2004). We used Tukeys post hoc tests (HSD) for unequal sample sizes to examine significant effects in the GLM. All data were tested for normality using Kolmogorov–Smirnov tests, and non-parametric tests were used for data that did not fulfill the assumptions of normality.

We calculated the stress responsiveness of each individual as the difference between the corticosterone concentration in the baseline sample and those at 60 min, because the majority of birds had their highest corticosterone concentrations at this time. We calculated the total corticosterone response by adding the mean corticosterone concentration exposure between 3 and 30 min to the mean exposure between 30 and 60 min. The total response is expressed as nanogram corticosterone per milliliter and hour. Total stress responses and stress responsiveness could only be calculated for birds where all the required samples were available. In the text, r represents correlation coefficients from Pearson's product moment correlations, and R_s are correlation coefficient from Spearman rank correlations. We used Statistica for Windows software (Statsoft, Inc. 2004) for all analyses.

3. Results

3.1. Effects of month, latitude, and year for stress-induced corticosterone

We tested for an effect of month, latitude, and year on stress-induced corticosterone concentrations in a general linear model. In the model, corticosterone concentrations at 30 and 60 min were included as repeated measures dependent variables, and year, month, and latitude were included as independent variables. There was a significant effect of month ($F_{3,122} = 7.97$, $p < 0.001$) on stress-induced corticosterone concentrations. This effect was due to an increase in corticosterone concentrations across the study period (Fig. 1). Post hoc tests showed no significant difference between samples taken in December and January ($p = 0.12$), while there was significant increases from January to February ($p < 0.001$), and from February to March ($p = 0.01$).

There was also a tendency of an effect of year ($F_{1,122} = 3.22$, $p = 0.08$), and corticosterone concentrations tended to increase from the first to the second year.

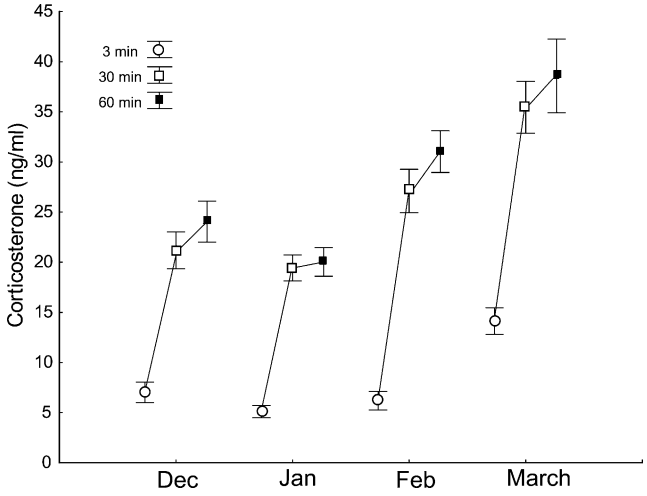

Fig. 1. Seasonal variation in baseline (3 min) and stress-induced (30 and 60 min) corticosterone in free-ranging house finches. Values are monthly means and standard error of means from two seasons and three populations. The sample sizes of each month are for 3, 30, and 60 min, respectively, December: 40, 40, 37; January: 57, 55, 50; February: 29, 34, 34; March: 19, 19, and 16.

We found a significant interaction between year and latitude ($F_{2,122} = 9.46$, $p < 0.001$), thus the year-to-year variation in corticosterone concentrations was different across latitudes. In the first year, stress-induced corticosterone concentrations were lowest at the southern latitude, and the second year they were highest at this latitude (Fig. 2). We also found a significant effect of sample time ($F_{1,122} = 4.85$, $p < 0.029$): corticosterone concentrations increased between the 30 min (23.14 ± 2.3 ng/ml) and 60 min (26.27 ± 2.3 ng/ml) sample. There was no significant effect of latitude on stress-induced corticosterone concentrations ($F_{2,122} = 0.23$, $p = 0.79$).

