Chronic Noise Exposure has Context-dependent Effects on Nestling Stress Physiology in Tree Swallows (*Tachycineta bicolor*)

by

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ABSTRACT

Anthropogenic noise is increasing in intensity and scope, resulting in changes to acoustic landscapes and largely negative effects on a range of species. In birds, noise can mask acoustic signals used in a variety of communication systems, including parent-offspring communication. As a result, nestling birds raised in noise may have challenges soliciting food from parents and avoiding predators around the nest. Given that passerine nestlings are confined to a nest and therefore cannot escape these challenges, noise may act as a chronic stressor and alter the stress physiology of nestlings. If noise acts as a chronic stressor during development, nestlings may exhibit negative effects such as reduced immune function and alterations to the development of the HPA axis. Negative effects may also persist into adulthood and ultimately reduce reproductive success.

In my study, Tree Swallow (*Tachycineta bicolor*) nestlings were raised in either continuous, white noise or in the absence of this added noise. I tested whether noise exposure affected baseline, stress-induced, and integrated feather corticosterone (the main glucocorticoid in birds) levels, as well as cell-mediated immune responses and the ratio of heterophils to lymphocytes (H/L ratio), as measures of immune function. Given that stress and immune responses may vary with the competitive environment, I also examined how nestling responses to noise varied with nestling size.

I found that, overall, exposure to noise did not alter nestling stress physiology or immune responses. However, lighter nestlings raised in noise exhibited lower baseline, higher stress-induced, and lower integrated CORT than their heavier counterparts. Lighter nestlings raised in noise did not, however, exhibit compromised cell-mediated immune responses or increased H/L ratios.

Overall, my findings suggest that noise can affect the stress physiology of developing birds; however, these effects may depend on developmental conditions and the presence of other environmental stressors, such as competition. My findings suggest that competitively disadvantaged nestlings may be more vulnerable to the effects of noise and highlight why populations are not uniformly affected by noise. Further research is needed to understand how noise-induced alterations to the HPA axis during development affect fitness and reproductive success over time.

LIST OF ABBREVIATIONS USED

GC	Glucocorticoids
HPA	Hypothalamic-pituitary-adrenal
CRH	Corticotropic releasing hormone
AVT	Arginine vasotocin
ACTH	Adrenocorticotropic hormone
CORT	Corticosterone
PHA	Phytohemagglutinin
H / L	Heterophils / Lymphocytes
S.E.	Standard error
kHz	Kilohertz
Hz	Hertz
dB	Decibel
SPL	Sound pressure level
μl	Microlitres
ml	Mililitre
t	T-test statistic
p	Statistical probability
F	F-test statistic
Km	Kilometers

R.P.M.	Rotations per minute
°C	Degrees celcius
R^2	Coefficient of determination
mm	Milimetre
μg	Micrograms
PBS	Phosphate buffered saline
r	Correlation coefficient
n	Sample size
WBC	White blood cells
GLMM	Generalized linear mixed-effect models
LMM	Linear mixed-effect models
ng	Nanograms
g	Grams
β	Beta coefficient
AICc	Akaike Information Criterion
h	Hour
CBG	Corticosterone binding protein

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CHAPTER 1 : GENERAL INTRODUCTION

Anthropogenic noise and acoustic signals

Anthropogenic noise is increasing in intensity and scope due to urbanization and the globalization of transportation networks, which are growing faster than the human population (Barber et al. 2010, McGregor et al. 2013). Anthropogenic noise is not just restricted to populated areas, but has also expanded into natural, protected areas (Barber et al. 2011), resulting in changes to acoustic landscapes and largely negative effects on both terrestrial and marine species (Kight and Swaddle 2011, McGregor et al. 2013). While negative effects are widespread, noise is especially problematic for species that rely on acoustic signals for communication. Noise can mask or attenuate acoustic signals, especially if the frequency of ambient noise overlaps with the frequency of the signal (Rosa and Koper 2018). This masking may reduce the distance at which the signal can be detected and the amount of information that can be interpreted (Shannon et al. 2016, Rosa and Koper 2018).

If noise masks signals used in communication, individuals may experience disruptions to behaviours such as foraging, predator avoidance, mate attraction, and territory defense (Francis and Barber 2013, Halfwerk and Slabbekoorn 2013). For example, noise can mask acoustic cues used by predators to find prey, thus reducing their foraging efficiency (Francis et al. 2009, Siemers and Schaub 2011). Conversely, noise can also mask alarm calls or sounds created by predators, thus increasing predation risk for prey (Templeton et al. 2016). In turn, prey species may exhibit chronic hypervigilance (Meillère et al. 2015) at the expense of other activities (e.g. foraging; Quinn et al. 2006), as well as increased predation rates (Francis and Barber 2013). Noise may also mask songs and calls used to attract mates and defend territories (Habib et al. 2007). This may reduce an individual's ability to assess mate quality and to defend high-quality territories, and may consequently reduce reproductive success (Halfwerk et al. 2011, Halfwerk and Slabbekoorn 2013). Evidently, masking of acoustic signals has the potential to reduce

fitness through disturbances to communication networks, which in turn, can lead to population-level changes (Shannon et al. 2016, Rosa and Koper 2018).

Animals may counter the negative effects of noise, however, by modifying their behaviour in ways that reduce the masking effect of noise (Kight and Swaddle 2011, Francis and Barber 2013). Some animals may avoid or leave noisy areas in favour of quieter habitats (Francis and Barber 2013). For example, one third of a bird community on its fall migration avoided a stopover area where traffic noise was increased experimentally (Ware et al. 2015). Animals may also restrict their vocal activity to periods of time when the habitat is quieter (Francis and Barber 2013). European Robins (*Erithacus rubecula*) switch to vocalizing during the night in areas where day-time noise is pervasive (Fuller et al. 2007). However, altering the spatial and temporal dynamics of communication networks based on variable noise sources may be difficult or impossible for some species and at certain life stages. For example, young birds are often confined to a nest during development and so cannot evade noise surrounding their nest, nor can they afford to be selective about when they communicate with parents. So, rather than leaving the area or shifting the timing of the vocalizations they use to acquire food, nestlings may call longer, louder, and/or at higher frequencies (e.g. alter timing, amplitude, and frequency; Brumm 2004, Brumm and Slater 2006, Leonard and Horn 2008) in noise, thus reducing effects of acoustic masking and improving signal transmission. Nestlings may also increase overall vigilance towards parents arriving to the nest with food and potential predators in the environment, thus increasing the chances of missing important signals from parents and behaving conspicuously in the presence of a predator (Ruppli et al. 2012, Dreiss et al. 2013).

Regardless of these adaptations, ambient noise can be problematic to populations of breeding birds. Noise can reduce the density, as well as the diversity of species breeding in a particular area (Slabbekoorn and Ripmeester 2008, Francis et al. 2009). Those that persist in breeding in noisy areas may lay fewer eggs and fledge fewer young compared to birds breeding in quiet areas (Halfwerk et al. 2011). The mechanism by which noise reduces reproductive success is not entirely clear, however (Kight and Swaddle 2011). One possible mechanism is that noise interferes with communication between parents and their young, which disrupts feeding and predator avoidance

behaviours (Francis and Barber 2013, Halfwerk and Slabbekoorn 2013) and ultimately reduces reproductive success.

Parent-offspring communication in birds: Begging and predator avoidance

During the breeding season, parent birds and their young rely on acoustic signals to communicate essential information, such as hunger levels within a brood and the presence of nearby predators (Godfray 1991, Wright and Leonard 2002). Parents produce contact calls as they are approaching the nest with food and nestlings respond by engaging in begging displays, which typically entail calling, posturing towards parents, and gaping (Wright and Leonard 2002). A nestling's ability to hear and respond to parents arriving with food affects its ability to compete with nestmates and attain resources (Godfray 1995, Leonard et al. 2000). As well, the parent's ability to hear nestling begging signals is important in moderating provisioning (Leonard et al. 2003). Parents use begging displays to determine which nestlings to feed and to gauge feeding rates to the brood (Godfray 1995, Horn and Leonard 2008). As well, feeding rates are often higher for broods that call at higher rates and for longer durations, compared to broods that call at lower rates and for shorter durations (Horn and Leonard 2008).

Parent birds and their young also rely on acoustic cues to communicate information regarding the presence of predators in the environment. When parents detect a predator near the nest, they produce alarm calls that trigger nestlings to crouch in the nest and cease calling (Haskell 2002). These cues are essential in ensuring nestlings remain inconspicuous to predators. Alarm calls not only indicate the presence of a predator, but often hold information on predator identity (Haskell 2002) that may elicit different predator-avoidance behaviours in nestlings (Platzen and Magrath 2004, Suzuki 2011). Thus, communication between parents and their young about the presence of predators is important in triggering predator avoidance behaviours which are likely to reduce the risk of predation. Overall, the acoustic signals used in parent-offspring communication encode salient and complex information necessary for feeding and predator avoidance, however, anthropogenic noise can mask these signals.

Disruptions to parent-offspring communication in noise

Cues associated with parents arriving to the nest with food or signalling the presence of predators may be masked by noise, causing nestlings to respond inappropriately in both contexts. If nestlings are unable to detect parents' arrivals because noise masks contact calls, nestlings may fail to beg when parents arrive with food (Leonard and Horn 2012, Lucass et al. 2016). This failure reduces nestlings' ability to compete with nestmates for food and results in missed feedings (Lucass et al. 2016). Similarly, if parents are unable to hear nestling begging calls or discriminate between nestlings within a nest (Leonard and Horn 2005), they may underestimate nestling hunger levels and decrease rates of provisioning (Schroeder et al. 2012, Lucass et al. 2016). If parents reduce feeding rates, nestlings may exhibit reduced growth rates, fledging success, and recruitment into the breeding population (Schroeder et al. 2012, Potvin and MacDougall-Shackleton 2015, Lucass et al. 2016; but see below). Fledging success and recruitment can also be reduced in noise if parental alarm calls produced in response to nearby predators are masked. If nestlings are unable to detect parental alarm calls, they may fail to crouch in the nest and attenuate begging in the presence of a predator (McIntyre et al. 2014, Templeton et al. 2016). Elevated begging can attract predators to the nest, so the failure to respond appropriately to alarm calls can greatly increase nest predation and thus reduce nestling survival (Haff and Magrath 2011).

