White blood cells as indicators of adult condition and parental effort in European starlings, *Sturnus vulgaris*

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Abstract

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Parental care requires that substantial energy be allocated towards offspring that would potentially be used to maintain the adult's own health. Female birds tend to provide most of the care, but a male's assistance increases fledging success. The stress of parental care can take its toll on the bird with decreases in body condition and potential changes in parental effort. White blood cells (WBC) tend to increase in response to both stress and disease, and a high heterophil/lymphocyte (H/L) ratio in particular indicates stress in birds. This study aims to examine how WBC counts and H/L ratios vary with adult condition and parental effort in breeding European starlings (*Sturnus vulgaris*). European starlings are a facultatively polygynous passerine that typically exhibits social monogamy and bi-parental care. It was predicted that both WBC counts and H/L ratios would be negatively correlated with adult condition. It was also examined whether these two measures of stress were correlated with parental effort. WBC counts were not correlated with body condition or parental effort, but males tended to have lower counts than females. Males also tended to have lower H/L ratios than females. H/L ratios were negatively correlated with body condition in both sexes. H/L ratios were also not correlated with most forms of parental effort, but female H/L ratios tended to be negatively correlated with clutch size.

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Introduction

All organisms undergo fluctuating levels of stress throughout their lives as they struggle to obtain resources to survive and reproduce (Batt et al., 1992). Animals that exhibit some degree of parental care are particularly vulnerable to increased stress during the breeding season (Bókony et al., 2009). They are already pushing their physiological limits to ensure the survival of both themselves and their young, and expend significantly more energy at this time than during the non-breeding season (Bókony et al., 2009).

In birds, breeding is one of the most costly activities in their annual cycle, comparable only to molting and migration (Gill, 2007). Consequently, breeding individuals are more vulnerable to stress and disease than are non-breeding individuals (Gill, 2007). Adults must find their optimal working capacity, balancing parental care with self-care (Daan & Drent, 1980). Males will remain with their female partners to rear offspring if the benefits outweigh the cost of parental care (Krohne, 2001). In socially monogamous passerines, both a male and female typically work together in raising the offspring; this biparental care is considered essential to their reproductive success (Krohne, 2001). Studies have shown that in socially monogamous species, the efforts of a single parent are generally insufficient to providing offspring care, resulting in a reduced fledging success (Hall, 1999; Krohne, 2001).

In avian species with biparental care, females typically provide more parental care than do males (Krohne, 2001; Gill, 2007). As such, they are more likely to incur higher stress levels. Norris et al. (1994) showed that female great tits (*Parus major*) were often more susceptible to infections than were males due to the extra energy they expended on parental care.

There is typically visual evidence when a bird is infected with a disease, but examining them externally does not always provide the full picture. Occasionally, the birds harbouring those types of infections will appear healthy for a time while the agent of disease undergoes a latent period, as was found in waterfowl (Shawky & Schat, 2002). Internal examinations of the gut contents and organs are often more conclusive in discerning disease (Davis et al. 2008), but result in the organism's death. Sampling blood however is less invasive, relatively easy to do, and its components are an excellent indicator of a bird's overall health (Maxwell, 1993; Davis et al. 2008).

Leukocytes are otherwise known as white blood cells (WBCs). They typically increase in response to the presence of infections and disease, as reviewed by Maxwell (1993). WBCs also fluctuate in response to non-infectious stress such as malnutrition and environmental constraints in the form of climate change, habitat degradation, and pollution (Batt et al., 1992; Maxwell, 1993). Breeding birds often have significant increases in WBCs during this stressful period (Circule et al., 2012, Maxwell, 1993). Breeding adults with high WBC counts tend to be in poor body condition, likely due to breeding stress, as reviewed by Gladbach et al. (2010). Adult body condition and plumage brightness have been shown to decrease during the nestling period (Bryant, 1988; Daan & Drent, 1980). It has also been shown that breeding female birds with high WBC counts tend to lay smaller eggs and have decreased clutch size (Gladbach et al., 2010; Bókony et al., 2009). Therefore, WBC counts are a good indicator of the stress and health of an organism, as well as parental investment. However, leukocytes increase rapidly in the bloodstream in response to stress, within minutes to an hour. It is critical that blood be sampled quickly after capture, and that the sampling procedure be the same for all individuals (Davis et al. 2005).

