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Sex Ratios and the City: Is parental corticosterone a mediator of the relationship between urbanization and offspring sex allocation?

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Overview

When and how mothers adjust the sex ratio of their broods has puzzled researchers since Fisher proposed his theory of equal allocation in 1930. Since then, several new hypotheses have emerged, including the Trivers-Willard hypothesis, which states that mothers can adjust their sex allocation according to their own condition and the environmental conditions they face during breeding. When testing this hypothesis in the past, research has frequently used avian systems and focused on how natural variation in weather, food availability and quality, and predation pressure impact individual and environmental quality and contribute to shaping sex allocation trade-offs. In the context of global change, we know that anthropogenic effects and urbanization can alter all of the above characteristics, which presents animals with novel challenges in optimizing their brood sex allocation. Urbanization can have positive effects on individual condition, such as milder weather and increased food abundance, as well as negative impacts on condition, including increased noise, light, and chemical pollution. Research has examined how urban living influences individual body condition but has not yet explored whether urbanization shapes offspring sex allocation decisions. Corticosterone (CORT)—the primary glucocorticoid in many vertebrates, including birds—is linked to changes in body condition and may play a role in adaptive sex allocation. Due to its role in mediating energetic trade-offs within the body and linking an organism's external and internal environments, CORT is a possible mechanism that links urbanization and adaptive sex allocation. Previous research has found connections between changes in CORT and body condition for animals experiencing urban environments, as well as links between CORT and sex ratio allocation. However, these relationships are inconsistent across study systems, and to date, no research has examined the links between urbanization, individual condition, CORT, and adaptive sex allocation within the same study. Furthermore, research that has examined CORT's influence on sex ratios and parental condition focused solely on maternal CORT. In species where both parents care for the young, paternal CORT or the similarity of maternal and paternal CORT levels may also play a role in ensuring that offspring demand matches parental condition and care quality.

In Chapter One, I introduce the foundations for this research, including sex allocation theory and the impacts of urbanization on wildlife. Chapter Two reviews the state of prior work on the relationships between urbanization, individual condition, parental hormones, and sex ratio allocation. Using a field-collected dataset, Chapter Three explores whether sex allocation in European starlings (Sturnus vulgaris) differs for animals across an urban to rural gradient. We did not find an effect of urbanization or parental condition on investment in the more costly sex (males). Instead, we found that the similarity of maternal and paternal CORT levels within a breeding pair increased the likelihood of successfully fledging costly sons, suggesting that parental similarity, rather than the condition of any individual parental alone, plays a key role in shaping offspring sex allocation outcomes. Starlings are considered an urban-adapted species, which could explain why breeding under more urbanized conditions did not alter sex allocation in our study. Finally, in Chapter Four, I discuss future considerations for how global change may alter sex allocation in animals and make additional suggestions for future work to expand on my findings.

Chapter One – Background and Relevance

Introduction to Sex Allocation Theory

The study of sex allocation theory is a complex field that has evolved over the years as our understanding of ecology has improved and as the world has changed. We begin with Fisher's equal allocation theory (1930). According to Fisher, parents should invest equally in the sexes to gain the largest fitness advantage. This theory assumes that males and females make equal contributions to the next generation because every offspring has one mother and one father. If there is an excess of sons, they will have few opportunities to mate, which confers a greater fitness advantage to parents producing daughters. On the other hand, if there is an excess of daughters, sons can now have more than one mate, which confers the fitness advantages to parents producing males. As a result, the ratio should be unbiased and balance out to be 1:1, males to females, at the population level. However, if one sex is more costly than the other to produce or raise, the observed population ratio can shift from equal production to equal investment. Parents should invest more in the less costly sex to ensure that overall resource investment is equal between the sexes (Fisher 1930).

While foundational, Fisher's theory did rely on key assumptions that are not always met in nature. Hamilton (1967) was among the first to point out conditions that violated the assumptions of Fisher's equal allocation theory. A significant oversight is that Fisher's theory does not account for within-group interactions that differentially affect the sexes. For example, in species where one sex supports or helps group members more than the other, such as in cooperative breeding or other altruistic behaviors, mothers should produce more of the helping sex as it should increase their direct fitness by ameliorating some of the adverse effects of offspring care. Alternatively, if siblings of one sex are competing for mates within the group, mothers should produce more of the sex that is not competing to alleviate competition and increase the likelihood of their offspring's reproductive success (Komdeur 2012).

Trivers-Willard (1973) further modified this theory by showing that mothers could be selected to adjust the sex of their offspring in response to environmental variation. The costs and benefits of producing each of the sexes can fluctuate under different environmental conditions, leading parents to favor one sex over the other depending upon their current environment and their own intrinsic condition. When mothers are in poor condition (e.g., with poor body condition or facing low food availability), they should invest in the less costly sex, and when they are in better condition, they should invest in the more costly sex. This adaptive sex allocation or bet-hedging could lead to biased offspring ratios within a brood. However, at the population level and through time, these biases should cancel out (Trivers and Willard 1973).

Research often uses avian systems to test these hypotheses. Female birds are the heterogametic sex and can contribute either a Z or W chromosome to the offspring, thus manipulating the primary sex ratio (Navara, Kristen J. 2013). Furthermore, birds represent a wide variety of mating systems and levels of parental care, which provides many situations to study if and when sex ratio biases occur. Finally, because they are oviparous, avian embryos develop independently of their mother. Mothers only have one chance to hedge their bets according to their condition and the environmental conditions. This "sealed bid" provides opportunities to study how the timing of changes in condition or environmental predictability will affect sex ratios and allows researchers to isolate manipulations of the embryo from the mother. Overall, avian systems provide excellent opportunities for testing sex allocation theory.

Impacts of Urban Life

Many different environmental conditions can affect individual condition—including weather, food availability and quality, disturbance frequency, or predator density—and shape offspring sex ratios. For example, common grackles (*Quiscalus quiscula*) bias the sex ratio of their broods towards the less expensive sex early in the season when they experience harsher weather and reduced food availability (Howe 1977). Additionally, lesser spotted eagles (*Aquila pomarina*) increased production of the more expensive sex in years with increased prey availability and rainfall and more of the less expensive sex under poor conditions (Väli 2012) In addition to the variation in environmental conditions that organisms have likely experienced throughout their evolutionary histories, contemporary anthropogenic habitat modifications have also altered the availability of resources for organisms worldwide. Increased human population density on nearly all continents has led to more developed and paved surfaces, increased pollution, decreased natural vegetation, and have altered microclimate, to shift the environmental conditions that organisms face in urban environments compared to rural ones (Seress and Liker 2015)

Living in urban areas can have adverse effects on avian species. Urban areas suffer from several types of pollution, including chemical, noise, and light, which can negatively affect avian health (Seress and Liker 2015; Shanahan et al. 2013). Chemical pollution in the air, soil, and water can change biogeochemical and nutrient cycles and primary production (Pickett et al. 2011). Also, insectivorous birds can suffer the effects of bioaccumulation of heavy metals such as lead (Seress and Liker 2015). Light and noise pollution both alter behavior. Artificial light at night can affect reproductive phenology and daily activity schedules (Deviche and Davies 2014). This altered phenology may be beneficial by creating extended periods of activity for increased foraging opportunities. However, it can also be detrimental due to interactions with competitors and predators that a species may not normally encounter (Seress and Liker 2015; Visser and Spoelstra 2014). Noise pollution masks important acoustic signals which birds use to communicate a wide variety of information. Masking those signals can impact individuals' ability to warn each other about potential threats and attract mates. Eventually, the noise pollution can cause them to modify their song to communicate effectively. Excessive noise can also impact birds' health through increased stress and hearing damage (Gil and Brumm 2013).

Urban environments do not have exclusively adverse effects, however. They tend to have altered meteorological conditions, including increased precipitation and higher temperatures (Berry 2008). The elevated temperature in urban areas, often referred to as the urban heat island effect, results in urban areas being several degrees warmer than rural or forested areas, and urban areas retain heat for longer periods (Pickett et al. 2011). Increased precipitation and temperature can encourage increased vegetation growth or extended growing seasons, which can be beneficial by increasing insect abundance and foraging opportunities. The milder temperatures can also increase overwinter survival, improve post-winter body condition, and reduce annual population size fluctuation. They can also alter vegetation phenology and lead to asynchrony among trophic levels (Seress and Liker 2015), but supplemental food sources available in urban environments may buffer the impacts of this asynchrony.

Many of the factors discussed above have indirect effects on avian species through changes to food abundance and type. However, food provided by humans can also directly affect birds living in urbanized areas. Providing supplemental food, such as seed and suet at feeders, can help improve the nutritional condition of wintering birds, increase body mass, and may increase survival. However, these effects are not always consistent, and researchers have found that urban birds can be leaner than their rural counterparts (Amrhein 2013). The consequences of birds consuming human foods and refuse rather than more natural foods like seeds or insects are varied, but some research has shown that their diet is nutritionally poorer (Ottoni, de Oliveira, Young 2009). While increased availability may improve body condition and survival, it can also increase the spread of infectious diseases, reduce body mass and survival of nestlings, and increase reliance on anthropogenic food sources (Ottoni, de Oliveira, Young 2009; Seress and Liker 2015). Overall, the impact of urbanization on avian species is context-dependent but often acts through altered behavior and physiological changes in individual health and condition.