It is important to note, however, that in some populations, feeding rates, growth rates and fledging success are not compromised in noisy areas, despite apparent disruptions to communication (Leonard and Horn 2008, Leonard et al. 2015, Brischoux et al. 2017, Injaian 2018a). For example, Tree Swallow (*Tachycineta bicolor*) parents raising broods in noisy conditions exhibited similar feeding rates to parents raising broods in relatively quieter conditions (Leonard and Horn 2008). Furthermore, nestlings exposed to experimentally-added white noise during development exhibited similar growth rates to control nestlings, despite apparent disruptions to parent-offspring communication (Leonard and Horn 2008). This suggests that there may be another mechanism by which noise affects nestlings other than noise-induced changes in parental behaviour (Injaian et al. 2019). Another possibility is that noise causes physiological

stress in nestlings, either directly by inducing fear or indirectly by masking signals and increasing the difficulty of soliciting food and avoiding predators (Kight and Swaddle 2011, Halfwerk et al. 2011, Mulhollen et al. 2018, Kleist et al. 2018).

Nestling stress physiology and benefits of the stress response

If noise causes stress, nestlings may respond with elevated levels of glucocorticoids (i.e. stress hormones; hereafter "GCs"; Sapolsky et al. 2000), which are controlled by the hypothalamic-pituitary-adrenal (hereafter "HPA") axis. The HPA axis responds to the environment and is activated in periods of allostasis, the process through which an individual maintains physiological and behavioural stability in response to changing environmental conditions (McEwen and Wingfield 2003). Upon activation of the HPA axis, GCs are secreted from the adrenal glands to enhance and mediate the stress response. GCs bind to receptors that influence the expression of various behavioural and physiological processes that are regulated through negative feedback (reviewed in Sapolsky et al. 2000).

There is evidence that nestlings experience a hyposensitive period after hatching where their GC responses to stress is minimal (Schwabl and Lipar 2002). The duration of the hyposensitive period is likely species-specific, but precocial young can typically respond to stressors a few days after hatching (Saito et al. 2000), while semi-precocial and altricial young may not respond until the later stages of development (Wada et al. 2007, Tilgar et al. 2009). The HPA axis sensitizes as nestlings age and, consequently, stress responses intensify throughout development and into early adulthood to cope with challenges in the environment and to promote behaviours important to survival (Schwabl and Lipar 2002, Wada et al. 2007).

Short-term increases in stress hormones allow individuals to cope and respond to everyday stressors, as well as to periods of acute stress, by regulating energy storage and mobilization (Sapolsky et al. 2000, McEwen and Wingfield 2003). For example, GCs play an important role in metabolism and maintaining plasma glucose levels (Sapolsky et al. 2000). Specifically, when glucose levels drop below the homeostatic norm, GC levels increase to help convert protein and lipids to carbohydrates, which can be readily used for

energy (Brillon et al. 1995). For this reason, GCs increase in response to food-related stress, and help regulate begging behaviour (Kitaysky 1999, 2001). That GCs mobilize stored energy also helps to facilitate behaviours such as shivering in cold climates and, panting and wing-spreading in warmer climates, thus helping nestlings thermoregulate (Sapolsky et al. 2000, Fairhurst et al. 2012). Finally, GCs redirect energy allocation towards immediate survival in life threatening situations (Sapolsky et al. 2000, Landys et al. 2006). For example, GC levels increase during predation events, which promotes survival by inducing freezing behaviour in the presence of a predator (Tilgar et al. 2010, Ibanez-Alamo et al. 2011). Thus, stress hormones are modulated to help individuals respond to every day and seasonal stressors related to short-term energy deficits, as well as to acute perturbations in the environment (Bonier et al. 2009a). There may, however, be negative consequences if stress responses are sustained long-term.

Cost of chronic stress

Long-term exposure to extreme stressors can result in a state where energy demands exceed energy income (i.e. allostatic overload), and consequently, cause physiological damage (McEwen and Wingfield 2003). Even relatively short-term elevations of GCs may suppress other physiological functions and have permanent effects on nestling HPA axis responsiveness, behaviour and ultimately long-term fitness (Sapolsky et al. 2000, McEwen and Wingfield 2003). For example, stress hormones suppress the synthesis, proliferation, and efficacy of immune cells (Sapolsky et al. 2000). Suppression of immune responses results in increased vulnerability to parasites and infections, and reduced fitness into adulthood (Dhabhar 2009). GCs also mobilize stored energy (Sapolsky, 2000) and so chronic elevation may result in reduced growth and body condition (McEwen and Wingfield 2003). Chronic stress may also cause neuronal death and consequently alter cognitive development (Potvin et al. 2016). This may result in reduced cognitive abilities (Kitaysky et al. 2003), as well as changes to begging calls and adult song, which may affect future communication with conspecifics (Spencer et al. 2005, MacDougall-Shackleton et al. 2009, Potvin et al. 2016). As well, chronic stress early in life may alter the development of the HPA axis, causing birds to be hypersensitive to stressors in adulthood (Spencer et al. 2009). Hypersensitivity in

adulthood can cause a shift from territorial and parental behaviour (e.g. incubation and feeding effort) to self-maintenance (Angelier 2009, Spencer et al. 2010), which can result in decreased reproductive success (Wingfield et al. 1995, Vitousek et al. 2018). Chronic stress can have short- and long-term effects on nestlings that can be transmitted across generations through reduced parental behaviour (Schoech et al. 2011).

If long-term exposure to noise causes chronic stress, nestlings may be especially vulnerable because they are confined to the nest during development and cannot escape the stressor. As a result, they may suffer effects on immunocompetence, cognitive development, and future territorial and reproductive behaviour (reviewed by Schoech et al. 2011), with differential effects depending on early life experiences (Blas 2005, Spencer et al. 2009, Zimmer et al. 2013).

Developmental stress across and within broods

Effects of developmental stress on HPA axis responsiveness

While stress responses are largely heritable (Stedman et al. 2017), early life experiences and stressful conditions during development can affect the structure and functioning of the HPA axis, which can alter endocrine function and consequently, an individual's response to future challenges (Blas 2005, Spencer et al. 2009, Zimmer et al. 2013). Specifically, stress during development can alter the development of the HPA axis in ways that increase GC secretion, for example by increasing the production of hormones (corticotropic releasing hormone (CRH)/arginine vasotocin (AVT) from the hypothalamus, adrenocorticotropic hormone (ACTH) from the pituitary, or CORT from adrenal glands) or reducing sensitivity to negative feedback, making individuals hypersensitive to future stressors (Meanley 2001). In contrast, stress during development can also alter the development of the HPA axis in ways that lower GC secretion and increase the efficiency of terminating stress responses, for example by reducing the production of hormones, notably, AVT (Rich and Romero 2005), or increasing sensitivity to negative feedback (Meanley 2001).

The plasticity of the HPA axis allows for early experience to program physiological phenotypes (e.g. stress reactivity) to suit the environmental conditions that

individuals are most likely to encounter during their life (i.e. developmental programming; Monaghan 2008, Angelier and Wingfield 2013). For example, Love and Williams (2008) subjected European starling (*Sturnus vulgaris*) nestlings to pre-natal and post-natal stress by injecting CORT into yolks of freshly laid eggs (mimicking reduced maternal quality) and decreasing maternal provisioning via feather-clipping. Nestlings exposed to pre-natal yolk GCs had reduced stress responses at fledging, presumably because elevated pre-natal yolk GCs is a predictive signal that their mother or post-natal environment is low-quality and there is a selection for downregulated HPA responsiveness to avoid deleterious effects of elevated GCs. Conversely, nestlings raised by feather-clipped mothers, who provided less food, exhibited elevated HPA responsiveness at fledging, presumably in an attempt to overcome the unpredictable decrease in the quality of the post-natal environment and increase in sibling competition (e.g. via mobilization of energy stores and increased begging; Kitaysky 1999, 2001). Therefore, developmental stress may have programmed the HPA axis of nestlings within this population variably, so that nestling stress reactivity was suited for their developmental environment (Monaghan 2008). Thus, early experiences and developmental stress have the potential to program physiological phenotypes, resulting in variable stress responses across nestlings (Spencer et al. 2009, Zimmer et al. 2013).

Sources of developmental stress in nestlings

Nestling survival is particularly affected by their ability to acquire resources from parents and to compete with nestmates for these resources (Wright and Leonard 2002). As such, reduced food availability and competition for resources are likely to be significant sources of developmental stress in nestlings and may affect HPA responsiveness to future challenges (Blas et al. 2005, Wada et al. 2009, Vitousek et al. 2017). The ability of a nestling to acquire resources may depend on food availability in their environment. For example, periods of inclement weather may affect the abundance of prey and forging abilities (McCarty and Wrinkler 1999, Winkler et al. 2013). Reduced food availability is associated with increased nestling GC levels in a range of species (Kitaysky 1999, Pradosudov and Kitaysky 2006, Crino et al. 2020).

The ability of a nestling to acquire resources also depends on their competitive environment, which can vary across and within broods. Nestlings in larger broods often obtain less food (Dijkstra et al. 1990), and so may exhibit smaller body sizes and greater sibling competition compared to nestlings in smaller broods (Leonard et al. 2000, Neueschwander et al. 2003, Kozlowski and Ricklefs 2011). As a result, nestlings in larger broods experience greater developmental stress compared to nestlings from smaller brood (Saino et al. 2003, Blas et al. 2005, Vitousek et al. 2017). For example, Saino et al. (2003) increased or decreased brood sizes of Barn Swallows (*Hirundo rustica*) by one nestling and found that nestlings from enlarged broods had lower immunocompetence, weighed less, were in poorer body condition, and had higher GC levels compared to nestlings in reduced broods.

Furthermore, the likelihood of obtaining food from parents depends on an individual's competitive ability, which varies within a brood. Nestlings often hatch asynchronously, resulting in a size hierarchy (Cotton et al. 1999). Smaller nestlings may be less able to outcompete larger nestmates for food and so may be more vulnerable to chronic food restriction (Godfray 1995, Leonard et al. 2003, Fresneau et al. 2018) and often greater developmental stress than larger nestlings (Kitaysky et al. 1999, Tarlow et al. 2001, Walker et al. 2005b). For example, Walker et al. (2005b) found that smaller, food-deprived Magellanic penguins (*Spheniscus magellanicus*) nestlings have higher GC levels compared to their larger, healthier counterparts.

Elevating circulating stress hormones may help mediate behavioural and physiological responses to food-deprivation and increased competition for resources, for example by increasing begging intensity and mobilization of stored energy (Kitaysky 1999, Sapolsky et al. 2000). However, if food restriction and competition for resources are long-lasting and GC responses are sustained long-term, the development of nestling neural systems which underlie their responses to stress may be altered (Blas et al. 2005, Spencer et al. 2009, Zimmer et al. 2013). Given that nestlings across and within broods may be affected differently by food availability and sibling competition, nestling HPA responsiveness and consequently, responses to stressors like noise, within a population can be variable based on factors such as brood size and body size.