3.2. Variation in total stress responses and stress responsiveness

We defined the total stress response as the average corticosterone exposure during the hour that a bird was captured and handled. There were significant effect of month ($F_{3,110} = 8.6$, $p < 0.001$) and year ($F_{1,110} = 4.11$, $p = 0.045$) on total stress responses. Total stress responses (ng/ml h) increased into the spring (December, 16.8; January, 14.8; February, 21.9; March, 26.2), and were higher in the second year (2001/2002, 18.3; 2002/2003, 21.5). We found no significant differences between latitudes ($F_{2,110} = 0.69$, $p = 0.50$) whereas the interaction between latitude and season was significant ($F_{2,110} = 6.62$, $p = 0.002$). The reason for this was that total stress responses increased in the second year compared to the first at the southern latitude, while remaining similar at the other two latitudes (Fig. 2).

We defined the stress responsiveness as the difference between baseline and 60 min corticosterone concentrations.

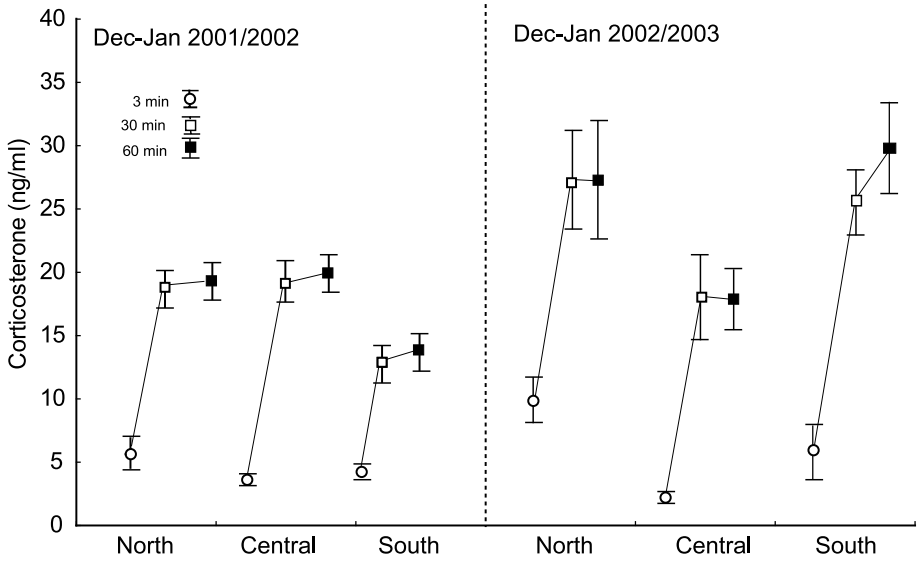

Fig. 2. Latitudinal variation in baseline (3 min) and stress-induced (30 and 60 min) corticosterone concentrations in house finches. Values are means and standard errors of early winter (December–January) samples from two study years (2001/2001, 2002/2003) and three study populations. The northern population was at latitude 42° (Itacha, NY), the central population at 40° (Princeton, NJ), and the southern population at 30° (Atlanta, GA). For 2001/2002 sample sizes were the following for north (33, 32, 32) central (11, 13, 13), and south (12, 11, 12) for 3, 30, and 60 min, respectively. For 2002/2003 sample sizes were the following for north (14, 16, 15), central (8, 8, 8), and south (19, 21, 19).

We found no significant effect of month ($F_{3,115}=1.26$, $p=0.29$), latitude ($F_{2,115}=0.55$, $p=0.57$), or year ($F_{1,115}=2.72$, $p=0.10$) on stress responsiveness. The interaction between latitude and year was significant ($F_{2,115}=3.44$, $p=0.035$). This effect was due to an increase in stress responsiveness in the second winter at the southern latitude (Fig. 2).

3.3. Effects of month, latitude, and year on baseline corticosterone

Baseline corticosterone concentrations showed a positive correlation with sampling month (1–4) in the southern ($R_s=0.52$, $n=49$, $p<0.001$) and northern latitude ($R_s=0.28$, $n=62$, $p=0.03$). On the central latitude, this correlation was not significant ($R_s=0.08$, $n=34$, $p=0.63$).