Although the vast majority of the literature suggests that WBC counts are indicative of phenotypic condition (Maxwell, 1993; David et al., 2008; Gross & Siegel, 1983), it is not always the case. Krams et al. (2012) found that there was no correlation between body condition and the fluctuation of WBC profiles. Siegel (1980) found that some birds may have an increase in WBC counts as a preventative measure for future disease, if they are already undergoing stressful conditions. However, this was the only study to have found this. Adult birds cannot afford to become ill during breeding season with so many energy demands to meet (Krams, 2012). However, this preventative measure of increasing WBC numbers actually tends to lower resistance to certain diseases, despite the fact that it can provide temporary energy and increase specific defenses (Siegel, 1980).

There is also an abundance of literature suggesting that WBC counts are correlated with avian parental care (Daan & Drent, 1980; Bókony et al., 2009; Gladbach et al., 2010). How it is correlated depends heavily on how beneficial the life history trade-offs are in the bird’s situation, and they will alter their behaviour to ensure highest possible fitness (Bókony et al., 2009; Gladbach et al., 2010). Species with longer life spans will have greater fitness if they focus on their own survival as opposed to that of their nestlings, since they will have many attempts to breed in the future (Daan & Drent, 1980; Bókony et al., 2009). This would likely lead to a decrease in parental care, such as offspring provisioning rates, as stress and WBC counts increase. It could also lead to a decrease in the number of offspring per breeding attempt, potentially lowering the bird's reproductive success. However, those species with shorter life spans or that do not have an annual breeding cycle will focus on ensuring nestling survival, which may lead to a positive correlation between WBC counts parental care indicators like offspring provisioning rates (Daan & Drent, 1980; Bókony et al., 2009).

WBCs consist predominantly of heterophils (H) and lymphocytes (L), which are particularly in tune to changes in stress and health of the bird (Harmon, 1998; Krams, 2012). Their combined numbers tend to make up between 70-90% of all circulating leukocytes in the blood stream (Maxwell, 1993; Harmon, 1998). Other primary leukocytes include monocytes, eosinophils and basophils, which are all granulocytes (Maxwell, 1993). Monocytes are large and stored in the spleen (Stabler et al., 1994), eosinophils deal with allergenic foreign matter (Maxwell, 1987), and basophils are the least common leukocyte which deal with ectoparasites (Maxwell, 1995). Heterophils are the avian equivalent of the mammalian neutrophil and serve essentially as defenses against microbial pathogens (He et al., 2003). They are granulocytes, and tend to cluster within inflamed tissue where they use phagocytosis to destroy foreign antigens (Harmon, 1998). Lymphocytes are agranulocytes that vary in size, and are composed of natural killer cells used to combat tumors and viruses (Schat et al., 2006). Heterophils tend to increase with stress, while lymphocytes tend to decrease (Gross & Siegel, 1983). Birds will tend to have high H/L ratios in high stress conditions (Gross & Siegel, 1983).

European starlings (*Sturnus vulgaris*) are a passerine bird that is highly robust and behaviourally complex (Latham & Latham, 2011). It is not native to North America, but has found its place in urban areas throughout the continent, thriving to the point of being abundant while not overly problematic (Latham & Latham, 2011). Starlings have done so well in their new range because they are lacking natural predators, competition, and parasites (Torchin et al., 2003). They are small, scavenging birds with a mixture of purple and green plumage that is often iridescent on many parts of the body, serving as a mechanism for mate choice (Feare 1984). Starlings are often labelled as facultatively polygynous, but are typically social monogamous (Sandell et al. 1996). This simply means that both parents incubate the eggs and care for their offspring (Gill, 2007). However, males tend to provide less care overall, and females tend to contribute more to feeding the nestlings (Feare, 1984). Females lay approximately 3-7 eggs per clutch, and nestlings fledge when they are 21-24 days old (Higgins et al., 2006). Adults typically have two broods over the nesting period, but do not necessarily remain together for the second brood (Higgins et al., 2006). Starlings are ideal as a model species for examining avian stress levels during the breeding season; they are common, robust and easy to handle.

There were three main objectives to this study. The first objective was to determine if WBC counts were correlated with adult body condition and offspring provisioning rates in European starlings. Second, I also determined whether H/L ratios were correlated with either adult condition, offspring provisioning rates, or other indicators of parental effort (see methods). It was predicted that there would be a negative correlation between WBC counts and body condition. Similarly, I predicted that H/L ratios would be negatively correlated with condition. I did not predict how WBC counts or H/L ratios correlated with provisioning as there could be two possible outcomes: either the adults provisioned less due to their high stress levels, or their high stress levels were due to their high provisioning effort. The third objective was to determine if males and females differ significantly in either their WBC counts or H/L ratios. I predicted that males would have lower WBC counts and H/L ratios than females, as they tend to provide less care to their offspring (Feare, 1984).