Purpose of study

Most research on the effects of noise on animal populations has focused on adults. However, noise exposure during development can not only disrupt parent-offspring communication but may also cause chronic physiological stress during a life stage at which individuals are most vulnerable. In fact, physiological stress in nestlings may be one of the reasons why some avian populations suffer reduced breeding success in noise (Kight and Swaddle 2011, Halfwerk et al. 2011, Mulhollen et al. 2018, Kleist et al. 2018). As well, adverse effects of noise stress on nestlings (e.g. compromised immune responses and altered HPA axis development) can extend into adulthood and be transmitted cross-generationally. Alterations to stress hormones can serve as an indicator that noise may be negatively affecting breeding birds and their young, even if declines in individual fitness or population numbers are not immediately evident (Dantzer et al. 2014). Therefore, it is crucial to understand the effects of chronic noise on the physiology of free-living, developing animals. Such information will be useful in establishing whether conservation efforts should be targeted towards mitigating the effects of noise during nestling development (McGregor et al. 2013).

Thus, the purpose of my study is to determine whether noise exposure during development acts as a chronic physiological stressor to wild Tree Swallow nestlings. In chapter two, I examine whether GC levels of nestlings reared in experimentally-added noise differ from those reared in the absence of noise. I also determine the effects of noise on nestling immune function and test whether GCs mediate these effects. Given that early life experiences may cause stress responses to vary within and across broods, I also examine if and how measures of stress and immune responses vary in response to nestling developmental factors, such as brood size and body size. In chapter 3, I discuss the implications of my results, and how my study fits into the broader framework of the effects of noise on avian populations. I also provide suggestions for future research.

Study species

Breeding biology

Tree Swallows readily accept artificial nest-boxes and so their nesting habitat can be easily manipulated, making this species a useful model for studying the effects of noise. Male Tree Swallows arrive to breeding grounds in Nova Scotia in early May, survey for favourable nest-sites, and begin defending the nest cavity. Females generally arrive later and, after pair formation, begin building the nest. Egg-laying occurs throughout May and June, and clutch sizes range from 2-7 eggs, depending on clutch initiation dates and resource availability (Leonard and Horn 2000, Winkler et al. 2011). Incubation begins after the penultimate egg is laid and lasts for 13-14 days (Clotfelter et al. 2000). Eggs hatch asynchronously over 1-3 days, with earlier-laid eggs hatching before later-laid eggs (Leonard and Horn 1996). Nestlings vary in size within broods, with large broods having greater size variation among nest-mates than small broods (Leonard and Horn 2000). Both parents contribute equally to nest defence, removal of fecal sacs, and feeding nestlings until they fledge between 18 and 22 days after hatching (Michaud and Leonard 2000).

Nestling begging and sibling competition

Tree Swallow parents arriving to the nest with food produce contact calls that elicit a begging response from nestlings (Leonard et al. 1996). Nestlings advertise hunger levels and compete with nestmates for food by producing begging calls and engaging in postural begging (e.g. gaping, stretching towards parent; Leonard et al. 2003) and parents preferentially feed nestlings that beg first, reach higher, are closer to the parent (Leonard and Horn 1996), and call at higher rates (Leonard and Horn 2001a). Begging intensifies as hunger increases and parents respond by increasing the provisioning rate to the brood (Leonard and Horn 2001a,b). Tree Swallow nestlings in larger broods do not receive equal per capita feeding rates compared to nestlings from smaller broods, despite parents modulating their foraging effort in response to brood size (Leonard et al. 2000). As a result, nestlings in larger broods often experience greater sibling competition for

resources and may respond by increasing begging intensity (Leonard and Horn 1998, Leonard et al. 2000).

Parent-offspring communication in noise

Noise disrupts parent-offspring communication in Tree Swallows (Leonard and Horn 2005, 2008, 2012, Leonard et al. 2015). In noise, Tree Swallow nestlings are more likely to miss the arrival of parents with food (Leonard and Horn 2012) and parents are less responsive to begging calls (Leonard et al. 2015). Therefore, Tree Swallow parents in noise may fail to discriminate between nestlings of varying hunger levels and so are less able to preferentially feed nestlings (Leonard and Horn 2005). Also, Tree Swallow nestlings in noise are more likely to miss parental alarm calls, which may cause nestlings to fail to attenuate begging and crouch in the nest when predators are nearby (McIntyre et al. 2014). Tree Swallow nestlings raised in noise have calls of greater amplitude, narrower frequency ranges and greater minimum frequencies compared to nestlings raised in quiet nests, an effect which persists after noise has been removed (Leonard and Horn 2008). These changes in call structure appear to restore communication with parents, thus increasing parental discrimination of begging calls and preferential feeding in noise (Leonard and Horn 2005, Leonard et al. 2015).

CHAPTER 2 : EFFECTS OF NOISE ON NESTLING STRESS PHYSIOLOGY

2.1. INTRODUCTION

Anthropogenic noise is becoming increasingly ubiquitous and is expected to rise globally due to the pervasiveness of noise-producing infrastructure (Shannon et al. 2016). Noise has a range of effects on an array of species across taxa (Kight and Swaddle 2011, McGregor et al. 2013), and may be especially detrimental to species that rely on acoustic signals for communication. One particular communication system that seems to be affected by noise is that used by parent birds and their offspring. Indeed, noise can disrupt signals used by nestling birds to solicit food from parents (Leonard and Horn 2005, 2008, 2012, Leonard et al. 2015) and to avoid predators around the nest (McIntyre et al. 2014, Templeton et al. 2016). This may result in reduced reproductive success in some populations living in noise (Halfwerk and Slabbekoorn 2013). Indeed, species breeding in noisy areas may lay fewer eggs and fledge fewer young (Halfwerk et al. 2011, Schroeder et al. 2012, Mulhollen et al. 2018, Kleist et al. 2018), suggesting that noise is negatively affecting breeding birds and their nestlings, although the mechanism for these effects is not entirely clear (Kight and Swaddle 2011). A proposed explanation for reduced breeding success is that long-term noise exposure induces physiological stress in nestlings by interfering with food solicitation (Leonard and Horn 2005, 2008, 2012, Leonard et al. 2015) for example, or causing fear by masking cues associated with nearby predators (Kight and Swaddle 2011, Francis and Barber 2013, Kleist et al. 2018).

Short-term elevations in stress hormone levels may be helpful to nestling birds by facilitating begging (Kitaysky et al. 2001), as well as vigilance towards parents arriving with food and/or warning of nearby predators (McEwen and Wingfield 2003). However, if stress responses are sustained, there may be physiological costs such as reduced growth (Schwabl and Lipar 2002, McEwen and Wingfield 2003), and immunosuppression (Sapolsky et al. 2000, Saino et al. 2003), as well as altered cognitive (McEwen and Wingfield 2003, Kitaysky et al. 2003) and HPA axis development (Spencer et al. 2009, Zimmer et al. 2013, Zimmer and Spencer 2014). Adverse effects of chronic stress on nestlings, such as altered HPA axis and cognitive development, can also extend into

adulthood and be transmitted cross-generationally, via reduced mating success and parental behaviour, for example (Schoech et al. 2011).

Relatively little is known about the effects of chronic noise exposure on the stress physiology of developing birds, and where research does exist, the results have been inconsistent. For example, some studies have found little to no effect of noise on nestling corticosterone (dominant glucocorticoid in birds; hereafter, "CORT") levels (i.e. Angelier et al. 2016, Flores et al. 2019). Indeed, one study reported that noise exposure reduced nestling stress-induced CORT levels and improved nestling condition (Crino et al. 2013). However, other studies have concluded that noise alters the stress physiology of nestlings by either increasing (i.e. Crino et al. 2011, Injaian et al. 2018a, 2019) or decreasing (i.e. Kleist et al. 2018, Heathcote 2019) CORT levels. Furthermore, it is not always clear that variation in nestling CORT levels is attributable to noise exposure per se, because noise is often associated with other aspects of human developments (e.g. mechanical vibration, chemical pollution, human presence, and/or visual disturbance; Crino et al. 2013). In fact, among studies attributing nestling stress to noise exposure, only Injaian et al. (2018a, 2019), conducted a controlled experiment, where these confounding variables could be eliminated. Here they found that noise increased CORT levels in Tree Swallow nestlings. Clearly, more research is required to elucidate the effects of ambient noise on nestling stress physiology. This may help us to understand the ways in which noise reduces avian reproductive success and, how and at which life stages the effects of noise should be mitigated (McGregor et al. 2013).

The overall goal of my study was to determine whether noise exposure during development acts as a physiological stressor to Tree Swallow nestlings. Specifically, I examined whether baseline (a measure of an individual's baseline stress), stress-induced (a measure of an individual's response to acute stressors), and integrated feather (a measure of average stress overtime) CORT levels differed between nestlings raised in noise compared to control nestlings.

I also examined whether individuals reared in noise exhibited compromised immune responses compared to control nestlings. Specifically, I assessed their T-cell-mediated swelling responses to an injected mitogen (i.e. phytohemagglutinin, hereafter, "PHA")

and the relative proportion of heterophils and lymphocytes (hereafter, "H/L ratio"). Chronic stress is associated with reduced swelling responses (Smits et al. 1999) as well as increased numbers of heterophils and decreased number of lymphocytes (Davis et al. 2008). Reduced swelling responses are indicative of compromised cell-mediated immune responses (Tella et al. 2008) and elevated H/L ratios are indicative of the increased ability to cope with infection by injury (via heterophils) and reduced ability to cope with infection by disease (via lymphocytes; Minias 2019).

Given that competitive environments across and within nests are often variable (Neueschwander et al. 2003, Leonard et al. 2000, 2003, Kozlowski and Ricklefs 2011) and could affect the development and programming of the HPA axis (Spencer et al. 2009, Zimmer et al. 2013), the effects of noise exposure on stress physiology may not be uniform across all nestlings. For this reason, I also examined if and how nestling stress and immune responses varied with factors such as brood size and body size.

If noise acts as a physiological stressor to Tree Swallow nestlings, I expected nestlings exposed to noise during development would exhibit an increase in baseline, stress-induced, and integrated CORT levels (Injaian et al. 2018a, 2019, Beauregard et al. 2019), reduced swelling responses (Ilmonen et al. 2003, Saino et al. 2003), and elevated H/L ratios (Minias et al. 2019) compared to control nestlings. I also predicted that the effects of long-term noise exposure on nestling stress physiology and immunocompetence would be greater for lighter/smaller nestlings in a brood and for nestlings in larger broods as a result of developmental stress from food restriction and sibling competition.

2.2. METHODS

Study site

My study was conducted at four sites (45°5'0"N, 64°22'0"W) in the Gaspereau Valley of Nova Scotia, Canada. Birds at these sites nest in boxes measuring 30 x 15 x 15 cm that are erected on poles approximately 1.5 m above the ground and at least 5 m apart. In 2018, 32 of 93 nest boxes were occupied while in 2019, 30 of 91 nest boxes were occupied. See Leonard and Horn (1996) for a more detailed description of the study sites.