Glucocorticoids: A Mechanism to Link Environment and Condition

These habitat alterations can impact individual body condition, and one of the primary pathways for these alterations is through glucocorticoids. In birds, corticosterone (CORT) is the primary glucocorticoid, and it serves primarily metabolic functions. It mediates trade-offs within the body, maintains homeostasis, and links an organism's external and internal environments. Activation of the hypothalamic-pituitary-adrenal (HPA) axis produces CORT. Following the release of corticotropin-releasing hormone from the hypothalamus and adrenocorticotropic hormone by the anterior pituitary, the adrenal cortex creates and releases CORT (Schoech, Rensel, Heiss 2011).

We often split CORT concentrations into two categories: baseline and stress-induced. Baseline concentrations are continually circulating within the body and drive the metabolic role of this hormone.

They mediate daily activities and predictable life-history stages (Landys, Ramenofsky, Wingfield 2006). In contrast, stress-induced concentrations arise in response to acute stressors and allocate resources to immediate needs for escape or survival (Landys, Ramenofsky, Wingfield 2006; Sapolsky, Romero, Munck 2000). Under these circumstances, there is a significant increase in circulating concentrations from baseline, including more than 10-fold changes in concentration. This stress-response serves a different purpose than baseline concentrations. It causes the diversion of energy away from routine functions and towards muscle function, the immune system, cognition, and brain activity while inhibiting reproduction and decreasing appetite and digestion (Sapolsky, Romero, Munck 2000). The circulating concentrations affect which receptors CORT binds to, and consequently, the function it serves.

As a result of its diverse effects, CORT plays various critical roles throughout an individual's lifetime. Its exact impacts and concentrations are highly variable and dependent on an array of internal and external contexts but can link together seemingly disparate processes, for example, adaptive sex allocation and urbanization. Due to its role in mediating energy expenditure, CORT is linked to individual body condition (Love et al. 2005a) and external food availability, and in turn both body condition and food availability can shape sex allocation (Trivers and Willard 1973). As a result, researchers have proposed that CORT may play a direct role in sex allocation (Navara 2013). The challenges presented by urban environments are related to changes in CORT concentrations (Bonier 2012) and body condition, but these urban challenges have not been linked to sex ratio biases. While differences in CORT are a likely mediator of the relationship between urbanization and sex allocation, this relationship has not yet been explored.

Statement of Problem/Purpose

While many studies have examined segments of the relationship between CORT, body condition, urbanization, and sex ratio biases, none have explored all of these elements within the same study system and at the same time. My thesis addresses the following questions:

(1) Do European starlings (*Sturnus vulgaris*) vary their secondary sex ratio across an urban to rural gradient?

(2) Do maternal or paternal body condition and/or corticosterone levels differ along an urban to rural gradient and reflect differences in the sex ratio of broods?

(3) Is the similarity of paternal and maternal body condition/corticosterone levels reflected in the sex ratio of the brood?

Due to the increased number of stressors associated with urban living, we predict that urban parents would produce a higher proportion of daughters than rural ones. We also predicted that there would be a positive correlation between maternal or paternal CORT and the proportion of daughters produced and a negative correlation with maternal or paternal body condition regardless of habitat. Finally, we expected that pairs that are more similar in their condition and/or CORT would produce a lower proportion of daughters. By integrating physiological mechanisms with ultimate changes in brood sex allocation across an urban to rural gradient, we hope to draw new conclusions about the impacts of urbanization on avian species and learn how this urban adapter flourishes under anthropogenic conditions.

Chapter Two - Literature Review

Several studies have evaluated the effects of living in urban environments on the body condition of birds. While some researchers have not found a relationship between body condition and urbanization (Foltz et al. 2015), others have found both positive and negative effects on body mass and condition. With consistent access to anthropogenic food, we might predict that urban individuals would be in better condition. Auman *et al.* (2008) found that adult male silver gulls (*Larus novaehollandiae*) in urban areas had greater body mass and were in better condition than male gulls from rural areas, but the relationship was absent in female silver gulls. Conversely, when studying a passerine commonly found in urban areas, the house sparrow (*Passer domesticus*), many researchers have found a negative relationship between body mass and urbanization (Bókony et al. 2012; Liker et al. 2008; Meillère et al. 2015; Strubbe et al. 2020).

It has been challenging to find a consistent relationship between body condition and urbanization. Body condition is often measured in the literature using a scaled body mass index where body mass is divided by some measure of body size, such as tarsus length. Some researchers have found no difference in scaled mass index between urban and rural house sparrows (Bókony et al. 2012; Meillère et al. 2015). Others have been able to identify a negative relationship between the amount of urbanization and scaled mass index, meaning that birds in more urbanized areas were both smaller, leaner, and in worse body condition (Liker et al. 2008; Strubbe et al. 2020). Regardless of their competing findings on the relationship between body condition and urbanization, researchers have been able to agree that the smaller body size is probably not due to adult nutritional stress, reduced access to food, or short-term responses to high food predictability, but rather to urban habitats providing inadequate nutrition during nestling growth and development (Liker et al. 2008; Meillère et al. 2015). Other studies have supported this conclusion. For example, researchers found urban European starling (*Sturnus vulgaris*) nestlings smaller in body mass at day six and day twelve than suburban nestlings (Mennechez and Clergeau 2006). While it is difficult to conclude the direct effects of urbanization on adult body mass or condition, it does seem to impact offspring size and quality.

The relationship between urban environments and adult body condition remains equivocal, and as a result, any physiological mechanisms behind this relationship also remain unknown. Fluctuations in baseline CORT concentrations are a possible cause. Baseline CORT concentrations manage energy allocation and mediate trade-offs between investment in reproduction and survival (Patterson et al. 2014a) Most prior studies have attributed the smaller offspring size of urban birds to inadequate nutrition through a poor quality diet, but it could also be decreased parental provisioning (Liker et al. 2008; Meillère et al. 2015; Mennechez and Clergeau 2006) If urban life is more energetically challenging, baseline CORT levels may be altered during the breeding season to decrease investment in reproduction through reduced egg and offspring size and reduced parental care activities like provisioning nestlings. A recent meta-analysis found that the relationship between CORT concentrations and urbanization depended on several factors such as life-history stage and urban adaptability and was inconsistent across species (Injaian et al. 2020).

Some studies investigating adult body condition along the urban to rural gradient have included CORT concentrations in their analyses with mixed results. Researchers found a decrease in body mass but not condition in house sparrows and found no relationship between CORT levels and urbanization (Bókony et al. 2012). However, the researchers sampled the birds during the non-breeding season, obscuring the relationship between reproductive investment, body condition, and urbanization. During a longer-term study over four years, researchers did not find a consistent relationship between urbanization, CORT, and body condition but did find that both CORT and body condition varied between habitats during some years (Foltz et al. 2015). Finally, researchers found that body mass, body condition, and feather CORT (which provides a retrospective view on HPA axis activity) were inversely related to the amount of built-up area. However, these relationships were detected at different spatial scales of analysis, and, as a result, it may be problematic to relate them (Strubbe et al. 2020). Establishing a clear connection among CORT, body condition, and urbanization is challenging, but we should not discount the role the CORT may be playing in translating or mediating the impacts of urbanization on nestlings.

Changes in maternal CORT can affect nestling growth and development during both pre- and post-natal stages. Mothers deposit CORT and other steroid hormones into the yolk and albumen during egg production (Downing and Bryden 2008; Engelhardt and Groothuis 2005; Navara, K. J. and Pinson 2010; Schwabl 1993). As offspring absorb the yolk and albumen, the CORT they contain is also absorbed and can significantly impact nestling development. For example, elevated CORT in the egg has been shown to lead to a later hatch date, decreased the rate and loudness of embryonic vocalizations (Rubolini et al. 2005), and decreased embryonic growth (Parolini et al. 2019) in yellow-legged gulls (*Larus michahellis*), lower hatchability and mass at hatching in barn swallows (*Hirundo rustica*) (Saino et al. 2005) and house wren (*Troglodytes aedon*) nestlings that were lighter at hatching but were ultimately able to compensate for their decreased hatch weight before fledging the nest (Strange et al. 2016). Elevated pre-natal CORT can also shape begging activity, growth rate, HPA axis development, and immune function (Bowers et al. 2016; Hayward and Wingfield 2004; Rubolini et al. 2005; Tilgar et al. 2016). Researchers believe that this embryonic CORT exposure could have a “programming” effect on offspring, shaping the young’s behavior or physiology into adulthood to better match them with the environment they will experience (Schoech, Rensel, Heiss 2011).