Methods

Fieldwork

This study was conducted on the Saint Mary's University campus in Halifax, Nova Scotia, Canada (44º 37' 54.07" N, 63º 34' 47.09" W) between May and August 2012. Both first and second brood adults were included in this study. Nest boxes were placed on trees throughout the campus in early 2007. Adult starlings were caught in the nestbox with hinge traps (Stutchbury & Robertson, 1986) when provisioning their offspring. All adults were captured between days 5-12 of nestling period (or nestling development), where hatch day was considered to be day 0 (Clemmons & Howitz, 1990). Capturing adults any earlier could cause them to desert their nests and any later than 12 days of age could result in premature fledging of the young. This one week window to capture adults ensured that they were all undergoing similar breeding stress. The gender of adults was determined by the colour at the base of the mandible, which is blue in males and a light pink in females (Kessel, 1951; Feare, 1984). Females also have a ring around their iris (Kessel, 1951; Feare, 1984). Adults were then given a unique combination of coloured leg bands according to their gender, as well as a Canadian Wildlife Service band for identification. There were 24 females and 13 males caught, though not all individuals were able to be used for every test (i.e. three females were not bled).

Mass and tarsus length were taken for each adult to calculate their condition index. Mass was taken with a pesola spring scale to the nearest 0.5g. Three to five right tarsal measurements were taken with digital calipers (to the nearest 0.1mm), and an average was calculated. Condition was calculated by running a regression for adult males and females of mass against tarsal length. The residuals generated were used as an index of their body condition, where data points above the resulting line were considered adults in good condition and those below the line were in poor condition (Schulte-Hostedde et al., 2005).

Between 50-100μL of blood was sampled from the brachial vein of adults for DNA analysis for a different study. One drop of this blood was used to make a smear on a microscope slide, and two smears were made per individual. A smear was made by lightly pushing the blood drop along the microscope slide with another slide, creating a uniform distribution. The blood samples were taken immediately after banding and tarsal length measurements were done, within five minutes of capture. All adults were processed in this way to ensure the results were comparable. These blood smears were air dried for 2 hours before being fixed in 95% methanol. They were stained a few days later using a Hema III nuclear staining agent. This stain is preferred when heavy nuclear staining and details within the cytoplasm are required (Cole, 1943). Once dry, the slides were mounted with Permount to increase resolution for microscopic examination.

Offspring provisioning effort was considered the primary indicator of parental effort. Provisioning was indicated by the number of trips made to the nestbox by each parent per hour, divided by the number of nestlings in that brood. Provisioning watches were done on each nest on days 7-8 and 13-14 of the nestling period, over a one-hour period between 700h and 1100h. A final provisioning value was calculated for each parent by taking the average of days 7-8 and 13-14. Poor weather conditions do not tend to affect the offspring provisioning rates of starlings (Feare, 1984), but there were two days allotted to each watching period in case of storm conditions.

The observations were conducted with binoculars at approximately 10-30 metres away from the nest boxes to avoid disrupting the behavioural patterns of the adults. Sex of the provisioning adult was noted by their banding combination. Overall provisioning effort at the nestbox was recorded for each of male and female adults. Eight adults (2 males and 6 females) with blood smears and body condition measurements did not have any provisioning recorded, and were therefore excluded from provisioning statistical tests. Other measures of parental effort included clutch size, number of offspring fledged and proportion of offspring fledged. These were only tested for correlation with H/L ratios, and both first egg date and clutch size were only examined for females since they lay the eggs.

Microscope Analysis

The smears were examined using a Leitz Laborlux D light microscope under two different magnifications. First, 400x magnification was used to scan the slide and complete an overall white blood cell count per 10,000 red blood cells (Maxwell, 1993).

Oil immersion under 1000x magnification was used to identify the different types of the first 100 of these leukocytes (Maxwell, 1993). The identification process took approximately 1-2 hours per slide.

Granulocytes tend to be round in shape, with granules filling their cytoplasm (Stabler et al., 1994). Heterophils are the most common (Stabler et al., 1994). Once stained with Hema3, heterophils have colourless cytoplasm and small rod-shaped granules accompanied by a lobe nucleus (Maxwell, 1993). Eosinophils have cytoplasm that appears as pale blue, with round granules that stain bright pink or red, and which are larger than those of the heterophils (Maxwell, 1987). Basophils are the rarest of the granulocytes, and have a central nucleus that is generally obscured by their many large granules which stain dark blue or purple (Maxwell, 1995). The non-granulocytes are easier to identify. Monocytes can be distinguished by their large size and kidney-shaped nucleus. They tend to have an irregular shape and granular cytoplasm which can stain darkly (Stabler et al., 1994). Lymphocytes are common and are found in all sizes (Maxwell, 1993). They can be round or irregular in shape, each with a wide nucleus that leaves only a thin layer of cytoplasm around it (Maxwell, 1993).