Experimental set-up

Beginning in May, I checked nest-boxes every 2-3 days to determine clutch initiation date and clutch size. This allowed me to project the hatch date of each nest (14-15 days after last egg laid; Robertson et al. 1992) and age nestlings. Once the eggs hatched, nests were checked daily until 14 days post-hatch.

At hatch, I alternately assigned nests to an experimental (i.e. continuous white noise playback; N = 31) or control (i.e. no noise added; N = 31) treatment group. In addition to age, I also balanced treatments for brood size (mean ± S.E. = experimental: 5.03 ± 0.15 nestlings, control: 4.99 ± 0.17 nestlings; $t_{(62)} = -0.21$, $p = 0.83$, Welch Two Sample t-test). White noise playbacks were computer synthesized using Audacity version 2.1 (Free Software Corporation, Boston, MA, 1991) at a resolution of 16 bits and a sampling rate of 44 kHz. The noise ranged in frequency from 0 to 22 kHz, which includes the frequency range of nestling calls (Figure 2.1) and was played at 65 dB, which is at the upper end of ambient noise levels measured inside nest-boxes in the field (41 to 67 dB SPL; Leonard and Horn 2008). Previous studies have shown that exposure to white noise at this level does not affect nestling growth or reproductive success in this population (Leonard and Horn 2008). Experimental and control nests were exposed to a mean (± S.E) ambient sound pressure level (SPL) of 65 ± 0.8 dB and 55 ± 0.8 dB, respectively (Leonard et al. 2015).

Beginning 3 days post-hatch, I placed earbud speakers in the nesting material halfway between the front and back of the nest-box and, in experimental nests, I attached

the speakers to a MP3 player. I programmed the MP3 players to play white noise continuously for 24 hours/day and used a sound level meter (RadioShack CAT. NO. 33-2055) to set the correct volume for the desired sound intensity. I wrapped MP3 players inside plastic bags and pinned them to the underside of nest-boxes. To mimic experimental conditions, I also placed plastic bags at control nest-boxes. I changed batteries in MP3 players daily and, to control for experimenter disturbance, I visited control nests for the same amount of time. I played noise continuously from day 3 to day 14 post-hatch (see Leonard and Horn 2008).

To identify the heaviest and lightest nestling in each brood, I weighed all nestlings to the nearest 0.1 g on day 12 post-hatch. Then, I marked the heaviest and lightest nestling in each brood on the top of the head with non-toxic paint, so I could quickly identify and remove focal nestlings from the nest-box for sampling the next day. To record the final mass of nestlings and ensure that focal nestlings were still heaviest and lightest nestlings in a nest after sampling, I reweighed all nestlings on day 14 post-hatch. As part of the regular monitoring of the study population, I also banded nestlings with a Canadian Wildlife Service band at this time.

Sample collection: Blood and feathers

To measure instantaneous baseline plasma CORT concentrations, I collected blood from the heaviest and lightest nestlings on day 13 post-hatch. I randomly selected whether the heaviest or lightest nestling was bled first in each treatment group, then I alternated the sampling order within experimental and control groups. I individually removed nestlings from nest-boxes, punctured the caudal tibial vein (accessible in smaller birds and less vulnerable to hematomas; Campbell and Ellis 2013) using a 26 gauge needle and removed 40 µl of blood using heparinized microcapillary tubes. I set a timer to ensure I collected all baseline blood samples in less than 1.5 minutes after restraint, because plasma CORT in birds typically increases after 1.5 minutes of an acute disturbance such as handling (Romero and Reed 2005).

To measure stress-induced plasma CORT concentrations, I took an additional blood sample 30 minutes after capture when CORT levels typically plateau following an

acute disturbance (Wingfield et al. 1995). Between sampling periods, nestlings were placed in a cloth bag and kept in the shade approximately 15 m from the nest (Wingfield et al. 1995). In wild birds, stress hormones vary throughout the day (Dantzer et al. 2014) so I aimed to balance nests across treatment groups for sample collection times (mean ± S.E. = experimental: 10:59 am ± 0.36 hours, control: 10:50 am ± 0.32 hours; $t_{(62)}$ = -0.28, p = 0.84, Welch Two Sample t-test). I returned the nestling to the nest after sampling. I then waited at least 2 hours before sampling the second nestling. I immediately placed all blood samples on ice for a maximum of 2 hours until they could be transported to a lab at Acadia University (< 6 km from study sites).

To measure the cumulative effect of noise on nestling stress physiology, I also measured CORT in feather samples collected in the 2019 field season. Unlike blood samples, which measure instantaneous baseline and stress-induced CORT concentrations at the time of sampling, feather samples integrate both baseline and acute responses to stressors and reflect the average CORT individuals have secreted and metabolized throughout development (Dantzer et al. 2014). To sample feather CORT, I removed five contour feathers from the flanks of the heaviest and lightest nestling on day 14 post-hatch by gently pulling at the rachis with forceps (Fairhurst et al. 2013). I stored feather samples in paper envelopes. All sampling methods were approved by the Animal Care and Use Committee at Dalhousie University (protocol #18-051).

Assessment of nestling stress: Corticosterone determination

I centrifuged blood samples to separate plasma from red blood cells (11,500 R.P.M. for 10 minutes at 4°C) within 2 hours of sample collection. I stored plasma in microcentrifuge tubes at -80°C and feathers in envelopes at room temperature until being shipped to Gabriela Mastromonaco, curator of Reproductive Programs and Research at the Toronto Zoo, to determine CORT concentrations. All samples were analyzed at the end of the 2019 field season to reduce assay variation between years.

Plasma CORT was extracted using modifications of a triple diethyl ether extraction (Covino et al. 2017). Plasma extracts were evaporated in a 50°C water bath and reconstituted with 150 ml enzyme immunoassay buffer. Feather CORT was extracted

using a methanol-based technique (Bortolotti et al. 2008) that has previously been used on Tree Swallow nestlings (Fairhurst et al. 2013). After the addition of methanol, samples were incubated in a 50°C water bath for 24 hours. Methanol from feather extracts was evaporated in a fume hood and dried feather extracts were reconstituted with 150 ml of enzyme immunoassay buffer.

Plasma and feather CORT concentrations were determined using an enzyme immunoassay (previously described by Baxter-Gilbert et al. 2014). Dilutions of a known purified CORT extract produced a standard curve of CORT concentrations (pg) in relation to the amount of antibody bound (%). This standard curve was used to determine the unknown CORT concentrations in plasma and feather extracts. Plasma and feather extracts were measured on 7 and 2 plates respectively, and intra- and inter-assay variation were 4.4% and 9.8% respectively, which is similar to what is reported in other populations of Tree Swallows (e.g. Stedman et al. 2017). The sample size for the analysis of nestling plasma CORT was reduced due to occasional lysis of plasma samples during the centrifugation process (Table 2.1).

Assessment of nestling immune responses

T-cell mediated immune responsiveness/ Phytohemagglutinin (PHA) skin test

To assess nestling immune responses, I performed a PHA skin test (after Smits et al. 1999) on the heaviest and lightest nestling immediately following blood sampling on day 13 post-hatch. Prior to PHA injections, I measured the thickness of the right wing-web to the nearest 0.01 mm using a digital micrometer. Then, I injected 40 μg of PHA dissolved in 20 μl of sterile phosphate buffered saline (PBS) subcutaneously using a 26 gauge needle following methods by Smits et al. (1999). I returned nestlings to the nest immediately after PHA injections. To record nestling swelling response to PHA injections, I re-measured wing-web thickness 24 ± 2 hours later. To minimize measurement error, I measured each right wing-web three times and used the mean in analyses. I also established repeatability of wing-web measurements early in each breeding season ($r = 0.96$; Smits et al. 2001). Nestling swelling response is represented by the difference in the wing-web thickness before and after the PHA injection, with a

large swelling response indicating a strong immune response (Smits et al. 1999). It is sometimes practice to inject the left wing-web with the same volume of phosphate buffered saline (PBS) to serve as a control for the effect of injection. This, however, increases handling time and stress to the nestlings, and adding additional measurements may increase the coefficient of variation due to measurement inaccuracies (Smits et al. 1999). For these reasons, I did not do a control injection. The sample size for the analysis of nestling swelling responses was reduced because of inclement weather in which I did not disturb nests (n = 4), delays in the arrival of the PHA (n = 8) and brood loss (n = 1; Table 2.1).

White blood cell profiles

To assess nestling H/L ratios, I prepared a blood smear for each blood sample collected from nestlings on day 13 post-hatch. Immediately after collecting the stress-induced blood sample, I placed a small drop of blood from the microcapillary tube at the end of a pre-cleaned, beveled-edged microscope slide. To make the blood smear, I used the edge of a cover slip to make contact with the drop of blood at a 45° angle and to spread cells across the microscope slide (Campbell and Ellis 2013). This produced a thin layer of blood with "feathered" edges. I air-dried slides in the field and, upon returning to the lab, stained slides using a JorVet Dip Quick Stain Introductory Kit (Jorgensen Laboratories Inc., J0322) which is comparable to the Wright-Giesma staining method (standard in veterinary hematology; Walberg 2001). This stain allowed for discrimination between different types of white blood cells (hereafter, "WBC"). Staining procedures followed manufacturer recommendations (procedure WSGD-128, Sigma Chemicals, St. Louis, MO). Slides were kept in a closed box to avoid contamination and sun exposure until being sent to the Ontario Veterinary College at the University of Guelph for differential WBC counts. Elevated H/L ratios are associated with chronic stress and indicative of the increased ability to cope with infection by injury (via heterophils) and reduced ability to cope with infection by disease (via lymphocytes; Davis et al. 2008, Minias 2019). Blood smears were prepared for nestlings in the 2018 field season only (Table 2.1).

Statistical analyses

I created mixed models using the "lme4" package in R (RStudio team 2016). To determine whether nestling stress responses vary in response to treatment, I created separate generalized linear mixed-effect models (GLMMs) for baseline, stress-induced, and feather CORT levels using the "glmer" function (Love and Williams 2008, Angelier 2016, Crino et al. 2020). I ran these GLMMs with Gaussian distributions and log link functions. In some cases, CORT samples did not meet the minimum detection limit of CORT concentrations used in enzyme immunoassays (36/114 baseline plasma CORT samples and 8/60 feather CORT samples). Concentrations below the detection limit, were assigned 0.06 ng/ml for plasma CORT and 0.30 ng/g for feather CORT, which were the lowest concentrations of CORT detected among samples (as recommended in previous studies; e.g. Bauer et al. 2016, Stedman et al. 2017, Kleist 2018). Feather CORT concentrations are typically corrected by feather length, because the rate at which feathers grow may affect the rate of CORT deposition into feather tissue (Patterson et al. 2014). It was, however, difficult to accurately measure the feathers because of their small size and so instead, I used feather CORT concentrations corrected by total mass of feathers (ng CORT g $^{-1}$; e.g. Lodjak et al. 2015, Beauregard et al. 2019). To determine whether nestling immune responses vary in response to treatment, I created separate linear mixed-effect models (LMMs) for PHA swelling responses and H/L ratios using the "lme" function. H/L ratios were log-transformed to improve normality (Vennum et al. 2019). I examined normality and homoscedasticity of all models by using the Shapiro Wilk's test on residuals, and visual examination of residual plots and histograms.