To test for an effect of latitude and year on baseline corticosterone concentrations, we combined samples taken in December and January where the effect of sampling month was low. We found a significant increase in baseline corticosterone concentrations in the second year compared to the first, both at the southern- (from 3.1 ± 0.4 to 7.0 ± 0.8 ng/ml, $U_{33,15}=132$, $p=0.009$) and northern latitude (from 6.4 ± 1.3 to 9.8 ± 1.7 ng/ml, $U_{12,19}=26.5$, $p<0.001$). At the central latitude, baseline corticosterone concentrations did not change significantly between study years (2001/2002, 2.7 ± 0.4 ng/ml; 2002/2003, 2.1 ± 0.1 ng/ml; $U_{10,8}=31.5$, $p=0.30$).

In the combined data from December–January from both years, we found that baseline corticosterone concentrations were lower in the central latitude (2.4 ± 0.2 ng/ml), compared to the other two latitudes (north, 7.5 ± 1.0 ng/ml; south, 5.5 ± 0.6 ng/ml; Kruskal–Wallis ANOVA; $H_{2,97}=17.86$, $p<0.001$).

3.4. Effects of body condition, sex, and disease on corticosterone

There was a significant negative correlation between baseline corticosterone concentrations and a bird's condition (mass/tarsus) ($R_s=-0.21$, $p=0.02$, $n=117$): birds with higher baseline corticosterone concentrations were in poorer condition. We also found a significant positive correlation between an individual's fat score and baseline corticosterone concentrations ($R_s=0.25$, $p=0.002$, $n=151$): birds with higher baseline concentrations had larger fat loads. There was no significant relationship between a bird's pectoral muscle index and baseline corticosterone concentrations ($R_s=-0.06$, $p=0.45$, $n=162$). Stress-induced corticosterone concentrations at 30 and 60 min showed no significant correlation with either pectoral muscle index, body condition or fat load ($r<0.11$, $p>0.17$, $n<114$, in all tests).

In total, 23 out of 185 birds that we sampled showed symptoms of *Mycoplasma* infection. Infected birds tended to have increased baseline corticosterone concentrations (Kruskal–Wallis ANOVA: $H_{1,165}=2.85$, $p=0.09$, Fig. 3), and this difference was significant for corticosterone concentrations at 30 ($T=4.26$: uninfected, $n=148$; infected, $n=21$; $p<0.001$, Fig. 3) and 60 min ($T=2.1$: infected, $n=21$; uninfected, $n=137$; $p=0.04$, Fig. 3). Males and females showed no significant difference in corticosterone concentrations either at

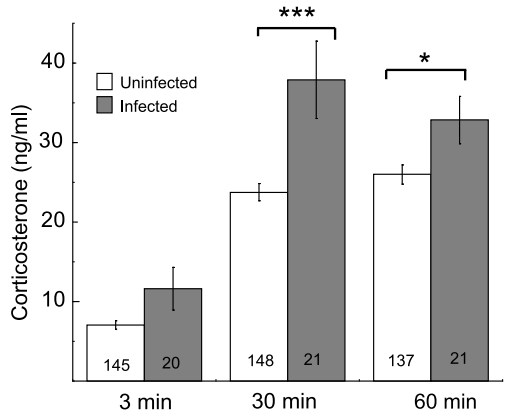

Fig. 3. Corticosterone concentrations of free-ranging house finches infected with *Mycoplasma* compared to uninfected house finches. Sample sizes of each group are noted in the graph and significant differences are illustrated with bars, ***$p < 0.001$, *$p < 0.05$.

baseline levels (K–W: $H_{1,165} = 0.54$, $p = 0.46$) or concentrations at 30 ($T = 0.12$, $p = 0.91$; females, $n = 71$; males, $n = 91$) or 60 min ($T = 1.14$, $p = 0.26$; females, $n = 67$; males, $n = 89$).

The prevalence of *Mycoplasma* infection was higher at the central latitude (23%, $n = 52$) compared to the south (7%, $n = 60$), and the north (9%, $n = 74$). The proportion of infected birds was higher the second winter (14%, $n = 111$) compared to the first (9%, $n = 75$). The prevalence of infected birds increase from December (6%, $n = 46$), January (10%, $n = 70$), and February (19%, $n = 46$) and remained high in March (17%, $n = 23$).