One way in which this matching could occur would be through a biased sex ratio that would match offspring needs with maternal care quality. Several studies have examined the relationships between CORT, maternal condition, and adaptive sex allocation. To explore the relationship between maternal condition and sex ratio in lesser black-backed gulls (*Larus fuscus*), Nager *et al.* (1999) experimentally manipulated maternal condition. They found that the sex ratio at hatching was female-biased in lower quality mothers but that supplementary feeding removed the bias. They also found that survival to fledging decreased in male offspring from poorly provisioned broods. Their results support adaptive changes to both the primary and secondary sex ratio in relation to maternal condition because female offspring in the species are more likely to survive and reproduce in the future (Nager et al. 1999). In a study on a cooperatively breeding species, the superb starling (*Lamprotornis superbus*), researchers also found an adaptive relationship between maternal condition and secondary sex ratio and extended their analysis to include environmental conditions. The relationship found in this study was the inverse of previous findings and assumptions. During years with increased precipitation, when mothers were in better condition, they produced more females. During drier years, when the mothers were in poorer condition, they produced more males. This inversion was likely due to females' greater variance in reproductive success, a predictor of possible future success and the mother's inclusive fitness. Daughters being the sex with potential greater reproductive output is a unique life-history trait of cooperatively breeding species (Rubenstein 2007). These results also support adaptive sex allocation based on maternal condition and habitat quality. However, neither of these studies included any measures of CORT in relation to condition.

Several studies have related elevated maternal CORT with a female-biased sex ratio (Geffroy and Douhard 2019). One of the first to do so was Bonier *et al.* (2007). They found that free-ranging, unmanipulated white-crowned sparrow females with high CORT levels produced more daughters than females with low hormone levels. Furthermore, they found that females with artificially elevated CORT levels produced more female embryos than control females. These results provided the first experimental evidence of a causal relationship between increased CORT concentrations and a primary sex ratio bias (Bonier, Martin, Wingfield 2007). While this study was among the first of its kind, it did not include any links between female condition, CORT, and sex ratio biases and only examined the trends during one breeding season.

More recent research has worked on addressing these gaps. For example, Riechert *et al.* (2013) examined the primary sex ratio of common terns (*Sterna hirundo*) over three years. They found no bias in the sex ratio during any year, despite variation in food availability and breeding success, and found no influence of CORT concentrations on the hatching sex ratio (Riechert, Chastel, Becker 2013). However, the researchers did not include any measurement of the secondary sex ratio, which may have been linked to the environmental conditions or maternal CORT levels. It is also possible that, because sons and daughters are very similar in size in common terns, it would not be adaptive to alter the sex ratio (Riechert, Chastel, Becker 2013).

Henderson *et al.* (2014) evaluated variation in maternal condition and CORT levels with changes in the offspring sex ratio of blue tits (*Cyanistes caeruleus*) over three years. When evaluating the population sex ratio, they found no bias in either the primary or secondary sex ratio in any year and no evidence of male-biased mortality (Henderson et al. 2014). However, this is expected based on the Trivers-Willard hypothesis (Trivers and Willard 1973). When evaluating the relationship between brood sex ratios and maternal condition, the researchers found mothers produced male-biased broods during one year when they were in superior condition. However, maternal CORT was not correlated to the sex ratio in any year. Furthermore, they found no relationship between maternal CORT and condition, which may explain the lack of correlation with sex ratio. Elevated maternal CORT still affected nestling quality and lead to lighter offspring, but there were no sex-specific effects (Henderson et al. 2014). Although these researchers did an excellent job relating maternal CORT, condition, and sex allocation, they did not measure any environmental variables or stressors. As a result, it is possible that in this species and environment, maternal CORT is not a strong predictor of the conditions offspring will experience, so adjusting the sex ratio based on CORT concentrations would not be adaptive, or there may be some other factor obscuring the relationship. While there has been an increase in research on the connections between maternal CORT, maternal condition, and adaptive sex allocation, the findings are often context-specific and difficult to generalize. Moreover, most studies have been experimental, performed under controlled conditions, and have not explored the three-way association between environmental stressors, maternal CORT, and sex ratios (Geffroy and Douhard 2019).

Almost all current research has focused on the impacts of maternal CORT and maternal condition on offspring care and quality. Few studies have considered paternal CORT's effect on nestling care and chick quality, and none have evaluated the impact on sex ratios. The relationship between elevated baseline CORT and male provisioning is unclear. The few studies which have examined correlations between natural variation and paternal care have given equivocal results, with some finding a positive relationship and others finding no connection. Experimental studies have rarely manipulated paternal CORT directly, and as a result, the findings are context-dependent and difficult to generalize (Lynn 2016). A few studies have connected paternal hormone levels, paternal care, and reproductive success. For example, in house sparrows (*Passer domesticus*), there is a positive correlation between paternal baseline CORT during breeding and the number of offspring fledged. Also, there is a negative correlation between stress-induced CORT and nestling provisioning in fathers (Ouyang et al. 2011). A recent study on common terns supported a similar relationship. Researchers found that males with moderately increased baseline CORT were more active, demonstrated higher foraging effort, and increased nestling attendance. They also found that more parental attendance during nestling provisioning led to increased fledging success (Riechert and Becker 2017).

An extension of the relationship between paternal CORT, nestling care, and fledging success that has received very little attention is the similarity of maternal and paternal CORT concentrations and its impacts on reproductive success. We are only aware of one study that has examined this relationship. Ouyang *et al.* (2014) found that baseline concentrations of CORT were highly correlated between members of a breeding pair of great tits (*Parus major*) and that they became more similar in pairs that remained bonded for multiple years. Moreover, they found that pairs that increased their similarity during the breeding season had the highest breeding success. Paternal CORT and the similarity of maternal and paternal CORT concentrations are related to nestling care and success. Consequently, it is possible that in species with biparental care, the secondary sex ratio could be affected by changes in these hormone levels, but there is a paucity of research in this area.

Early research into the sex ratios of European starlings found a significant female bias (Bradbury et al. 1997) . However, because the researchers used five-day-old chicks, some chick mortality occurred before the sex ratio was calculated. As a result, we can only consider this a bias in the secondary sex ratio, despite the authors finding no sex-biased mortality. The researchers examined several environmental and parental variables but found no significant relationships explaining the bias (Bradbury et al. 1997). Other studies have included physiological measures and have explored the relationship between CORT and sex ratio in European starlings. Love *et al.* (2005b) found that baseline CORT correlated negatively with energetic condition in breeding females and that artificially elevated CORT in the egg led to a female sex bias at hatching through male embryonic mortality. Furthermore, male nestlings were lighter, grew more slowly, and had a compromised immune response compared to control males. Female nestlings were unaffected by the elevated CORT (Love et al. 2005)

Love and Williams (2008) manipulated maternal quality and pre-natal CORT to create different combinations of quality and CORT exposure and then linked these factors to alterations in offspring quality and secondary sex ratio of European starlings. They found that broods with elevated pre-natal CORT produced sons that were lighter and begged for less time, while daughters were unaffected. While there was no effect of maternal quality on hatching success, it did affect nestling survival to fledging. Broods from low-quality mothers showed rapid, male-biased mortality that created a female-biased secondary sex ratio. Finally, low-quality mothers with a female-biased sex ratio could reduce their investment in current reproduction, maintain a higher body mass, and gain fitness advantages through increased survival and future reproduction (Love and Williams 2008). However, Rowland *et al.* (2007) found that if maternal rearing ability decreases after laying and pre-natal CORT is not changed, daughters are more sensitive and show reduced growth, but no sex-biased mortality occurs. It seems that if European starling mothers have elevated CORT while laying and transfer those concentrations to their offspring in the egg, it can result in a brood that can be altered through differential mortality to have a sex ratio that better matches the mother's ability to invest in her offspring. However, not many observational studies examine this relationship and connect it to environmental variables and challenges in European starlings, such as the effects of living in an urban habitat.

Chapter Three – Sex Ratios and the City: Is parental corticosterone a mediator of the relationship between urbanization and offspring sex allocation?

Introduction

The study of sex allocation theory is a complex field that has evolved as our understanding of evolutionary ecology has improved. Fisher's equal allocation theory (1930) suggests that parents should invest equally in the sexes to gain the largest fitness advantage; it assumes males and females contribute equally to the next generation because every offspring has one mother and one father. However, if one sex is more costly than the other to produce or raise, the observed population ratio can shift from equal production to equal investment in both sexes. Therefore, parents should invest more in the less costly sex to ensure that overall resource investment is equal between the sexes (Fisher 1930). Trivers-Willard (1973) expanded this idea after noting that mothers can adjust the sex of their offspring in response to environmental conditions to improve their fitness through increased survival and future reproduction. The costs and benefits of producing each of the sexes can fluctuate under different environmental conditions, leading parents to favor one sex over the other depending on their current environment and intrinsic condition. When mothers are in poor condition (e.g., with poor body condition or facing low food availability), they should invest in the less costly sex, and when they are in better condition, they should invest in the more costly sex. This adaptive sex allocation—or bet-hedging—could lead to biased offspring ratios within a brood based on the conditions at the time of breeding. However, these biases should cancel out at the population level and through time (Trivers and Willard 1973).