Statistical Analysis

All data were analyzed using GraphPad Prism software (version 5.04). Data sets were tested for normality using a D'Agostino and Pearson omnibus normality test. Normally distributed data were analyzed with parametric statistics that included Pearson correlations. For those that were not normal, non-parametric Spearman correlations were used. All tests were two-tailed and results were considered to be significant when $P \leq 0.05$. WBC counts for males and females were compared via a Mann-Whitney U test to determine if there was a significant difference between sexes. WBC counts were plotted against adult body condition indices separately for males and females to determine if there was a correlation between the two. The same was done for WBC counts and offspring provisioning rates.

H/L ratios for males and females were compared in the same manner as the overall WBC counts. H/L ratios were plotted against condition to determine if there was a correlation for each of males and females. The same was done for provisioning and H/L ratios. Only females lay eggs, so female H/L ratios were plotted against both first egg date (only for first brood) and clutch size. Therefore, mean pair H/L ratios vs. number fledged and mean pair H/L ratios vs. proportion of young fledged were both examined, as well as mean pair H/L vs. offspring provisioning rate. It was also examined whether males were more or less likely to pair with females having high H/L ratios.

Results

There was a large range in residuals generated by the regression plot of mass against tarsus length, indicating high variation in body condition for both males and females. Males body condition residuals ranged from -5.32 to 7.07, while females ranged from -5.39 to 7.01. There were 32 adults caught in total, with 14 pairs and 4 singles. Among the four singles there were three females and one male, all late brood adults. The late brood females all had normal provisioning values (2.25, 2.33, 4.33), but had poor body condition values (-3.89, -3.93, -5.39). However, the late brood male had no provisioning, and his body condition value was 0.121.

WBC Count Correlations

There was no significant correlation found between WBC counts and adult body condition in either males ($r = 0.0731$, $n = 13$, $P = 0.81$) or females ($r_s = 0.3117$, $n = 21$, $P = 0.17$). WBC counts did not correlate with provisioning effort in either males ($r_s = 0.0913$, $n = 11$, $P = 0.80$) or females ($r_s = 0.1000$, $n = 16$, $P = 0.71$). There was also no correlation between body condition and provisioning effort for males (rs = -0.2557, n = 11, P = 0.45) or females ($r = -0.1990$, $n = 18$, $P = 0.43$). Offspring provisioning rates ranged from 0.125-3.25 in males and 1.5-4.33 in females. Males tended to have lower WBC counts than females, and with less variation among the counts (Mann-Whitney U = 89.5, $n_1 = 13$, $n_2 = 21$, $P = 0.10$; Figure 1). Females had more variable WBC counts than did males.

H/L Ratio Correlations

H/L ratios and adult body condition were significantly negatively correlated in both males ($r = -0.8325$, $n = 13$, $P = 0.0004$; Figure 2) and females ($r = -0.7384$, $n = 21$, $P = 0.0001$; Figure 3). However, H/L ratios were not significantly correlated with provisioning effort in either males ($r_s = 0.1050$, $n = 11$, $p = 0.75$) or females ($r = 0.1595$, $n = 16$, $P = 0.55$). Female H/L ratios tended towards a significant correlation with clutch size ($r = -0.3728$, $n = 21$, $P = 0.096$; Figure 4). Mean pair H/L ratios were not correlated with either the number of young fledged ($r_s = 0.0329$, $n = 10$, $P = 0.95$) or the proportion of young fledged ($r_s = -0.1953$, $n = 10$, $P = 0.58$). Mean H/L for each pair was also not correlated with a pair's total provisioning effort per nestling ($r_s = 0.3222$, $n = 10$, $P = 0.37$). Males with high H/L ratios were not more likely to pair with females based on female H/L ratios ($r_s = 0.4788$, $n = 10$, $P = 0.17$). Males tended to have lower H/L ratios than females ($t = 1.726$, $df = 32$, $P = 0.09$; Figure 5). Male H/L ratios also varied less than those of females (Mean $\pm$ SE = 0.84 ± 0.20 vs. 1.47 ± 0.26 respectively).