3.5. Variation in body condition, pectoral muscle index, and fat load

We tested for differences in body condition with a general linear model in which latitude, year, and month were included as dependent variables. There was a significant effect of latitude on body condition ($F_{2,124} = 56.28$, $p < 0.001$). House finches had low body conditions (body mass (g)/tarsus length (mm)) at the southern latitude (0.82 ± 0.01) intermediate body condition in the north (0.90 ± 0.01) and body conditions were high in the central population (0.98 ± 0.01). There was also a significant effect of year ($F_{1,124} = 6.39$, $p = 0.012$): body conditions were reduced the second study year (0.88 ± 0.01) compared to the first (0.92 ± 0.01). We also found significant variation in body condition between months ($F_{3,124} = 2.75$, $p = 0.046$). Body conditions were low in the coldest months January (0.88 ± 0.01) and February (0.89 ± 0.02) and higher in December (0.90 ± 0.01) and March (0.93 ± 0.03).

There were significant differences between latitudes also in pectoral muscle index scores (1–5) (Kruskal–Wallis ANOVA: $H_{2,161} = 49$, $p < 0.001$). Birds at the southern latitude had lower pectoral muscle index scores (2.64 ± 0.08) compared to those in the central (3.47 ± 0.08) and northern latitude (3.38 ± 0.06). There was no difference in pectoral muscle index between years (K–W: $H_{1,161} = 0.43$, $p = 0.98$).

Fat scores (1–5) differed between latitudes (K–W: $H_{2,152} = 35$, $p < 0.001$). Birds in the northern latitude had higher fat scores (2.38 ± 0.06) compared to both the central (1.36 ± 0.18) and the south (1.33 ± 0.13). Fat scores also changed between years (K–W: $H_{2,152} = 7.1$, $p < 0.01$), and became higher (1.91 ± 0.10) in the second winter that was colder compared to the first (1.69 ± 0.10).

4. Discussion

We found that both baseline and stress-induced corticosterone concentrations in house finches significantly increased across the study period, and the first significant increase between subsequent months took place in February. Generally, the baseline and stress-induced corticosterone changed in parallel. Therefore, the capacity to respond to a stressor, or stress responsiveness did not change, but rather the total magnitude of the stress response. The pattern of increased corticosterone concentrations observed at the beginning of the breeding season in house finches is similar to the patterns found in other studies of passerines and also in other vertebrates (Hegner and Wingfield, 1986; Romero, 2002). One reason for this increase could be that competition for mates becomes more intense in the beginning of the breeding season.

We found no overall latitudinal patterns in corticosterone concentrations. Instead, corticosterone concentrations showed different latitudinal patterns in the two study years. In the first study year, corticosterone concentrations were lowest on the southern latitude, while they were higher in populations further north. The latitudinal pattern this year was similar to that found in earlier studies (Holberton and Able, 2000; Silverin et al., 1997; Wingfield et al., 1995). In the winter, increased corticosterone concentrations at high latitudes could reflect a greater preparedness to respond to the cold and unpredictable winter climate in the north. This may be important because the energetic demands of house finches are increased in cold temperatures (McEwen and Wingfield, 2003; Salt, 1952). Our finding that birds in the south had less well-developed breast muscles, lower body condition, and less fat reserves compared to birds in the north, also indicate that birds at southern latitudes were physiologically less well-prepared to over-winter compared to birds in populations further north. Interestingly, in the second year when the winter was colder we found a different pattern. This year, corticosterone concentrations became elevated at the northern latitude, and a rather dramatic increase in corticosterone concentrations occurred at the southern latitude, where stress

responsiveness significantly increased. Although absolute temperatures in the south were higher than those in the north, the increased corticosterone in the south this year could perhaps reflect a higher level of cold-stress experienced by the birds at this latitude because they were less well-prepared for a cold winter.

Corticosterone concentrations in birds can also be affected by short-term temperature changes (Romero et al., 2000). For example, corticosterone levels could be expected to be elevated in the morning, if temperatures the night before trapping have been low. In this study, birds were captured at several different trapping days per month, thus any effects of short-term temperature changes would not have affected all our samples in the same way. It is also possible that corticosterone concentrations were affected by the presence of predators at the trapping sites (Scheuerlein et al., 2001), or some of other variable that was not measured in this study.