Many different environmental conditions can affect individual condition—including weather, food availability and quality, disturbance frequency, or predator density—and shape offspring sex ratios. For example, common grackles (*Quiscalus quiscula*) bias the sex ratio of their broods towards the less expensive sex early in the season when they experience harsher weather and reduced food availability (Howe 1977). Lesser spotted eagles (*Aquila pomarina*) favor the more expensive sex in years with increased prey availability and rainfall (Väli 2012). In addition to the environmental variation that organisms have experienced throughout their evolutionary histories, recent anthropogenic change has drastically altered the availability of resources for organisms worldwide. Increased human population density on nearly all continents has led to altered environmental conditions in urban environments compared to rural ones that organisms face (Seress and Liker 2015). Urban areas are higher in chemical, noise, and light pollution (Seress and Liker 2015; Shanahan et al. 2013) which can negatively affect the health and behavior of organisms (Deviche and Davies 2014; Gil and Brumm 2013; Pickett et al. 2011; Seress and Liker 2015; Visser and Spoelstra 2014). Urban environments may also have altered meteorological conditions—including increased precipitation and higher temperatures through the urban heat island effect (Berry 2008; Pickett et al. 2011). In some avian species, the urban heat island effect is linked with increased overwinter survival and improved post-winter body condition (Seress and Liker 2015). Urban environments may also have altered food availability through a decline in insect prey (Boyes et al. 2021) and supplemental seed and suet at feeders. The effects of urbanization on animal condition are complex; for example, birds consuming human foods and refuse may have nutritionally poorer diets (Ottoni, de Oliveira, Young 2009). In contrast, feeders can improve the nutritional condition of wintering birds and increase adult body mass and survival (Amrhein 2013), but they also increase the spread of infectious diseases and reduce nestling body mass and survival (Ottoni, de Oliveira, Young 2009; Seress and Liker 2015). Overall, the impact of urbanization on wildlife is context-dependent but often acts through altered behavior and physiology to affect an individual's health and condition.

Habitat alterations that result from urbanization can impact parental condition—and thus offspring sex allocation—and one of the primary mechanisms linking the external environment to an animal's intrinsic condition are glucocorticoids. In birds—a taxon commonly used to explore endocrine mechanisms and sex ratio allocation—corticosterone (CORT) is the primary glucocorticoid. One of this hormone's primary functions is metabolic regulation. Baseline CORT maintains homeostasis, links an organism's external and internal environments, manages energy allocation, and mediates trade-offs between investment in reproduction and survival (Landys, Ramenofsky, Wingfield 2006; Patterson et al. 2014b; Sapolsky, Romero, Munck 2000). The relationship between urbanization, body condition, and CORT has been challenging to describe. While some studies find a positive relationship between urbanization and parental body mass (Auman, Meathrel, Richardson 2008), others find a negative impact of city living on body mass (Liker et al. 2008; Strubbe et al. 2020) or no impact of urban life on adult body condition at all (Bókony et al. 2012; Foltz et al. 2015; Meillère et al. 2015). However, a consistent conclusion across avian studies is that urban nestlings are smaller than their rural counterparts (Biard et al. 2017; Liker et al. 2008; Meillère et al. 2015; Mennechez and Clergeau 2006). Furthermore, a recent meta-analysis suggests that the relationship between CORT and urbanization is inconsistent and may depend on life-history stage and how tolerant species are to urban conditions (Injaian et al. 2020). As a result, the links between urbanization, CORT, parental condition, and offspring sex allocation remain debated (Bókony et al. 2012; Foltz et al. 2015; Strubbe et al. 2020).

Avian systems have emerged as common and excellent models to understand sex ratio allocation. Female birds are the heterogametic sex and can contribute either a Z or W chromosome to the offspring, thus manipulating the primary or embryonic sex ratio (Navara 2013). Furthermore, birds represent a wide variety of mating systems and levels of parental care, which provides many situations to study if and when sex ratio biases occur. Finally, because they are oviparous, mothers only have one chance to hedge their bets according to their intrinsic and environmental conditions. This "sealed bid" provides opportunities to study how the timing of changes in condition or environmental predictability will affect secondary or fledging sex ratios (Love and Williams 2008).

In birds, maternal condition can affect both the primary and secondary sex ratio. The primary sex ratio is the one a mother lays, while the secondary sex ratio is the ratio of males to females in the offspring that survive to fledge. In some cases, the primary and secondary sex ratio can differ through differential mortality of young in early life. Mothers are thought to bias their primary sex ratio through CORT and other steroid hormones deposited into the yolk and albumen of the egg (Downing and Bryden 2008; Engelhardt and Groothuis 2005; Navara and Pinson 2010; Schwabl 1993). Maternal CORT can also significantly impact nestling development and survival to match offspring needs with maternal care quality (Bowers et al. 2016; Hayward and Wingfield 2004; Parolini et al. 2019; Rubolini et al. 2005; Saino et al. 2005; Schoech, Rensel, Heiss 2011; Strange et al. 2016; Tilgar et al. 2016). Sex-specific mortality due to changes in parental care and provisioning during periods of lower environmental quality and poor maternal condition creates a bias towards the sex with lower cost (Nager et al. 1999; Rubenstein 2007). Elevated maternal CORT can cause primary sex ratio biases within a season (Bonier et al. 2007, Love et al. 2005), but the relationship between secondary sex ratios and maternal condition is more complicated (Henderson et al. 2014; Riechert, Chastel, Becker 2013). Elevated CORT has been related to female-biased secondary sex ratios and to smaller sons with reduced growth rates and immune response in European starlings (*Sturnus vulgaris*), where males are the slightly larger and more costly sex (Love et al. 2005; Love and Williams 2008). It seems that if mothers have elevated CORT while laying, it can result in a brood with one sex that is predisposed to a higher and more rapid rate of mortality through physiological or behavioral changes. This sex-biased mortality leads to a sex ratio that better matches the mother's ability to invest in her offspring (Love and Williams 2008). Despite the secondary sex ratio being a critical indicator of a mother's future fitness, we understand less about how maternal condition shapes secondary sex allocation relative to primary sex allocation.

For species that show biparental care, maternal condition is not the only factor likely to influence differential offspring mortality and the secondary sex ratio; paternal condition too may play a role in determining sex ratios. However, few studies have considered paternal CORT's effect on nestling care and chick quality, and none have evaluated the impact on secondary sex ratios. As a result, the relationship between elevated baseline CORT and male provisioning is unclear (Lynn 2016). In house sparrows (*Passer domesticus*), there is a positive correlation between paternal baseline CORT during breeding and the number of offspring fledged. Also, there is a negative correlation between stress-induced CORT and nestling provisioning in fathers (Ouyang et al. 2011). The results of a recent study on common terns supported a similar relationship: males with moderately increased baseline CORT were more active, demonstrated higher foraging effort, and increased nestling attendance (Riechert and Becker 2017). They also found that more parental attendance during nestling provisioning led to increased fledging success (Riechert and Becker 2017). Nevertheless, we know little about the role of paternal condition in shaping secondary sex ratios. If paternal condition shapes offspring sex ratios too, it opens up the possibility that the similarity of maternal and paternal condition may impact reproductive success. We are only aware of one study on this topic (Ouyang et al. 2014) that showed that similarity in baseline CORT in pairs of great tits (*Parus major*) increased their breeding success. Paternal CORT and parental similarity in CORT can shape nestling care and survival; thus, they could also shape the secondary sex ratio.

Here, we explore how maternal condition, paternal condition, and similarity among parents in condition shape sex ratios. While many studies have examined segments of the relationship between CORT, body condition, urbanization, and sex ratio allocation, none have explored all of these elements within the same study system and at the same time. We use an urban-adapted bird—the European starling—to explore how city living shapes sex ratios relative to rural life. This species shows biparental care, where both mothers and fathers will brood and provision their young. In addition, this species shows size dimorphism where males are larger than females (Cabe 2020), so sons are the costlier sex to raise. Sons also have higher reproductive potential than females—as they can have multiple female mates or extra-pair paternity—making high-quality sons a preferred investment to increase parental fitness (Smith and Von Schantz 1993). We would thus expect broods with a secondary sex ratio that is female-biased when parents are in poor condition. Otherwise, the ratio should be unbiased or male-biased. We seek to address the following questions with our work: (1) Do starlings vary their secondary sex ratio between urban versus rural habitats? (2) Do maternal or paternal body condition and/or CORT shape differences in the sex ratio of broods? (3) Is the similarity of paternal and maternal body condition and/or CORT reflected in the sex ratio of the brood? Due to the increased number of stressors associated with urban living, we predict that urban parents would produce a higher proportion of daughters than rural ones. We also predicted that there would be a positive correlation between maternal or paternal CORT and the proportion of daughters produced and a negative correlation with maternal or paternal body condition regardless of habitat. Finally, we expected that pairs that are more similar in their condition and/or CORT would produce a lower proportion of daughters. By integrating physiological mechanisms with ultimate changes in brood sex allocation across an urban to rural gradient, we hope to draw new conclusions about the impacts of urbanization on avian species and learn how this urban adapter flourishes under anthropogenic conditions.