I included a binary treatment factor (i.e. 'experimental' or 'control') as a fixed effect in all models. To examine whether the competitive environment between and within nests affected nestling stress and immune responses to treatment, I included 'brood size' (number of nestlings at hatch) and 'nestling size' as fixed covariates in all models. In preliminary analyses, I considered nestling size as either mass on day 14 post-hatch or as a binary factor (i.e. 'heavy' or 'light'). However, the experience of the smallest nestling in a brood of three, for example, is likely to be different than the experience of the smallest nestling in a brood of seven, thus these measures may not

represent the competitive environment within a nest. Indeed, nestling mass decreased (β = -0.47, S.E.: 0.20, p = 0.04, Linear regression) and mass difference between the heaviest and lightest nestling increased (β = 0.25, S.E.: 0.11, p = 0.03, Linear regression) with brood size. To better account for competitive environment within nests, I used the mass difference between each focal nestling and the brood mean on day 14 post-hatch as a measure of nestling size. It is worth noting that 34 of 124 nestlings originally ranked the heaviest and lightest nestlings in their brood on day 12, were no longer the heaviest and lightest nestlings in their brood on day 14 post-hatch. Of these nestlings, 24 were originally classified as "the heaviest" and 10 were classified as "the lightest". Of the 24 originally ranked as the heaviest, 15 had decreased by one rank and nine had decreased by two ranks. Of the 10 originally ranked as the lightest, nine had increased by one rank and one had increased by two ranks. In one these cases, this involved a reversal where the lightest nestling became the heaviest in a brood of three. With the exception of this, the heavy focal nestlings remained heavier than the light focal nestlings in each nest.

To control for variation in nest site quality and repeated sampling within nests, I included 'nest ID' as a random effect in all models. Although I alternated the order in which I sampled heavy and light nestlings within experimental and control groups, the second nestling sampled in each nest was exposed to an acute disturbance ~2 hours before when the first nestling was removed and so may have slightly elevated plasma CORT levels compared to their previously-sampled nestmate. To control for this effect, I also included 'sample order' (i.e. first or second nestling sampled) as a random effect in the final model for baseline and stress-induced plasma CORT.

Plasma and feather CORT concentrations, swelling responses and H/L ratios may also vary temporally by year and sample collection date, especially if weather conditions across years or within a breeding season are variable (Lifjeld et al. 2002, Fairhurst et al. 2012, Crino et al. 2020). This is because inclement weather may affect food availability and nestling thermoregulation (McCarty and Winkler 1999, Winkler et al. 2013). To control for temporal variation in stress and immune responses, I included 'sample collection date' as a predictor in all models, and 'year' as a predictor in models for baseline and stress-induced plasma CORT concentrations, and swelling responses. I

omitted 'year' from the feather CORT and H/L ratio models because data were collected in one year only for each procedure. To determine whether immune responses are affected by nestling stress physiology, I included baseline, stress-induced, and/or feather CORT as predictors in the linear mixed-effect model for nestling swelling responses and H/L ratios. I used a *dredge* function in R to create and rank model sets of covariate combinations and exclude non-significant predictors. To select optimal models, I used Akaike Information Criterion corrected for small sample sizes (AICc) and model weights.

Because results showed that nestling baseline CORT concentrations and immune responses varied by year and sample collection date (see Results), I examined the effect of weather on stress and immune responses in post-hoc analyses to understand this temporal variation (Fairhurst et al. 2012, Crino et al. 2020). I also wanted to understand whether some of the observed effects of noise could be increased or decreased by environmental conditions. I downloaded all weather data from the Environment Canada Weather Station in Kentville, NS (< 20 km from study sites). I created separate GLMMs for baseline, stress-induced, and feather CORT (gaussian distribution, log link) and LMMs for swelling responses and log-transformed H/L ratios. To determine the effects of immediate weather conditions on stress responses, I included temperature (°C) and wind speed (km/h) from the hourly reading closest to blood sampling time as predictors in separate models (Crino et al. 2020). I did not measure precipitation at the time of sampling because I did not sample when it was raining. To examine effects of weather conditions during development on stress and immune responses, I included the average of minimum daily temperatures (°C), maximum daily temperatures (°C), total daily precipitation (mm), and maximum daily wind gust speeds (km/h, related to mean daily wind speed; Weggel 1999) over the 13 days prior to sampling (i.e. duration of development) as predictors in separate models (Fairhurst et al. 2012, Crino et al. 2020). I included 'nest ID' as a random factor in all models and 'sample order' as a random factor in GLMMs for plasma CORT concentrations. Means are presented ± S.E.

2.3. RESULTS

Baseline and stress-induced plasma corticosterone concentrations

Stress-induced plasma CORT concentrations were significantly higher than baseline plasma CORT concentrations (baseline: 1.06 ± 0.13 ng/ml, stress-induced: 12.85 ± 0.95 ng/ml, $t_{(53)} = -12.26$, $p < 0.001$, Welch Two Sample t-test; Figure 2.2). Neither baseline nor stress-induced CORT concentrations differed significantly between treatments (baseline: $F_{1,64} = 0.16$, $p = 0.65$, stress-induced: $F_{1,67} = 0.02$, $p = 0.66$; Table 2.2, Figure 2.2). There was, however, a significant interaction between treatment and competitive environment within nests for both baseline and stress-induced CORT concentrations (Table 2.2). Specifically, baseline CORT concentrations increased with an increasingly positive difference in nestling mass relative to the brood mean (i.e. nestlings are heavier relative to the mean) in the experimental treatment but not in the control treatment ($F_{1,64} = 8.85$, $p = 0.01$; Table 2.2, Figure 2.3). In contrast, stress-induced CORT concentrations decreased with an increasingly positive difference in nestling mass relative to the brood mean in the experimental treatment but not in the control treatment ($F_{1,67} = 8.37$, $p = 0.004$; Table 2.2, Figure 2.4).

Integrated feather corticosterone concentrations

Feather CORT concentrations did not differ significantly between treatments ($F_{1,27} = 0.10$, $p = 0.74$; Table 2.2, Figure 2.5). There was, however, a significant interaction between treatment and competitive environment within nests (Table 2.2). Specifically, feather CORT concentrations increased with an increasingly positive difference in nestling mass relative to the brood mean in the experimental treatment and decreased with an increasingly positive difference in nestling mass relative to the brood mean in the control treatment ($F_{1,29} = 14.52$, $p < 0.001$; Table 2.2., Figure 2.6).

Assessment of nestling immune responses

T-cell mediated immune responsiveness/ Phytohemagglutinin (PHA) skin test

Overall, swelling responses did not differ significantly between treatments ($F_{1,23}$ = 1.07, p = 0.22; Figure 2.7). Across treatments, there was a significant interaction between brood size and the difference in nestling mass relative to the brood mean (Table 2.3). Specifically, as brood size increased, swelling responses increased with an increasingly positive difference in nestling mass relative to the brood mean ($F_{1,19}$ = 4.91, p = 0.04; Figure 2.8).

Baseline CORT concentrations and feather CORT were significant predictors of nestling swelling responses (Table 2.3). Specifically, nestling swelling responses increased in relation to baseline CORT concentrations ($F_{1,19}$ = 7.55, p = 0.01) and feather CORT concentrations ($F_{1,19}$ = 7.14, p = 0.01; Figure 2.9).

White blood cell profiles

Nestling H/L ratios did not differ significantly between treatments ($F_{1,17}$ = 0.01, p = 0.88; Figure 2.10) or in response to nestling size within nests (Table 2.3). Measures of nestling CORT concentrations were included as predictors in the full model of nestling H/L ratio, but H/L ratios did not vary in response to nestling CORT.

Year and weather effects on stress and immune responses

Nestling baseline CORT concentrations varied by year and sample collection date (Table 2.2). Specifically, baseline CORT concentrations were higher for nestlings in 2018 than 2019 ($F_{1,64} = 5.68$, $p = 0.02$; Figure 2.11) and in both years, concentrations increased significantly throughout the breeding season ($F_{1,64} = 8.01$, $p = 0.001$; Figure 2.12). The variable 'year' was omitted from the final model for nestling swelling responses because feather CORT was a significant predictor and was only collected in 2019. However, nestling swelling responses varied significantly by year in post-hoc analyses. Specifically, swelling responses were stronger for nestlings in 2018 compared to 2019 (2018: 0.71 ± 0.01 mm, 2019: 0.64 ± 0.03 mm; $t_{(49)} = 4.32$, $p = <0.001$, Welch Two Sample t-test; Figure 2.13). Finally, H/L ratios varied by sample collection date with H/L ratios decreasing significantly throughout the breeding season ($F_{1,17} = 4.57$, $p = 0.05$). However, this relationship was mainly driven by nestling H/L ratios in nests initiated late in the breeding season. These values were identified as influential according to Cook's distances and after the removal of influential values (n = 4), there was no significant relationship between H/L ratios and sample collection date.

To help explain the temporal variation observed above, I examined the effect of weather on stress and immune responses in post-hoc analyses. Nestling baseline but not stress-induced CORT concentrations increased significantly in response to temperature ($F_{1,69} = 3.92$, $p = 0.05$) and wind speed ($F_{1,69} = 17.09$, $p < 0.001$) at the time of sampling (Figure 2.14). CORT levels did not vary significantly in response to average daily minimum and maximum temperatures, average daily precipitation or average daily maximum wind gust speed in the 13 days prior to sampling. However, swelling responses decreased in response to increasing average daily minimum temperatures and to increasing average daily precipitation (temperature: $F_{1,57} = 9.44$, $p = 0.002$, precipitation: $F_{1,57} = 7.89$, $p = 0.01$) in the 13 days prior to sampling (Figure 2.15).