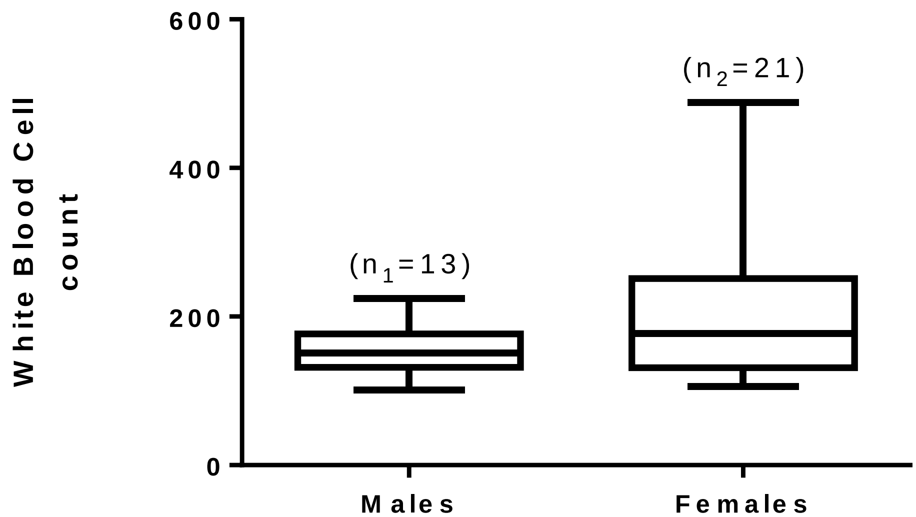

Figure 1. Comparison of male and female WBC counts per 10,000 erythrocytes (U = 89.5, P = 0.10). Sample size is in brackets.

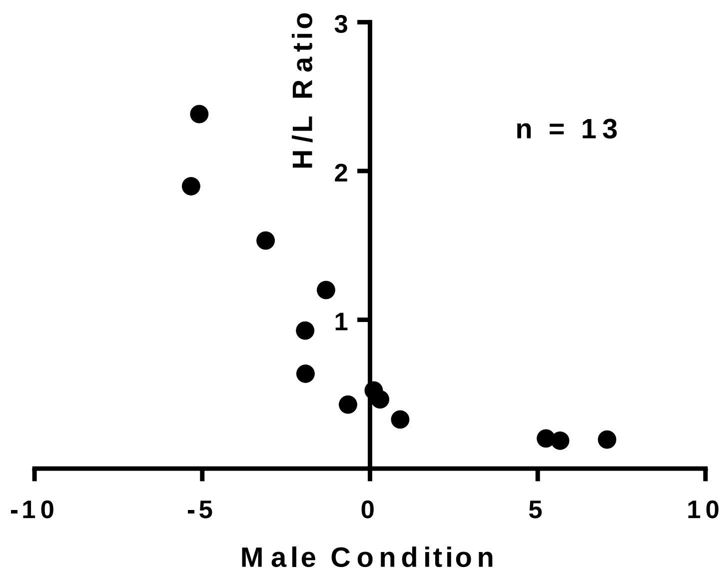

Figure 2. Male body condition plotted against H/L ratios (r = -0.8325, P = 0.0004).

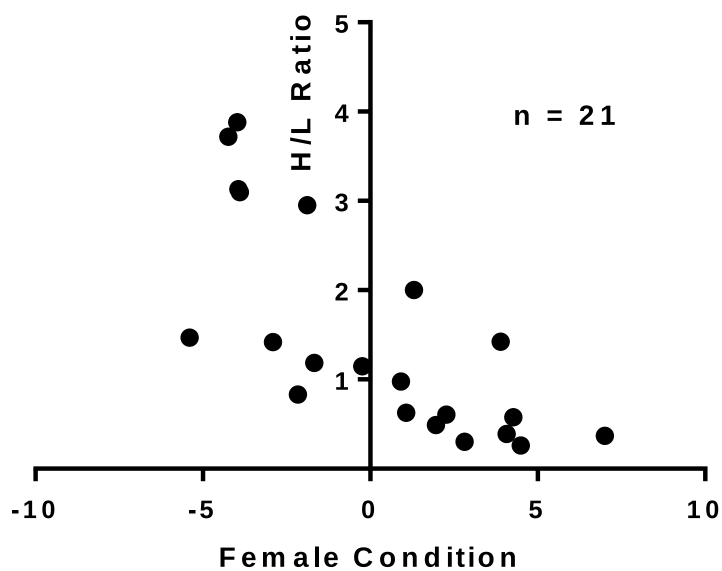

Figure 3. Female body condition plotted against H/L ratios (r = -0.7384, P = 0.0001).