Materials and Methods

Study System

European starlings are an urban-adapted species commonly found across a gradient of urbanized and rural areas (Blair 1996). They are secondary cavity nesters and readily breed in nest boxes, traffic lights, stadium lights, and tree cavities (Cabe 2020) making them an excellent study species for this work. We monitored breeding starlings at two study sites across an urban to rural gradient. The urban site is a small farm located in the midst of a metro-Atlanta suburb with over 2,500 people per square mile (Acworth, Georgia USA). The distance to the nearest restaurant is < 1 mile, and the distance to the nearest highway is < 0.5 miles (i.e., within the home range of a starling). Paved and built structures surround it, with some vegetation throughout, and the site experiences relatively high human disturbance. The rural site is located at a farm surrounded by fields and vegetation, with a few houses and roads dispersed throughout (Taylorsville, Georgia USA). The population density at the rural site is below 150 people per square mile, the distance to the nearest restaurant is > 10 miles, and the distance to the nearest highway is > 2 miles. The sites make a strong comparison because both are farm properties, but they differ primarily in the degree of urbanization within the breeding home range of a starling.

In addition to the qualitative differences in study sites described above, the level of urbanization at each site was quantified using a semi-automated imaging processing software called ‘UrbanizationScore’ based on the methods of Liker et al. (2008). Briefly, the software downloads a satellite image of a 1 km^2 area around a given geographical coordinate. It then scores the proportion of vegetation, buildings, and paved surfaces within each 100 m^2 cell of the image. Next, these scores are used to calculate the landscape-cover variables. The variables are then combined using principal component analysis to generate an ‘urbanization score’ for each study site. Finally, the sites are then ranked along a relative urban to rural gradient according to their score such that lower values are less urbanized (Czúni, Lipovits, Seress 2012; Lipovits, Czúni, Seress 2015; Seress et al. 2014). Our rural site was given a score of -2.24, while our urban site was given a score of 2.24, indicating a clear difference in the degree of urbanization between the sites that supports our qualitative impressions.

Field Sampling

We monitored approximately 40 nest boxes mounted on posts at each site every few days until hatching during the 2020 and 2021 breeding seasons (March to June). When nestlings were between four and thirteen days old, we trapped both parents to collect a blood sample for hormone analyses. To catch the parents, we set up a Van Ert trap at the nest box entrance and waited for the parent to return and spring the trap by entering the nest box. We monitored nest boxes closely to rapidly remove the parent from the nest box upon capture. We collected a baseline blood sample in under 3 minutes of disturbance (mean ± SE = 157 ± 5.3 seconds), weighed birds with a Pesola scale, and measured their tarsus length. Body condition scores were calculated as the residuals of a linear regression between tarsus length and body mass, where higher values indicate better body condition (Ardia 2005). After sampling was complete, we fit each individual with a uniquely numbered USGS aluminum band. When the chicks reached 16 –17 days old, they were also fitted with a permanent USGS aluminum band, and we collected morphometric measurements and a blood sample from the nestlings for molecular sexing.

All blood samples were collected by puncturing the brachial vein using a 25G needle. Whole blood was collected in heparinized capillary tubes and then transferred to a 1.5 ml Eppendorf microcentrifuge tube. A small amount of whole blood was transferred to a Whatman™ FTA™ card (Cytiva, Marlborough, MA, USA) and stored at room temperature for later genetic analyses. The remaining whole blood was centrifuged for 5 minutes. The plasma was kept and transported on ice until it could be stored at -80ºC.

Hormone assay

We measured baseline CORT concentration using a commercially available enzyme immunoassay (EIA) kit according to the manufacturer's protocol (Arbor Assays DetectX Corticosterone Kit). This protocol was validated for our samples, where serial dilutions (1:25, 1:50, 1:100, and 1:200) of pooled plasma yielded similar hormone concentrations (mean 4.3% difference in CORT across dilutions). We selected a 1:25 dilution to ensure that baseline samples with low corticosterone concentrations still fell within the range of the standard curve. Briefly, plasma samples were thawed before combining 10 µl plasma and 10 µl of dissociation reagent and incubating at room temperature for 5 min. Then we diluted the samples 1:25 in assay buffer. We included a standard curve (78.125–10,000 pg ml−1) and two blank controls on each plate. Next, 50 µl of each sample and standard were added to the wells of the plate. Then 25 µl of Conjugate and 25 µl of Antibody solution were added to every well. The plate was incubated at room temperature for 1 hour while being shaken at 500 RPM. Next, each plate was washed, 100 µl of TMB substrate was added to all wells, then the plates were incubated for another 30 minutes at room temperature. Finally, 50 µl of stop solution was added to all wells, and the absorbance was analyzed at 450 nm with a plate reader (BioTek ELX808). Circulating CORT concentrations were calculated from the absorbance relative to the standard curve and expressed in ng ml−1. We ran samples in duplicate and within six months of collection. The intra-assay coefficient of variation (from duplicate samples) was 4.56%, and the inter-assay coefficient of variation (from a pooled sample with an aliquot run on every plate) was 10.3%.

Molecular sexing

To determine the sex of the fledged nestlings, we took a 1.2mm punch from each FTA card. DNA was extracted from the punches using the DNEasy Blood & Tissue Kit (QIAGEN, Hilden, Germany) following a slightly modified protocol (Joint Experimental Molecular Unit 2013). Briefly, the punch was placed in 180 µl of Buffer ATL then incubated for 10 minutes at 94ºC. Then 20 µl of proteinase K was added, and the solution was incubated for one hour at 56ºC. The remaining steps were completed according to the manufacturer's protocol. Extracted DNA was stored at -20ºC until ready for analysis using polymerase chain reaction (PCR). Primers used for PCR were P2/P8, which target the CHD gene (and its homolog) located on Z and W sex chromosomes and are widely used for genetic assays of sex in birds (Griffiths et al. 1998). PCR was carried out in a total volume of 25 µl using the KAPA HiFi HotStart PCR kit (KAPA Biosystems, Cape Town, South Africa). The thermal cycle profile for the PCR was initial denaturation at 95ºC for 3 minutes, then 35 cycles of 98ºC for 20 seconds, 61ºC for 15 seconds, and 72ºC for 15 seconds, followed by a final extension at 72ºC for 5 minutes. PCR products were separated by electrophoresis for 60–75 minutes at 75V in a 2% agarose gel stained with Midori Green Direct DNA stain. Because these birds have a Z/W chromosomal sex determination, two bands indicate a female, and one indicates a male.

Statistical Analyses

During the study, we monitored 36 nests that fledged their young and for which we could calculate the secondary sex ratio of surviving nestlings. We compared mean nestling body mass within each site and found that males do tend to be larger than females at our urban site, but similar to females at our rural site (urban males = 75.27 ± 1.62 g, urban females = 62.29 ± 3.10 g, $t_{10.273} = -3.71$, $p < 0.01$; rural males = 73.13 ± 1.17 g, rural females = 70.53 ± 0.99 g, $t_{86.482} = -1.70$, $p = 0.09$). For each nest, we calculated the proportion of the fledged offspring that were daughters, such that a nest that produced only daughters had a value of 1.0, and a nest of only sons had a value of 0.0. Nests that fledged no offspring due to predation, abandonment, or other factors were excluded from the dataset. The mean survival rate at the brood level of young that hatched was 0.78 at our rural site and 1.00 at our urban site when considering only nests that fledged at least one nestling. If we include nests that failed due to abandonment, predation, or disease we found that the rural mean survival rate is 0.54 and the urban mean survival rate is 0.33. We only considered fledged offspring in our models as they are the most ecologically relevant and represent the parents' fitness through possible contributions to future generations.

First, we used a generalized linear mixed model (GLMM) with a binomial error structure and logit link function to determine if the site impacts the proportion of daughters produced. The response variable was the proportion of fledglings that were daughters, and the fixed effects were site and the nesting attempt number (as starlings can have two broods in one year, and parental quality is related to nesting attempt (Hoffmann, Postma, Schaub 2015; Verboven and Verhulst 1996). We also included a random intercept for nest ID to account for the non-independence of multiple breeding attempts by the same pair. The total number of fledged offspring was used for the weight parameter in all models.