2.4. DISCUSSION

Overall, Tree Swallow nestlings raised in added experimental noise did not exhibit altered stress physiology compared to nestlings that were not. However, noise appeared to alter nestling stress physiology in relation to size. Specifically, in noise, nestlings that were lighter relative to the brood mean (hereafter, "lighter nestlings") showed decreased baseline CORT and increased stress-induced CORT compared to nestlings that were heavier relative to the mean (hereafter, "heavier nestlings"). There was, however, no relationship between nestling size and baseline or stress-induced CORT levels in the control treatment. In noise, nestlings that were lighter also showed decreased integrated feather CORT concentrations, while in the control treatment they exhibited increased feather CORT compared to their heavier counterparts. These results suggest that the effects of noise may be context-dependent, with differential effects depending on nestling size and potentially, competitive ability, within a nest. Despite potential alterations to the HPA axis, nestlings in noise did not exhibit reduced cell-mediated immune responses or increased H/L ratios.

Noise and nestling corticosterone

Contrary to my predictions, noise exposure did not affect CORT levels in nestling Tree Swallows, suggesting that noise alone does not alter their resting physiological state nor their ability to respond to acute stressors. This is in contrast to a number of previous studies showing that noise exposure in developing birds is associated with changes in CORT including both elevated (Crino et al. 2011, Injaian et al. 2018a, 2019) and reduced (Crino et al. 2013, Heathcote 2019, Kleist et al. 2018) levels. Indeed, another study on the effects of experimentally-added noise on nestling stress in Tree Swallows, found that nestling baseline and, to a lesser degree, stress-induced CORT levels were positively related to the amplitude of experimentally-added traffic noise surrounding nests (Injaian et al. 2018a, 2019).

There are a few explanations for why my study may differ from those finding an effect of noise on nestling CORT levels. Firstly, the noise I used in my experiment differed from the noise used in other experimental studies. Specifically, I used continuous white noise, while the other studies all used either recordings of highway noise (Crino et al. 2013, Angelier et al. 2016, Injaian et al. 2018a, 2019, Flores et al. 2019) and/or city streets (Flores et al. 2019). This could have several implications. Traffic noise may be less predictable, with sudden peaks in amplitude that may startle nestlings more so than continuous noise (Rich and Romero 2005, Rosa and Koper 2018). Conversely, because continuous white noise is more predictable, it may be easier for nestlings to habituate or physiologically desensitize to this noise than to traffic noise (Cyr and Romero 2009). If so, it might explain why I found no overall effect of noise.

Unfortunately, I cannot examine whether habituation occurred because nestlings were not sampled repeatedly throughout their development to determine if CORT levels decreased over time in noise (Walker et al. 2005a, 2006, Cyr and Romero 2009), as would be expected if they had habituated. It is worth noting, however, that nestling Tree Swallows did exhibit behavioral changes in response to the same noise playbacks used in my study (Leonard and Horn 2005, 2008, 2012, Leonard et al. 2015), which suggests that they are not habituating to the noise.

Secondly, it is not clear in some of these studies (Crino et al. 2011, Kleist et al. 2018, Heathcote 2019) that it is noise exposure per se that caused the change in physiology. These studies examined the effects of roads (Crino et al. 2011) or noise from oil and gas extraction fields (Kleist et al. 2018, Heathcote 2019) on nestling stress physiology. In these cases, it is difficult to disentangle the role of noise exposure on nestling CORT levels from confounding factors, such as mechanical vibration, chemical pollution, human presence, and/or visual disturbance (Crino et al. 2013, Mulhollen et al. 2018).

To summarize, it is not entirely clear why I did not see overall effects of noise on nestling stress physiology that have been observed in other studies. I did find, however,

that nestling competitive environment appeared to affect physiological responses to noise exposure in Tree Swallows.

The role of nestling competitive environment

I found a relationship between noise and CORT levels related to nestling size within the brood. Specifically, in the noise treatment only, baseline CORT increased, and stress-induced CORT decreased in relation to nestling size. Furthermore, in the noise treatment, feather CORT increased in relation to nestling size and in the control treatment, it decreased in relation to size.

Baseline corticosterone

Baseline CORT levels increased in relation to nestling size in noise, with the lightest nestlings in each brood showing lower baseline CORT levels than their heavier counterparts. This suggests that nestlings may respond differently to the same anthropogenic stimuli depending on body size and competitive stress (Crino et al. 2011, Dantzer et al. 2014).

Lighter nestlings are likely to experience fewer feedings and more intense competition for those feedings than their heavier siblings (Leonard et al. 2003). In noise, these challenges may be intensified because Tree Swallow parents are less responsive to nestling calls (Leonard et al. 2015) and less able to identify nestlings calling at a higher rate, which they preferentially feed (Leonard and Horn 2005). Therefore, lighter nestlings in noise may experience more stress associated with food solicitation, and possibly less food than heavier nestmates and nestlings in quiet environments. Lower baseline CORT levels may help them to avoid the deleterious increases in energy mobilization associated with higher CORT levels and allow them to redirect energy towards other functions such as growth (Love and Williams 2008, Zimmer and Spencer 2014). Indeed, lower levels of baseline CORT have been observed in nestlings raised in noisy conditions (Kleist et al. 2018, Heathcote 2019). For example, Chestnut-collared Longspur (*Calcarius ornatus*) nestlings exhibited lower baseline CORT with increasing proximity to noise-emitting

infrastructure (Heathcote 2019). Here, reducing baseline CORT was considered to be a protective response to a sub-optimal habitat (Heathcote 2019).

Although lower CORT levels in lighter nestlings may be beneficial in noise, lower baseline CORT levels can also indicate that individuals have exceeded their allostatic load and are unable to produce sufficient concentrations of baseline CORT to cope with chronic stressors or challenging environmental conditions (i.e. hypocorticism; Fries et al. 2005). This appeared to be the case in a study where nestlings in three cavity-nesting species raised in a noisy natural gas field exhibited lower baseline CORT levels than nestlings raised in quieter areas (Kleist et al. 2018). Hypocorticism is an indicator of serious physiological stress and often associated with downstream, negative fitness effects (Rich and Romero 2005, Cyr and Romero 2007). For example, in Kleist et al. (2018), nestlings in noise exhibiting hypocorticism also experienced reduced feather development and body size, suggesting that they were not successfully coping with environmental stressors. In my study, lighter nestlings raised in noise did not exhibit reduced cell-mediated immunity or the redistribution of immune cells, and can respond to acute stressors. Therefore, the lower levels of baseline CORT observed in these nestlings is likely a controlled downregulation of the HPA axis in response to chronic stress as opposed to hypocorticism (Fries et al. 2005) or physiological exhaustion (Rich and Romero 2005, Cyr and Romero 2007).

In contrast, heavier nestlings typically outcompete lighter nestlings for food (Leonard et al. 2003) and therefore likely have smaller allostatic loads and larger energy reserves than lighter nestlings (McEwan and Wingfield 2003, Pravosudov and Kitaysky 2006). As a result, heavier nestlings may be more able to elevate baseline CORT to respond to challenges in the environment without inducing phenotypic damage. Indeed, heavier nestlings in noise may benefit from elevated baseline CORT, which can facilitate increases in begging intensity and access to more food (Kitaysky et al. 1999, 2001, Leonard and Horn 2005). Similar to this, elevated baseline CORT is associated with greater body sizes in White-crowned Sparrow (*Zonotrichia leucophrys*) nestlings near roads (Crino et al. 2011). This is further evidence that increasing baseline CORT in response to anthropogenic noise may be beneficial in some situations (Boonstra 2013). Given my finding that elevated baseline CORT confers greater cell-mediated immune

responses, I can speculate that heavier nestlings in noise may be successfully coping with noise exposure.

Stress-induced corticosterone

Stress-induced CORT levels decreased in relation to nestling size in noise, with lighter nestlings showing higher stress-induced CORT levels than their heavier counterparts. This suggests that differences in body size and competitive stress may result in different responses to acute stressors in noise (Kitaysky 1999, Pravosudov and Kitaysky 2006, Love and Williams 2008).

As mentioned above, lighter nestlings in noise may experience more stress than their heavier nestmates and nestlings in quiet environments. HPA axis alterations that reduce baseline CORT in chronic stress can sometimes enhance stress responses to novel stressors (i.e. facilitation; Romero 2004), which may explain why lighter nestlings in noise exhibit greater stress-induced CORT despite lower baseline CORT. Similarly to my study, the nestlings in cavity-nesting species mentioned above that exhibited dampened baseline CORT levels in response to increasing noise exposure, also exhibited exaggerated stress-induced CORT compared to nestlings in quieter areas (Kleist et al. 2018). Higher levels of stress-induced CORT in nestlings exposed to noise have also been observed in a study on Tree Swallows (Injaian et al. 2018a). In both of these studies, nestlings exhibiting elevated stress-induced CORT were considered chronically stressed as a result of noise exposure. These results are consistent with the suggestion that lighter nestling in noise are under a greater allostatic load and therefore may exhibit physiological alterations consistent with chronic stress (McEwen and Wingfield 2003).

Heavier nestlings in noise had lower stress-induced CORT than lighter nestlings. This is not likely because their HPA axis has been downregulated, as would be expected in response to chronic stress, because these nestlings are able to produce greater baseline CORT concentrations compared to lighter nestmates (Cyr and Romero 2007, Crino et al. 2013). Instead, lower stress-induced CORT levels could be a result of a trade-off with baseline CORT levels (Vitousek et al. 2018). The reactive scope model proposes that over a particular threshold, CORT causes phenotypic damage (Romero et al. 2009). If

baseline CORT is high in response to a chronic stressor, an exaggerated stress response is likely to exceed this threshold. As a result, nestlings in noise may trade-off stress-induced CORT for baseline CORT and vice versa, in order to avoid crossing this threshold. In fact, in my study, there is support for this theory. That is, stress-induced CORT decreased with increasing baseline CORT in the noise treatment (β = -0.42, S.E.: 0.16, p = 0.01, Linear regression), suggesting a trade-off between the two. A similar trade-off between baseline and stress-induced CORT also has been observed in adult Tree Swallows during breeding (Vitousek et al. 2018). This is further evidence that mounting a strong stress-response may be more costly for heavier nestling than for lighter nestlings that have reduced baseline CORT in noise. With this said, despite the benefits of reducing stress-induced CORT for these nestlings in the presence of noise, this response could be costly in certain environments (e.g. high predator density; Vitousek et al. 2014) because acute stress responses allow individuals to escape life-threatening situations (Blas et al. 2007).

Integrated feather corticosterone

Feather samples integrate both baseline and acute responses to stressors over time (Dantzer et al. 2014). Measures of feather CORT may therefore more closely reflect a nestling's cumulative exposure to CORT during development than instantaneous measures of plasma CORT (Dantzer et al. 2014).