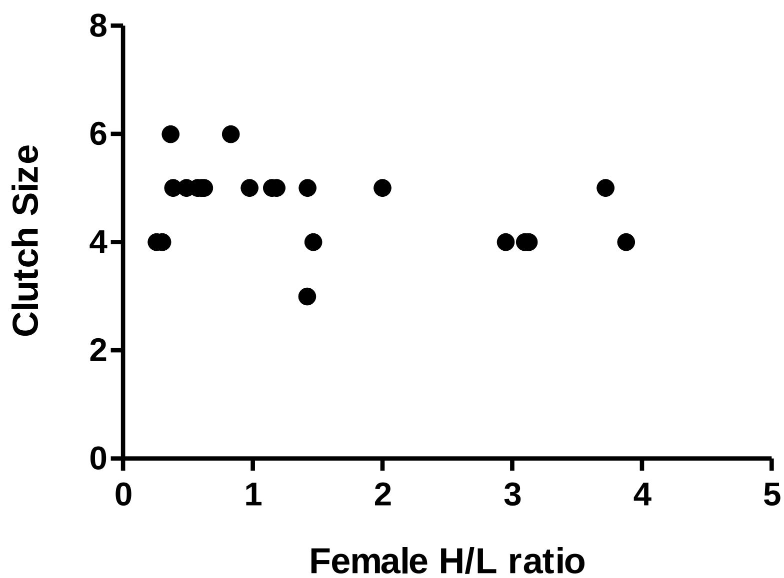

Figure 4. Female H/L ratios plotted against clutch size ($r = -0.3728$, $n = 21$, $P = 0.096$).

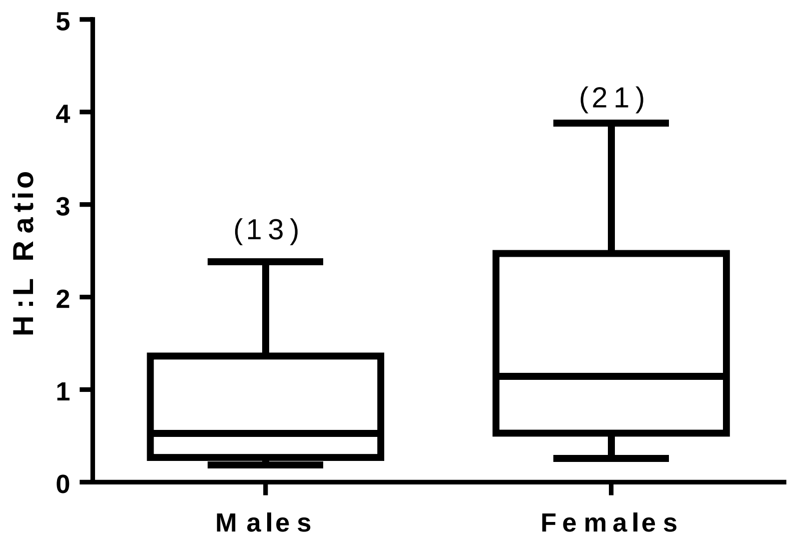

Figure 5. Comparison of male and female H/L ratios (Mean $\pm$ SE respectively: 0.84 $\pm$ 0.20 vs. 1.47 $\pm$ 0.26; $t = 1.726$, $df = 32$, $P = 0.09$). Sample size is in brackets.

Discussion

Overall WBC counts per 10,000 red blood cells were not correlated with adult body condition for either male or female European starlings. In Maxwell's (1993) review, it was documented that WBC counts tended to be negatively correlated with avian body condition, but that this is not always the case. Davis (2005) found a decrease in WBC counts with increased body condition, and some studies disregard overall WBC counts entirely and focus instead on the more specific H/L ratios (e.g. Shutler et al., 2004; Cirule et al., 2012; Ilmonen et al., 2003). For this study, the lack of correlation between WBC counts and adult condition may have been due to a threshold of condition beyond which an individual is unable to breed (e.g. Smart et al., 1968). If an adult is severely stressed or unhealthy, it is unlikely they will have the energy to procure a mate and care for offspring. Non-breeding adults were not included in this study, as only breeding individuals were captured. It is possible that overall WBC counts were higher than normal in 2012 due to the construction taking place on the Saint Mary's University campus during the breeding season. However, there appears to be few published studies on overall WBC counts in breeding wild passerines, so I am unable to make any conclusions on how the WBC counts found in this breeding population compares with other populations or other species.

H/L ratios and adult body condition were negatively correlated in our population of European starlings, supporting my prediction. It is now well-established that H/L ratios are positively correlated with stress in birds (Gross & Siegel, 1983; Vleck et al., 2000; Gladbach et al., 2010; Cirule et al., 2012). Therefore, the adults in our population having high H/L ratios were likely to be more stressed, and were also in significantly poorer condition than adults with lower H/L ratios. H/L ratios are a more reliable indicator of stress than are overall WBC counts, which can often be time and method sensitive (Davis, 2005; Gross & Siegel, 1983). H/L ratios are less susceptible to these two factors (Gross & Siegel, 1983). Therefore, H/L ratios are much more descriptive about the state of an individual's health and condition than WBC counts (Cirule et al., 2012).