Second, we used additional GLMM with a binomial error structure, logit link function, and random intercept to determine if maternal or paternal CORT or condition influenced the proportion of daughters produced. These analyses were completed with data from 2021 only (maternal n=22; paternal n=14) as we were not able to capture adults in 2020 due to COVID-19 field restrictions. We built two separate models—one for mothers and one for fathers—with the same fixed and random effects: fixed effects included the parental baseline CORT, parental body condition, site, and the number of fledged offspring. Individual ID was included as a random effect to account for multiple attempts by the same adult.

Finally, to determine the relationship between the similarity of parental CORT and condition on secondary sex ratios, we used a generalized linear model (GLM) with a binomial error structure and logit link. Due to the small sample size for this analysis (n=14 pairs), we calculated similarity in condition for breeding pairs two ways. First, we calculated a continuous similarity index for CORT concentration and body condition as the absolute value of the difference between the maternal and paternal values. Lower similarity index values indicate more similar parents regardless of whether the mother or father was in better condition. In addition to this continuous similarity index, we also calculated a simplified similarity index by classifying each pair as having high similarity or low similarity in relation to mean similarity in our study (mean similarity in CORT = 5.79; mean similarity in body condition = 7.05)—for this simplified index, the values below the mean were classified as high similarity and vice versa. We used two GLMs—one for each version of the similarity indexes described—to explore whether parental similarity shaped secondary sex ratios. The fixed effects in this model were the similarity of baseline CORT, the similarity of body condition, site, and the number of offspring fledged. We note that in the similarity analyses, there were two instances where one parent fledged two broods and thus was repeatedly included in the dataset. Because our sample size was too small to fit this model with a random effect of parent ID with only two instances of repeated samples, we did not include a random effect in the GLM. However, we note that the parent renested with a different individual both times. Our analyses' qualitative results and conclusions remained unchanged when we systematically excluded one repeated observation from the model. Thus, we do not believe that pseudo-replication is an issue biasing our results. All data analysis was completed in the R environment version 4.1.1 (R Core Team 2021) and the mixed-effects models were created using the package lme4 version 1.1.27.1 (Bates et al. 2015).

Results

When comparing the proportion of daughters produced between the urban and rural sites, we found no significant effect of site on the secondary sex ratio of starling broods (Table 1). However, the median proportion of daughters fledged at both the urban and rural sites was above the expected 50:50 ratio (Figure 1), suggesting that starlings at both sites tended to invest more in daughters. Nestling sex ratios did not differ with the number of prior breeding attempts by the parents (i.e., first versus second broods) (Table 1).

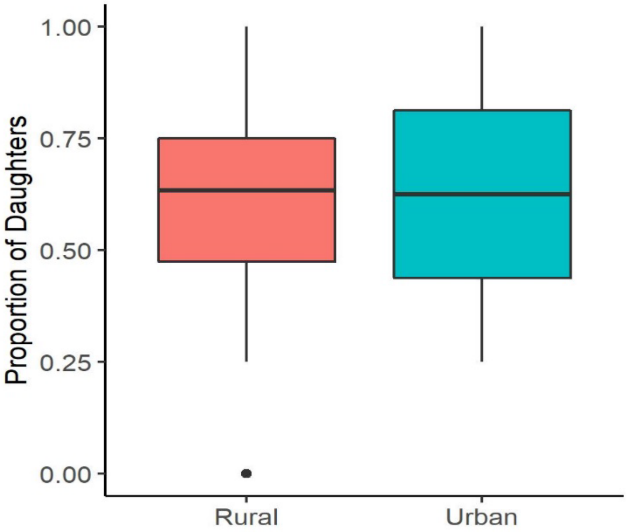

Figure 1. No difference in the proportion of daughters produced at the urban and rural sites. Median investment in daughters at both sites was above the expected 50:50 ratio. Mid-line represents the median, boxes are the first and third quartiles, and whiskers show minimum and maximum values

Table 1. Model estimates for the relationship between the proportion of daughters produced and site.

Fixed effects	**Estimate ± SE**	**z-value**	**p-value**
Intercept	0.78 ± 0.64	1.22	0.22
Site (Urban)	-0.05 ± 0.59	-0.09	0.93
Brood Attempt Number	-0.44 ± 0.49	-0.88	0.38
Random effects	**Variance**	**Std. Dev.**	
Nest ID and Year (intercept)	0.00	0.0	

Maternal CORT concentration and maternal body condition appear to have a slight positive correlation with the proportion of daughters produced (Figure 2A, 2B), but neither factor significantly influenced the secondary sex ratio (Table 2). Paternal CORT concentration showed little to no correlation with the proportion of daughters produced (Figure 2C) and was not a significant predictor. Similarly, paternal body condition was not a significant predictor of sex ratio in our model (Figure 2D; Table 2). We also found that site and the number of young fledged were uncorrelated with secondary sex ratios for both mothers and fathers (Table 2). Finally, individual parental CORT concentrations were not correlated with body condition score.

Table 2. Model estimates for the relationship between the proportion of daughters produced, maternal baseline CORT concentrations and condition, and paternal baseline CORT concentrations and condition.

	Maternal Model			**Paternal Model**		
Fixed Effects	**Estimate ± SE**	**z-value**	**p-value**	**Estimate ± SE**	**z-value**	**p-value**
Intercept	-0.32 ± 0.98	-0.33	0.74	-1.43 ± 1.35	-1.06	0.22
Baseline CORT	0.01 ± 0.03	0.452	0.65	-0.02 ± 0.09	-0.26	0.79
Body Condition	0.03 ± 0.08	0.41	0.67	-0.07 ± 0.06	-1.06	0.29
Site (Urban)	-0.20 ± 1.4	-0.14	0.89	-0.53 ± 1.17	-0.46	0.65
Number Fledged	0.16 ± 0.27	0.57	0.57	0.61 ± 0.43	1.40	0.16
Random Effects	**Variance**	**Std. Dev.**		**Variance**	**Std. Dev.**	
Individual ID (Intercept)	0	0		0	0	

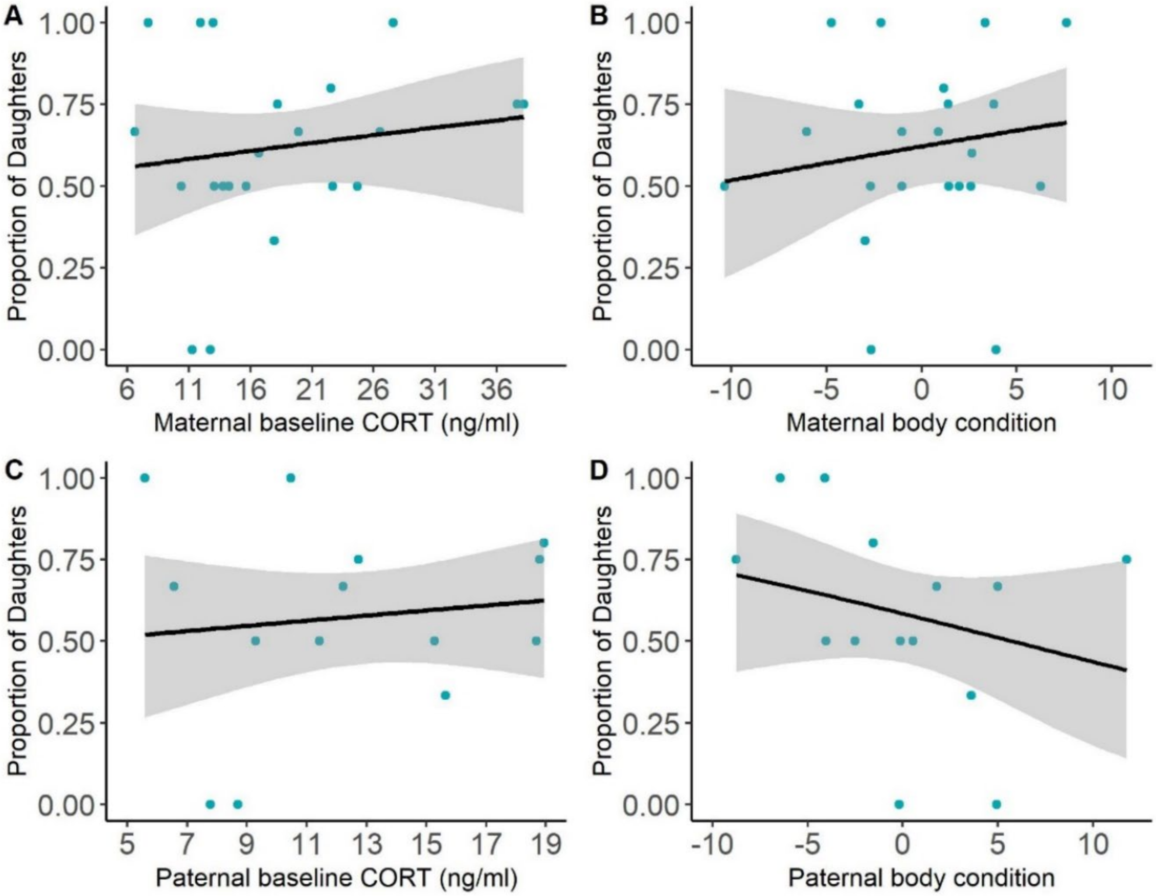

Figure 2. We found no relationships between parental condition and the proportion of daughters produced. Maternal baseline CORT concentration (A), maternal body condition (B), paternal baseline CORT (C), and paternal body condition (D) were all uncorrelated to the proportion of daughters fledged.