In control nests, lighter nestlings released greater levels of CORT over time than their heavier counterparts, which was not reflected in instantaneous measures of baseline or stress-induced plasma CORT. Higher CORT may facilitate increases in begging intensity (Kitaysky et al. 1999, 2001), which is beneficial for lighter nestlings in securing food over heavier nestmates. In fact, lighter nestlings often exhibit higher CORT compared to heavier nestlings in the absence of added noise (Tarlow et al. 2001, Walker et al. 2005b, Pravosudov and Kitaysky 2006, Wada et al. 2009) and this effect has been observed in feather CORT levels of Tree Swallow nestlings (Fairhust et al. 2013).

Conversely, in noisy nests, lighter nestlings released lower levels of CORT over time compared to their heavier counterparts. This is consistent with the idea that lighter nestlings may experience a greater allostatic load from sibling competition and noise

exposure and they exhibit alterations that reduce baseline CORT levels. Heavier nestlings that have a smaller allostatic load, may benefit more from higher levels of baseline CORT to cope with challenges of living in a noisy environment.

Overall, my results suggest that noise exposure may differentially affect individuals, which may be related to other stressors they are experiencing. This might cause individuals within the same nest to react differently to the same anthropogenic stressor. It should be noted that while this interpretation is seemingly the most parsimonious, there are several unknowns in this system that make it difficult to draw firm conclusions. More information is needed regarding mechanisms underlying physiological changes, repeatability of baseline and acute stress responses across time, and relationships between nestling stress physiology and fitness. Regardless, my results suggest noise effects may be context-dependent, which may also explain the variation in the results of previous studies on the impacts of noise on nestling stress responses.

Immune responses

Given that noise did not appear to affect nestling baseline, stress-induced, or integrated CORT levels overall, it is unsurprising that swelling responses to PHA or H/L ratios did not differ between treatments. With this said, swelling responses generally appeared to be affected by CORT levels in this study, with swelling responses increasing by 0.06 mm for every 1 ng/ml increase in baseline CORT and by 0.01 mm for every 1 ng/g increase in feather CORT. This result suggests that higher levels of baseline CORT are associated with greater cell-mediated immunocompetence (Tella et al. 2008) and may suggest that there is a cost to reducing CORT levels in this system. This is contrary to what I expected, given that elevated baseline and stress-induced CORT suppress the synthesis and proliferation of immune cells (Dhabhar 2009, Sapolsky et al. 2000) and nestlings with elevated CORT often have reduced swelling responses to PHA (Saino et al. 2003, Ilmonen et al. 2003, Loiseau et al. 2008). One explanation for this pattern is that elevated baseline and integrated CORT in this system are not pathological, but rather indicate that nestlings are successfully coping with and responding to stressors in their

environment and can invest energy in their immune system (i.e. "CORT-activity hypothesis"; Bonier et al. 2009a, Dhabar 2009).

I also found that lighter nestlings in larger broods in both noise and control treatments had weaker immune responses than heavier nestlings. As discussed above, lighter nestlings may be less able to compete with heavier nestmates for food, and this effect is likely exaggerated in larger broods where resources are limited and sibling competition is more intense than in smaller broods (Leonard et al. 2000, Neueschwander et al. 2003, Kozlowski and Ricklefs 2011). As a result, lighter nestlings in larger broods may experience suppression of cell-meditated immune responses as a direct consequence of stress-induced changes to immune cells or as an adaptive response to shift energy from the immune system towards other processes such as growth and development (Dhabhar 1995, Lifjeld et al. 2002, Tella et al. 2008). This result is consistent with previous studies finding that sibling competition and shortage of resources result in immunosuppression in nestlings (Lifjeld et al. 2002, Ilmonen et al. 2003, Saino et al. 2003).

Overall, these results show that higher baseline and integrated CORT confer stronger immune responses, which may suggest that elevated CORT levels observed in heavier nestlings exposed to noise are not necessarily deleterious but there may be costs associated with reduced CORT levels observed in lighter nestlings exposed to noise. Furthermore, nestling immune responses may be affected by their competitive environment, which reinforces that developmental conditions of nestlings within a population vary and this might be the basis for variable physiological responses to noise exposure.

Weather effects

I found that baseline CORT was higher in 2018 than 2019 and increased over the breeding season in both years. Similarly, swelling responses were greater in 2018 than 2019. These results suggest that nestling stress and immune responses are affected by environmental conditions both across and within breeding seasons.

Baseline corticosterone

Baseline CORT concentrations increased significantly with ambient temperature at the time of sampling. I found that temperatures at the time of sampling were significantly higher in 2018 than 2019 (2018: 19.41 ± 0.56 °C, 2019: 15.59 ± 0.42 °C; $t_{(58)} = 5.77$, $p < 0.001$, Welch Two Sample t-test; Figure 2.16) and increased significantly throughout breeding seasons ($\beta = 0.17$, S.E.: 0.06, $p = 0.005$, Linear regression; Figure 2.16). Together, this may explain the patterns I observed across and within seasons. CORT plays an important role in thermoregulation by facilitating behaviours such as panting or wing-spreading in warmer temperatures (Sapolsky et al. 2000, Fairhurst et al. 2012). Therefore, elevated CORT may help promote these behaviours and keep nestlings from overheating. Indeed, Tree Swallow nestlings in another population exhibited increased feather CORT in response to rising nest-box temperatures (Fairhurst et al. 2012). Elevated CORT also promotes shivering in cold weather (Sapolsky et al. 2000). However, in my study, it is more likely that CORT promoted cooling behaviours, because parental brooding and proximity to nestmates may buffer nestlings from the effects of cold temperatures (Fairhurst et al. 2012).

Weather conditions that reduce food abundance may also affect baseline CORT levels. Consistent with this, I found that baseline CORT concentrations increased significantly with wind speed at the time of sampling. Wind speeds were significantly higher in 2018 than in 2019 (2018: 11.05 ± 0.49 km/h, 2019: 5.16 ± 0.35 km/h; $t_{(58)} = 9.87$, $p < 0.001$, Welch Two Sample t-test; Figure 2.16), which may be another reason why baseline CORT was higher in 2018 than in 2019. High wind speeds that reduce the abundance of flying insects can decrease parental provisioning (McCarty and Wrinkler 1999). In response, nestlings may elevate baseline CORT to mobilize protein for short-term energy and increase begging intensity to signal hunger and compete for limited resources (Brillon et al. 1995, Sapolsky et al. 2000, Kitaysky et al. 2001).

Immune responses

Swelling responses decreased in response to increasing average daily minimum temperatures and average daily precipitation over the 13 days before sampling. Average daily minimum temperatures (2018: 6.32 ± 0.32 °C, 2019: 11.42 ± 0.07 °C; $t_{(58)} = -15.79$, $p < 0.001$, Welch Two Sample t-test) and average daily precipitation (2018: 2.80 ± 0.11 mm, 2019: 5.88 ± 0.27 mm; $t_{(58)} = -10.12$, $p < 0.001$, Welch Two Sample t-test) were significantly lower in 2018 than in 2019 (Figure 2.17). This may explain why swelling responses were higher in 2018 than 2019.

Sustained cold temperatures and precipitation likely impose stress due to reduced insect availability (McCarty and Wrinkler 1999, Winkler et al. 2013), and are consequently associated with nestling growth and survival (McCarty 2001, Cox et al. 2019). As such, an increase in nestling swelling responses in response to cold temperatures is unexpected (Lifjeld et al. 2002) and suggests that minimum temperatures may be related to other weather conditions that have a greater influence on cell-mediated immune responses. Indeed, average daily precipitation increased significantly with increasing average daily minimum temperatures ($\beta = 0.48$, S.E.: 0.05, $p = 0.01$, $p < 0.001$, Linear regression), and so it is possible that nestling swelling responses are more influenced by precipitation.

Nestling baseline CORT and swelling responses are affected by weather conditions at the time of sampling and over the course of development. Inclement weather is likely to have significant impacts on nestling thermoregulation as well as food availability, parental provisioning, and sibling competition (McCarty and Winkler 1999, McCarty 2001, Winkler et al. 2013), all of which are likely stressors to nestlings during development. Thus, the effect of noise on nestlings could vary with climatic conditions and be potentially greater in years with poor weather conditions.

Conclusion

In my study, noise appeared to have context-dependent effects depending on nestling size within a nest, suggesting that cumulative stress from exposure to noise and other stressors might cause individuals within the same nest to react differently to the same anthropogenic stressor. Specifically, lighter nestlings in noise had reduced baseline CORT, greater stress-induced CORT, and reduced integrated feather CORT compared to their heavier counterparts. I expected that if nestlings in noise were under chronic stress, they would exhibit reduced cell-mediated immune responses or increased H/L ratios, but this was not the case. However, light nestlings in noise exhibit physiological alterations consistent with those observed in chronically stressed nestlings exposed to noise in three other species of cavity-nesting birds (Kleist et al. 2018). As well, these nestlings may exhibit negative fitness effects that I did not measure (e.g. oxidative damage; Injaian et al. 2018b).

Furthermore, alterations to the HPA axis may be protective responses to increased allostatic loads in noise, however, future negative effects may ensue if alterations to the HPA axis persist long-term and physiological phenotypes programmed during development do not suit environments encountered by individuals across life stages (Monaghan 2008, Angelier and Wingfield 2013). The downregulation of baseline CORT observed in lighter nestlings in noise may be particularly harmful if sustained (Fries et al. 2005, Kleist et al. 2018). Chronically reduced baseline CORT has been associated with inflammation, disease susceptibility (Langgartner et al. 2015) and hypersensitivity to novel stressors (Uschold-Schmidt et al. 2012). If alterations that reduce baseline CORT persist into adulthood, individuals may also experience reduced exploration (Slatterry et al. 2012), and even reduced reproductive success (Dickens and Romero 2013), including reduced nestling growth rates (Bonier et al. 2009b, Vitousek et al. 2018) and hatching success (Kleist et al. 2018). This suggests nestlings exhibiting physiological alterations which reduce baseline CORT can bear negative reproductive effects in the future.

Similarly, the increased stress-induced CORT observed in lighter nestlings in noise is associated with reduced post-fledging survival (Blas et al. 2007) and the suppression of reproduction (Wingfield and Sapolsky 2003) if sustained. Reduced reproductive success as a result of elevated stress-induced CORT was observed in breeding Tree Swallows (Ouyang et al. 2011, Vitousek et al. 2014, 2018), as well as in a range of other species (Wingfield et al. 1995, Angelier et al. 2009).

With this said, little is known about the repeatability of individual CORT levels from development into adulthood in Tree Swallows (Stedman et al. 2017) and so I can only speculate about how alterations to stress physiology during development translates into long-term fitness. More research is needed to fully understand the repeatability of baseline and stress-induced CORT across life stages and how alterations to stress physiology during development affect future fitness and reproductive success across environmental contexts.