We found that individuals in poor body condition (body mass/tarsus) had high baseline corticosterone concentrations. Such negative associations with body condition is a pattern that has been found previously in house finches and also in other passerines (Duckworth et al., 2001; Wingfield and Kitaysky, 2002). Here, we also found a positive association between corticosterone and the amount of stored fat. Thus, individuals with high baseline corticosterone concentrations were in poor body condition, but had deposited large fat reserves. These two findings may appear contradictory since the amount of stored fat is sometimes used as an index of good health especially in migrating birds. Nevertheless, a positive relationship between fat reserves and corticosterone can be expected as glucocorticoids promotes the biosynthesis of fatty acids (Malheiros et al., 2003). In the winter, birds use fat reserves as an energy source to survive cold nights. A bird of poor quality or low social status can be expected to carry larger fat reserves because their food supply is less predictable (Ekman and Hake, 1990). Thus, in the winter season, a large fat store may indicate unpredictable conditions, or a poor condition.

In this study, we show that birds with symptoms of *Mycoplasma* infection had elevated stress-induced corticosterone concentrations. This could be a consequence of being infected, because the endotoxins of some bacterial infections can activate the HPA axis directly through interaction with glucocorticoid receptors (Weidenfeld et al., 1995). Alternatively, the elevated corticosterone concentrations in *Mycoplasma*-infected house finches could be because disease symptoms like impaired vision is a stressor. In a previous study, a tendency of elevated corticosterone concentrations were found in free-ranging house finches infected with coccodia (Duckworth et al., 2001). In other studies, we have found that the *Mycoplasma* infection can change several aspects of house finch behavior. Infected birds are associated with smaller feeding flocks, have longer feeding bouts and become more inactive (Hotchkiss et al., in press; Kollias et al., 2004). Because corticosterone concentrations were elevated in birds with *Mycoplasma* symptoms, we suggest that these behavioral changes could be mediated by corticosterone. Increased corticosterone concentrations could also have caused the increased heterophil to lymphocytes ratios (H/L) that have been recorded in *Mycoplasma*-infected birds (Davis et al., 2004).

We analyzed differences in corticosterone concentrations to determine if seasonal modulations of the stress response could influence *Mycoplasma* disease dynamics in this study system. We found that corticosterone concentrations showed a clear increase in February, when also the infection prevalence commonly increases (Altizer et al., 2004). Baseline and stress-induced concentrations changed in parallel, and it is not evident how such a change would affect the immunity of house finches. Baseline and stress-induced concentrations of corticosterone can have different effects on the immune system because they bind to different types of receptors (Romero, 2004). Thus, even though the increased stress-induced corticosterone concentrations could act to down-regulate immunity, the increased baseline levels that accompanied this change could in fact activate the immune system. This pattern was interesting, and further studies are needed to evaluate the effect of the observed changes in corticosterone concentration on the *Mycoplasma* infection.

Results from theoretical models have shown that the observed changes in *Mycoplasma* prevalence in house finches can be theoretically predicted by considering the seasonal changes in the number of susceptible individuals, social aggregation and the immunity of the house finch populations (Hosseini et al., 2004). Thus, if the observed up-regulation of the stress-response represents a down-regulation of immunity, with resources being allocated away from the immune system to meet other physiological demands, this could have important influence on disease dynamics.

So far, we do not know how an activation of the HPA axis affects *Mycoplasma* infections in house finches. In another study on house finches, treatment with the synthetic glucocorticoid dexamethasone was found to enhance viremia and prolong the infective period for two types of avian virus infections (Reisen et al., 2003). A suppression of the immune system could affect *Mycoplasma* growth in house finches both by a general increase in infection susceptibility or by reactivating latent infections. From laboratory studies where house finches have been experimentally infected with *Mycoplasma*, we know that birds that have recovered from the infection develop only partial immunity, and the majority of birds develop symptoms of disease after re-exposure (Sydenstricker et al., manuscript).

A challenge for future research will be to examine how interactions between the stress hormones of the

HPA-axis and the immune system will influence disease transmission. The *Mycoplasma* infection in house finches could provide one example of a wildlife epidemic where seasonal hormonal changes could play an important role for disease dynamics.

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