Our final models investigated the influence of parental similarity on sex allocation. The similarity of parental CORT concentrations exhibited a moderate positive correlation with the proportion of daughters produced, such that more dissimilar pairs appear to produce a female-biased sex ratio. However, the continuous similarity index was not a predictor of the secondary sex ratios (Table 3). When looking at our analysis with the simplified similarity index—whether each breeding pair had below or above average similarity—we found that lower than average CORT similarity scores were significantly positively correlated with the production of daughters (Table 4).

Table 3. Model estimates of the influence of similarity of maternal and paternal baseline CORT concentrations and body condition on proportion of daughters. Similarity is calculated as the absolute value of the difference between the maternal and paternal value.

Fixed effects	Estimate ± SE	z-value	p-value
Intercept	-1.55 ± 1.41	-1.102	0.27
Site (Urban)	-0.12 ± 1.30	-0.09	0.93
Baseline CORT Similarity	0.08 ± 0.06	1.25	0.21
Body Condition Similarity	-0.03 ± 0.07	-0.51	0.61
Number Fledged	0.49 ± 0.37	1.34	0.18

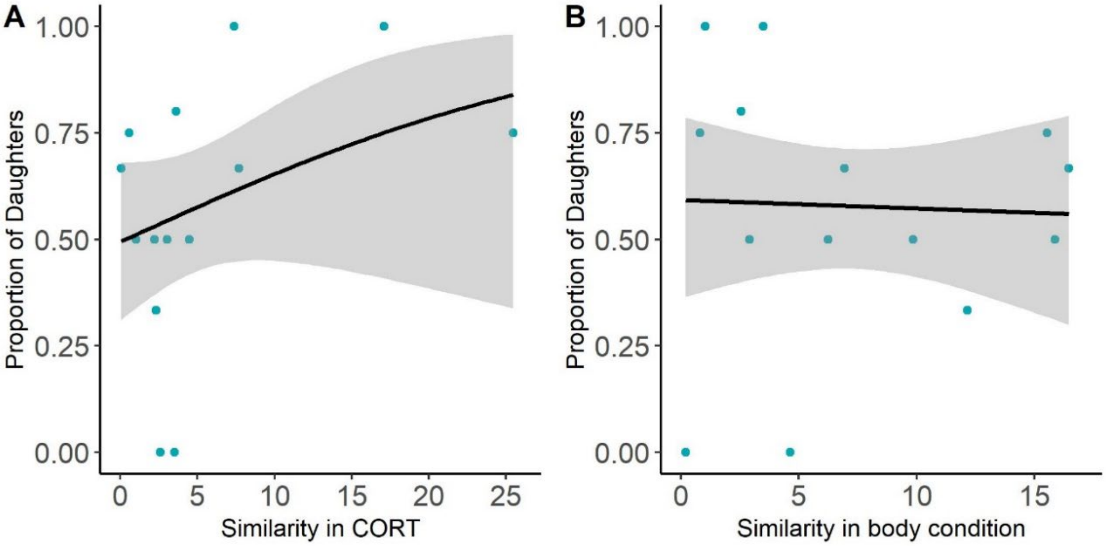

Figure 3. The similarity in parental baseline CORT concentrations (A) and the similarity of parental body condition (B) was not related to the sex ratio of the brood.

Table 4. Model estimates of the influence of similarity of maternal and paternal baseline CORT concentrations and body condition on the proportion of daughters. The similarity is calculated as above or below the average value.

Fixed effects	Estimate ± SE	z-value	p-value
Intercept	-2.00 ± 1.38	-1.46	0.15
Site (Urban)	0.41 ± 1.25	0.33	0.74
Baseline CORT Similarity (Low)	2.10 ± 0.97	2.16	**0.03**
Body Condition Similarity (Low)	-0.87 ± 0.83	-1.06	0.29
Number Fledged	0.62 ± 0.39	1.59	0.11

The similarity of parental body condition was not correlated with brood sex ratio and did not significantly influence the proportion of daughters fledged in either model (Table 3, 4). Finally, site and the number of fledged offspring from the nest did not influence the proportion of daughters produced in these models (Table 3, 4).

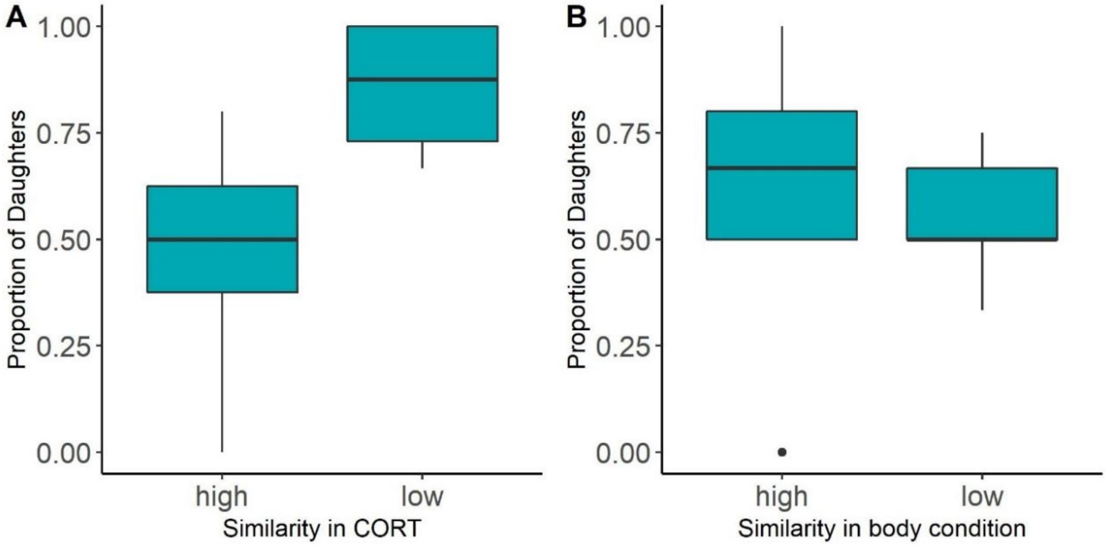

Figure 4. Pairs with below-average baseline CORT similarity produced a significantly higher proportion of daughters than pairs with above-average similarity (A), while high or low similarity in body condition did not affect secondary sex ratios (B). Mid-line represents the median, boxes are the first and third quartiles, and whiskers show minimum and maximum values.

Discussion

Our study did not find an effect of urbanization or parental condition on secondary sex ratios in starlings. Instead, we found that the similarity of parental CORT levels (but not body condition) increased the likelihood of successfully fledging sons, which are the more costly sex and provide greater possible future fitness. These findings suggest that parental similarity, rather than parental condition alone, may play a greater role in shaping offspring sex allocation outcomes for this urban-adapted species. This finding is consistent with prior research examining the effect of parental similarity on reproductive success (Ouyang et al. 2014). The results of this previous study on great tits showed that pairs with more similar baseline CORT stayed together for more seasons and had elevated reproductive success. One possible explanation for their result is that similarity in CORT represents similarity in investment or parental effort (Ouyang et al. 2014). Our results also supports this idea, as more similar pairs could produce more of the costly sex. If both parents are investing equally in their offspring, the chance of insufficient provisioning is reduced, resulting in less sex-specific mortality or additional overall resources for the more costly sex. It is important to note that, while this relationship exists, we only see a significant effect of similarity in CORT when grouping pairs by above and below average similarity. This discrepancy may result from our small sample size, which limited our power to detect a correlation between individual observations. To date, few other studies have explored how parental similarity in CORT shapes their reproductive success—more work is needed across other study systems to understand whether this pattern is common across taxa.

We were surprised to find no difference in sex ratio between the urban and rural sites, as we expected urban starlings to face more frequent disturbances than their rural counterparts, which could thus impact parental condition. One possibility is that as urban adapters, starlings may be coping well with the challenges presented in urban areas so that a shift in sex allocation is not required to preserve maternal condition. Interestingly, we did find that broods at both sites are above the expected equal allocation ratio of 1:1. Starlings in our study showed female-biased broods, which could indicate that European starlings follow equal investment rather than equal production strategies. If females are the less costly sex to produce, Fisher's theory would predict this bias regardless of the environment (Fisher 1930). Our findings are consistent with earlier work that showed a significant female bias in European starlings and no influence of environmental variables on sex ratios in this species (Bradbury et al. 1997). It is possible though that if we scale up from the individual brood level and look at the average in the population as a whole or track this pattern through time, the individual shifts in sex ratio may cancel each other out and the bias would disappear as predicted by the Trivers-Willard hypothesis. While comparing sex ratios across individuals did not reveal the pattern we expected, examining within-individual variation in sex allocation at each site may reveal a different relationship. Oddie and Reim (2002) did not find a relationship between brood sex ratios and several environmental characteristics when comparing between mothers, but they were able to find biases when examining sex ratios of the same female across years. It is possible that Trivers-Willard Hypothesis could still apply in this context but that the conditions in our urban site were not taxing enough or varied across years. While there was a quantitative difference in the urbanization level of our two study sites, our urban site was only moderately developed when compared with the conditions experienced in larger cities that starlings are known to inhabit. Expanding this work across a broader gradient of environmental conditions could yield more insight into the role of anthropogenic environments in shaping sex allocation.