Ilmonen et al. (2003) examined H/L ratios of the European pied flycatcher (*Ficedula hypoleuca*), a small passerine similar in size to the European starling. They noted a significant increase in H/L ratios when stress was experimentally increased by increasing brood size. The range of H/L ratios in this study is comparable to that found by Ilmonen et al. (2003), but are substantially higher than those found by Cirule et al. (2012) in Great tits (*Parus major*). Both my study and that of Ilmonen et al. (2003) examined H/L ratios of breeding adults, while Cirule et al. (2012) studied H/L ratios in wintering adults. It is possible that breeding is a more stressful activity than wintering in the annual cycle of passerines.

Neither WBC counts nor H/L ratios were correlated with offspring provisioning rates in European starlings. There are also few published studies examining the possible correlation between either WBC counts or H/L ratios and offspring provisioning. It is possible that stress does not tend to affect offspring provisioning because parents may put their all into ensuring nestling survival, if it is more beneficial for them to do so. Offspring provisioning comes at a cost to the parent's future survival, but once the nestlings have reached a certain age it is possible that it would be more reproductively costly to abandon the nest. For this study, provisioning watches began on day 7.

In this study, late brood female body condition was poor, whereas their offspring provisioning rates were average to high. This is likely because all of the late brood females were single and needed to provide for their nestlings on their own. They had to work harder than a paired female, and thus their body condition decreased as a result of their increased provisioning values. However, the late brood male was in average condition and had no provisioning value. Male European starlings do not tend to provision during the late brood (Feare, 1984). This is another example of why females tend to be under more stress during the breeding season than males.

There are some studies that use other indicators of parental investment, such as clutch size and fledging success (Gladbach et al., 2010; Ilmonen et al., 2003). In this study, female H/L ratios tended towards negative correlation with clutch size. Therefore, as the female's H/L ratio increased, her clutch size decreased. Similar results were found by Gladbach et al. (2010) in their study on Upland geese (*Chloephaga picta*), which are also socially monogamous. They found that both clutch size and egg size decreased with an increase in female H/L ratios. They also noted that females with larger clutch sizes tended to have lower WBC counts. However, fledging success in our population of European starlings did not seem to be correlated with H/L ratios, as was also found by Gladback et al. (2010).

As was predicted, males tended to have lower WBC counts and H/L ratios than females. It is possible that this finding is due to hormonal differences between the two sexes. Breeding males are territorial, and therefore undergo an influx of testosterone, which has been shown to have immune-suppressive effects in birds (e.g. Folstad, 1992; Greives et al., 2006). It is also possible that males simply incurred less stress than females during the breeding season. Both sexes care for their nestlings after they hatch, but it is often females that provide the majority of that care (Krohne, 2001; Gill, 2007). Male European starlings aid in both incubating the eggs and provisioning the offspring, and they also exert significant energy in constructing the nest alone (Feare, 1984). However, it is the females that lay the eggs, and after hatching they tend to provision the offspring more frequently (Feare, 1984), potentially resulting in a higher level of stress in females. This may have caused the higher WBC counts and H/L ratios.

This study has interesting implications that could lead to various possible future projects related to studying the European starling’s leukocyte profile. Since only breeding individuals were caught for this study, it would be beneficial to capture non-breeding adults in a future study. This would provide a comparison of breeding vs. non-breeding WBC data and allow for an evaluation of the potential condition threshold. A study is already being planned to examine the 2012 nestling WBC data to determine if H/L ratio and condition are correlated in first and last hatched nestlings within a brood.

It would also be interesting to compare the effects of different stressors by examining the WBC data. For example, winter is another stressful time for birds (Kessel, 1953; Gill, 2007). Starlings reside close by their breeding sites during the winter season and even begin their singing with snow still on the ground (Kessel, 1953). Comparing the WBC data of wintering and breeding starlings may provide insight into their stress levels during these different times of the year. Wintering and breeding are two of the most stressful times during the annual cycle of birds (Gill, 2007), so it is possible that similar patterns of WBC counts and H/L ratios would be found. However, it was previously found by Cirule et al. (2012) that passerines had substantially lower H/L ratios in winter than during breeding (Ilmonen et al., 2003; Maxwell, 1993).

Stress is often experimentally induced by hormones such as hydrocortisone and the adrenocorticotrophic hormone (ACTH), which are released by animals during times of high stress (Maxwell, 1993). After injection, the proportion of white blood cells changes due to the simulated stress (Maxwell, 1993). This technique has been widely used in the literature, particularly in regards to examining H/L ratios and their correlation with both body condition and social behaviour as reviewed by Gross and Siegel (1985). In that study, the behaviour examined included social stress in females when laying in groups, as well as stress levels when socializing with strange birds instead of familiar ones. Females who mixed with strangers were more stressed than those interacting with familiars (Gross & Siegel, 1985).