Another of our findings that contradicted our original prediction was that there was no significant effect of individual parental condition—either CORT or body condition—on the sex ratio of the brood. Some observational studies have also found no impact of maternal CORT on offspring sex ratio (Henderson et al. 2014; Riechert, Chastel, Becker 2013), but we know little about whether paternal CORT may act similarly to maternal CORT in adaptive sex allocation. One possibility is that there may be confounding factors that obscure the relationship between CORT and secondary sex ratios. For example, age and breeding experience, which we could not account for in our study, correlate with CORT concentrations and how individuals respond to stressors (Angelier et al. 2007; Heidinger, Nisbet, Ketterson 2006). There may also be another hormonal mechanism that would be a better predictor of investment when examined in concert with CORT concentrations. For example, prolactin is a candidate hormone that could shape sex ratios due to its role in stimulating parental care behaviors and how it interacts with CORT (Angelier and Chastel 2009). Finally, the CORT concentrations we measured may not fully capture the glucocorticoid phenotype for an individual. Accounting for within-individual plasticity in hormones could provide a more complete and accurate understanding of when parents are truly stressed and must change their behavior to conserve resources (Guindre-Parker 2020).

Like our findings with CORT, parental body condition also showed no relationship with sex allocation. Based on previous studies, the lack of relationship between maternal body condition and sex ratio is surprising. It is possible that we were unable to detect a relationship between body condition and sex allocation due to our sample size or because we did not sample animals under harsh enough environmental conditions. Our findings align with previous work that found only moderate bias with change in condition in Gouldian finches (*Erythrura gouldiae)*(Pryke, Rollins, Griffith 2011). It is also possible that variation in condition needs to be more drastic to induce a change in sex allocation. For example, Henderson et al. (2014) only found a significant change in the sex ratio of blue tits (*Cyanistes caeruleus*) in the year when mothers were in superior condition. Perhaps in our study, mothers were in similar condition to other years and did not bias their sex ratio. Longer-term research may be necessary to uncover the environmental or individual context when parental condition does shape altered secondary sex ratios. One limitation of our study is that the body condition index uses only morphological metrics, even though individual condition is more complex than size and weight alone.

While maternal and paternal condition did not correlate with their investment in one sex over the other, we did find that the similarity of parental CORT increased investment in the costly sex. This finding raises questions about how parental investment changes with condition for the sexes in starlings. While socially monogamous, European starlings do have extra-pair paternity at low rates (Smith and Von Schantz 1993) and males, as in many species, show higher variance in reproductive success. Therefore, mothers may experience more pressure to maintain their investment and produce a balanced ratio, regardless of their condition, whereas fathers may vary their investment in offspring care while still gaining fitness. This conclusion is in line with the findings that mothers behave as if they are committed to a certain amount or quality of parental care at the outset of breeding regardless of several environmental and individual variables (Fowler and Williams 2015). In contrast, it is quicker and easier for males to produce a new brood than females, and this difference in initial investment in reproduction means that when they are in worse condition, they can provide less support to their mate without risking their entire investment for the season. Future work could explore how parental similarity in CORT shapes investment in paternal care as a behavioral mechanism for the link between CORT similarity and sex ratios. For example, Oddie and Reim (2002) found that if the same female mated with a male in better condition in the next breeding season, their brood would be male-biased and that the inverse was also true. Perhaps a similar effect is occurring within our populations.

In conclusion, our research investigated whether urbanization altered secondary sex ratios in an urban-adapted species. We found that starlings at both our urban and rural sites showed similar and slightly daughter-biased secondary sex ratios, representing the less costly sex to produce. Future work should explore sex allocation across an expanded gradient of urbanization as many species—including starlings—breed in more urban areas than our urban study site. We also found that maternal and paternal condition were not correlated to sex allocation but that pairs were more successful at raising costly sons when they showed more similar baseline CORT. Our results suggest that for biparental species, the similarity of parental condition may be more important than the individual condition of either parent in shaping their reproductive investment and fitness. However, future work on parental similarity will be needed as very few studies have explored the role of similarity in shaping reproductive success. One exciting avenue for future research would be to explore whether parents are more likely to be similar—and thus more successful at raising costly young—under some environmental conditions but not others. Finally, future sex allocation research should expand upon our work to explore how urban-adapted and urban-avoiding species may respond to anthropogenic change differently. One possibility raised by our findings is that urban-adapted species like the European starling can cope with urbanization relatively well and thus may not adjust sex ratios along a gradient of urbanization. However, it remains less clear whether urban-avoiding species would show negative responses to our increasingly urbanized world.

Chapter Four – Future Considerations and Integration

Future Considerations

I found that while individual parental glucocorticoids were not implicated in mediating the impacts of urbanization on adaptive sex allocation, the similarity of parental hormone levels allowed parents to invest more in the costly sex (sons). The role of urbanization in shaping sex allocation remains equivocal based on my results. However, several factors constrain our conclusions, including a small sample size, which may make small effects challenging to detect. Furthermore, we were limited to one measure of body condition and physiological stress. The body condition index uses only morphological metrics, even though individual condition is more complex than size and weight. Additionally, while CORT concentrations taken from plasma in under three minutes are the standard for measuring baseline concentrations, they are only a snapshot of what the organism is experiencing and may not provide a complete representation of ongoing stress.

Future work could continue to build upon our findings by expanding the gradient to include a more urbanized site and creating a larger dataset to examine these trends. While both of our study sites show a qualitative and quantitative difference in the degree of urbanization experienced and offer an excellent comparison as they are both farm settings, it is possible that we do not capture the full range of urban habitats that starlings are able to use. As an urban adapted species, they maybe able to cope with the challenges of our current urban site, but would be challenged to successfully raise sons in a more drastically urbanized area, such as a city center. Additionally, it would be interesting to investigate within-individual variation in hormone concentrations and sex allocation over time to reveal trends that may be obscured when looking at the population as a whole. This long-term data set would also provide an opportunity to measure and track the effects of chronic stress on condition, breeding and sex allocation. Further studies should consider the interplay of other hormonal mechanisms, such as prolactin, and should incorporate additional measures of individual condition to create a more holistic representation. Finally, we observed anecdotally that urban mothers were more likely to abandon a nest after a disturbance during our work. We only considered baseline hormone concentration in our work, but including the degree of the stress response and some behavioral measures of reactivity in our analyses may reveal more about the complex interplay between urbanization, condition, glucocorticoids, and fitness that we have only just begun to investigate.

In addition to the observational research completed for this thesis, new field-based manipulations may help us tease apart the effects of urbanization, body condition, and CORT on brood sex ratios to better understand why the correlative results between parental CORT and body condition do not support my original hypothesis. Using a hormone-manipulation paired with a cross-fostering manipulation, it would be beneficial to ask the following questions: (1) Do urban and rural parents with experimentally elevated CORT bias their sex ratios similarly? (2) Does experimentally increasing or decreasing parental similarity in CORT concentration affect brood sex biases? (3) Do urban and rural parents with experimentally elevated CORT show differences in parental care strategies? (4) If rural parents raise an urban chick, is the chick more likely to fledge in a sex-dependent manner? These proposed manipulations would allow us to disentangle the threads linking parental CORT, sex ratio manipulation, and urbanization and provide a clearer understanding of what trends may exist.

Integration of the Thesis Research

This research project integrates techniques and theory from several biological disciplines including, animal behavior, molecular biology, endocrinology, ornithology, ecology and evolutionary biology. Briefly, our fieldwork is founded on ornithology and ecological techniques. This includes placing nest boxes for breeding birds to use and catching females and males off of the nest using a nest box trap. We are also incorporating endocrinological and molecular techniques: blood drawn in the field was used to measure the concentration of the hormone corticosterone in the plasma of the parents using an enzyme immunoassay. Blood taken from nestlings was used to determine the sex of the offspring through PCR and electrophoresis. Finally, Fisher's theory of equal investment and the Trivers-Willard Hypothesis, which create the foundation for this research, are based in animal behavior and evolutionary biology. This research spans and synthesizes several disciplines to take a unique approach to understand the impacts of urbanization and mechanisms that individuals may use to buffer its effects.

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