ACTH in particular affects heterophils and monocytes, leading to an increase in H/L ratios (Pardue & Thaxton, 1984). A future study could induce stress experimentally on adult starlings using these hormones. Doing so in a lab setting would allow for controls and provide more concrete support for the hypotheses proposed here. It could also be beneficial to examine the red blood cell counts in these future studies, to see how they vary with stress in comparison with WBC counts, as the literature suggests they tend to increase as well (e.g. Davis, 2005; Maxwell, 1993).

In conclusion, it has been shown by this study that breeding European starlings in poor condition have high H/L ratios. This significant negative correlation indicates the high level of stress and health risks that breeding inflicts upon adult birds. Not only does stress affect the individual adult, but it was also shown by this study to decrease clutch size, as indicated by the negative correlation between female H/L ratios and clutch size. It was interesting to compare this study with those on other avian species. Starlings are an introduced species that have proliferated here in North America, which makes them an important study species. It is necessary for us to look more closely at these behaviourally complex birds to understand how they have adapted to their new environment, and how they compare to native species. Some may consider starlings invasive or pests, but in truth they are beautiful and talented animals with such personality. It has been a pleasure to study them and to contribute to the fairly small niche of research about them coming from Canada.

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Appendix A. Summary of adult male statistics.

Nestbox	Band #	Residuals	WBC counts	H/L	Prov.
1 (EB)	872-15839	7.071246	184	0.197	
3 (EB)	872-15813	5.672212	103	0.188	2
4 (EB)	1272-10106	-3.110079	101	1.533	2.9
5 (EB)	942-94847	5.248355	158	0.205	3
6 (EB)	942-94829	-5.328209	156	1.9	2.833
8 (EB)	942-94836	0.9035963	148	0.33	2.708
9 (EB)	942-94848	-5.081888	169	2.384	2.125
11 (EB)	872-15996	0.3009434	187	0.467	2.125
16 (LB)	1342-36793	0.1217263	224	0.526	
17 (EB)	942-94830	-1.303392	126	1.2	0.125
39 (EB)	1292-04892	-1.928754	137	0.928	3.25
41 (EB)	942-94828	-1.919294	151	0.64	2.375
45 (EB)	942-94833	-0.646463	144	0.431	2

Appendix B. Summary of adult female statistics.

Nestbox	Band #	Residuals	WBC Counts	H/L	Prov.	Clutch size	Fledge	Prop Fledge
1 (EB)	1292-04830	7.008841	160	0.369		6	5	0.83
2 (LB)	1342-36773	-3.903139				4	3	0.75
3 (EB)	1292-04858	0.1088954			3.125	5	4	0.8
4 (EB)	942-94846	-2.155708	137	0.833	2.22	6	5	0.83
5 (EB)	1342-36731	4.497213	488	0.261	2.166	4	3	0.75
6 (EB)	942-94874	-3.973446	167	3.88	2.708	4	3	0.75
8 (EB)	1292-04891	0.9209571			2.125	3	3	1
9 (EB)	942-94834	-0.243863	217	1.15	4.25	5	4	0.8
11 (EB)	942-94879	-1.667469	119	1.185	1.875	5	4	0.8
12 (EB)	1292-04891	0.921	207	0.977	3.65	5	3	0.6
17 (EB)	1272-10152	1.956112	263	0.489	4	5	4	0.8
18 (EB)	942-94835	-1.879615	154	2.95	3	4	2	0.5
18 (LB)	1342-36751	-2.908546	177	1.42	3.5	3	2	0.67
19 (EB)	942-94831	1.309142	232	2		5	0	0
26 (EB)	1342-36773	2.814409	306	0.303		4	0	0
31 (EB)	942-94832	-4.243997	118	3.72		5	0	0
37 (EB)	1292-04887	4.073879	109	0.388	2.2	5	4	0.8
37 (LB)	1342-36712	-3.937887	178	3.13	4.33	4	3	0.75
38 (EB)	1342-36772	4.273578	125	0.576		5	0	0
39 (EB)	942-94827	3.897297	248	1.423	1.5	5	4	0.8
41 (EB)	1292-04820	1.067927	254	0.625	1.625	5	4	0.8
42 (LB)	1342-36730	-5.39678	106	1.468	2.25	4		
43 (LB)	942-94888	-3.891243	255	3.1	2.33	4	3	0.75
45 (EB)	942-94826	2.273445	176	0.608	3.2	5	5	1