2.5. TABLES

Table 2.1. Number of broods (N), mean, and standard error (S.E.) of baseline and stress-induced plasma CORT concentrations (ng/ml), feather CORT concentrations (ng/g), swelling responses (mm difference in wing-web thickness before and after PHA injection) and heterophil/lymphocyte (H/L) ratios for Tree Swallow broods in the experimental and control groups.

	Experimental			Control		
	N	Mean	S.E.	N	Mean	S.E.
Baseline plasma CORT	26	1.00	0.17	27	1.12	0.21
Stress-induced plasma CORT	26	12.74	1.46	27	12.95	1.26
Feather CORT	15	7.23	1.14	15	6.49	0.88
Swelling Response	22	0.68	0.02	27	0.67	0.03
H/L Ratio	9	0.95	0.17	11	1.00	0.14

Table 2.2. Final generalized linear mixed-effect models detailing the best predictors for baseline, stress induced, and feather CORT for Tree Swallow nestlings in relation to the experimental treatment, brood size, and nestling mass difference from brood mean (i.e. nestling size relative to other nestmates). Year and sample collection date were included as predictors in models for baseline and stress induced CORT. Conditional R^2 values are specified.

	β-estimate	S. E.	t-value	p-value	Model R^2
Baseline plasma CORT					0.65
Intercept	-13.440	3.862	-3.26	*<0.001	
Treatment	-0.135	0.301	-0.45	0.65	
Nestling mass difference from brood mean	0.033	0.087	0.38	0.70	
Brood size	0.214	0.114	1.87	0.06	
Sample collection date	0.076	0.023	3.26	*0.001	
Year	-0.812	0.343	-2.37	*0.018	
Treatment: Nestling mass difference from brood mean	0.313	0.118	2.64	*0.008	
Stress-induced plasma CORT					0.22
Intercept	2.399	0.769	3.12	*0.002	
Treatment	-0.108	0.244	-0.44	0.66	
Nestling mass difference from brood mean	0.087	0.051	1.71	0.09	
Brood size	0.017	0.079	0.22	0.79	
Treatment: Nestling mass difference from brood mean	-0.235	0.081	-2.89	*0.004	
Feather CORT					0.27
Intercept	2.159	1.725	1.13	0.26	
Treatment	0.243	0.719	0.34	0.74	
Nestling mass difference from brood mean	-0.255	0.082	-3.71	*0.002	
Brood size	-0.118	0.326	-0.36	0.72	
Treatment: Nestling mass difference from brood mean	0.348	0.091	3.81	*<0.001	

* denotes a significant variable term (p-value ≤ 0.05).

Table 2.3. Final linear mixed-effect models detailing the best predictors for nestling phytohemagglutinin (PHA) swelling responses and H/L ratios for Tree Swallow nestlings in relation to the experimental treatment, brood size, and nestling mass difference from brood mean (i.e. nestling size relative to other nestmates). Year and sample collection date were included as predictors in models for swelling responses and H/L ratios. Conditional R^2 values are specified.

	β-*estimate*	S. E.	t-value	*p- value*	Model R^2
Swelling response					0.37
Intercept	0.597	0.134	4.44	*<**0.001**	
Treatment	0.063	0.050	1.25	0.22	
Nestling mass difference from brood mean	-0.212	0.107	-1.98	0.06	
Brood size	-0.028	0.024	-1.14	0.26	
Baseline CORT	0.061	0.020	2.99	***0.007**	
Feather CORT	0.013	0.005	2.78	***0.012**	
Brood size: Nestling mass difference from brood mean	0.044	0.020	2.22	***0.039**	
H/L ratio					0.34
Intercept	-0.247	0.490	-0.50	0.62	
Treatment	-0.043	0.275	-0.16	0.88	
Nestling mass difference from brood mean	-0.072	0.070	-1.02	0.32	
Brood size	-0.001	0.094	-0.01	0.99	

* denotes a significant variable term (p-value ≤ 0.05).
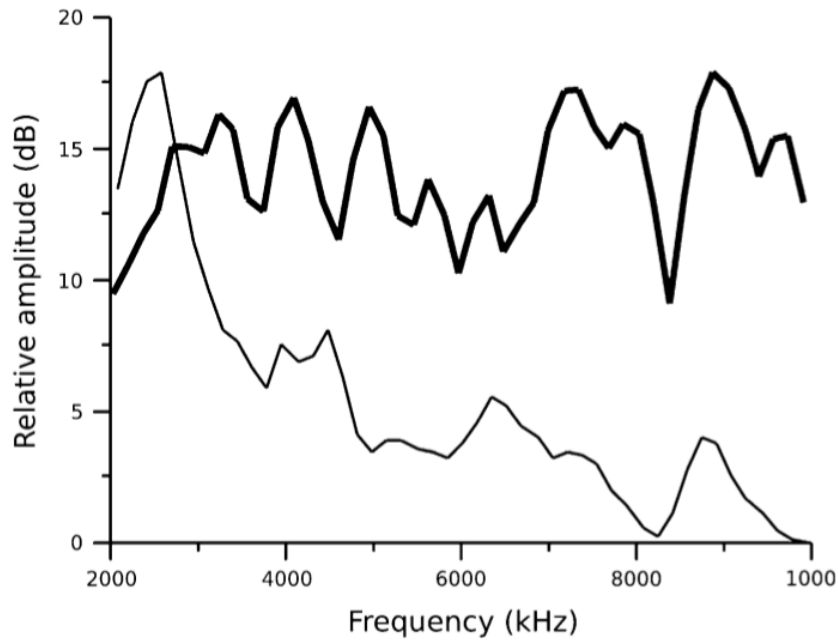
2.6. FIGURES

Figure 2.1. Sample spectra of ambient noise at an experimental nest (bold line) and at a control nest (light line). Spectra were calculated and produced in a previous study by Leonard and Horn (2008) on Tree Swallows using a filter bandwidth of 270 Hz for 10s of sound.
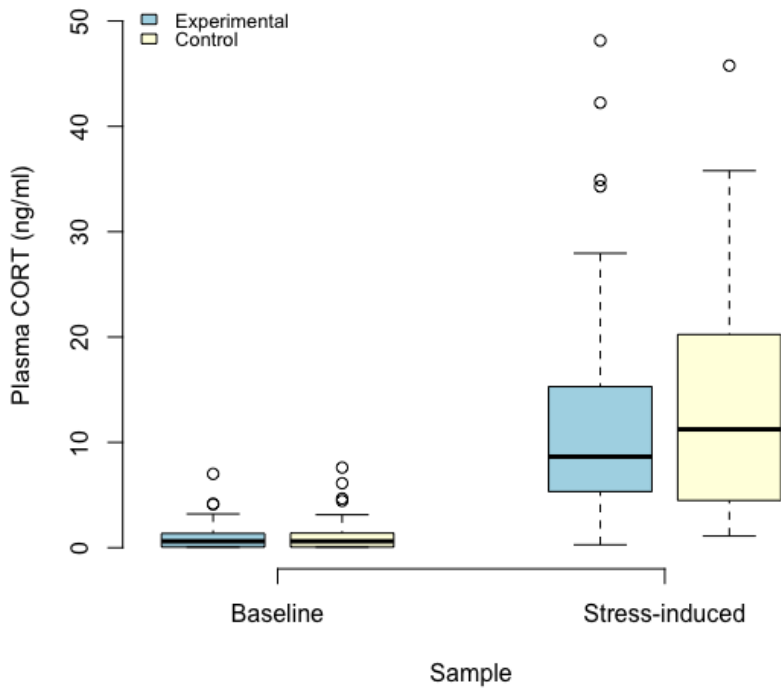
Figure 2.2. Boxplot of baseline and stress-induced plasma CORT concentrations (ng/ml) for Tree Swallow nestlings in experimental and control treatments on day 13 post-hatch. Boxes represent interquartile ranges of plasma CORT concentrations and the dark horizontal lines in the centre of boxes indicate the median. Whiskers represent the spread of plasma CORT values and circles represent outliers based on interquartile ranges.

Figure 2.3. Baseline plasma CORT concentrations (ng/ml) in relation to mass difference (g) from the brood mean (i.e. nestling size relative to other nestmates) for the heaviest and lightest Tree Swallow nestlings in experimental and control treatments on day 13 post-hatch. The x-axis is a continuum where negative values represent nestlings that are lighter relative to the brood mean, positive values represent nestlings that are heavier relative to the brood mean, and 0-values represent nestlings that are similar in mass to the brood mean.

Figure 2.4. Stress-induced plasma CORT concentrations (ng/ml) in relation to mass difference (g) from the brood mean (i.e. nestling size relative to other nestmates) for the heaviest and lightest Tree Swallow nestlings in experimental and control treatments on day 13 post-hatch. The x-axis is a continuum where negative values represent nestlings that are lighter relative to the brood mean, positive values represent nestlings that are heavier relative to the brood mean, and 0-values represent nestlings that are similar in mass to the brood mean.
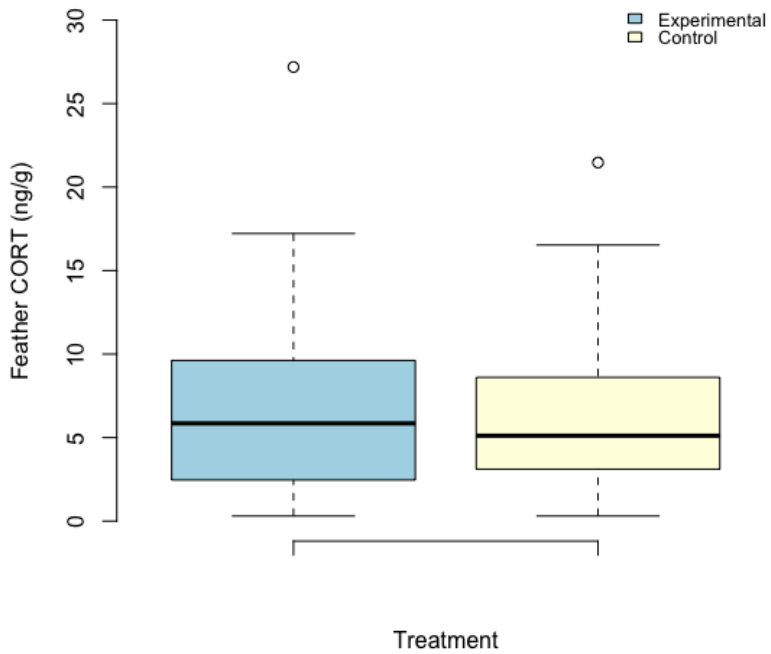
Figure 2.5. Boxplot of feather CORT concentrations (ng/g) for Tree Swallow nestlings in experimental and control treatments on day 14 post-hatch. Boxes represent interquartile ranges of feather CORT values and the dark horizontal lines in the centre of boxes indicate the median. Whiskers represent the spread of feather CORT values and circles represent outliers based on interquartile ranges.
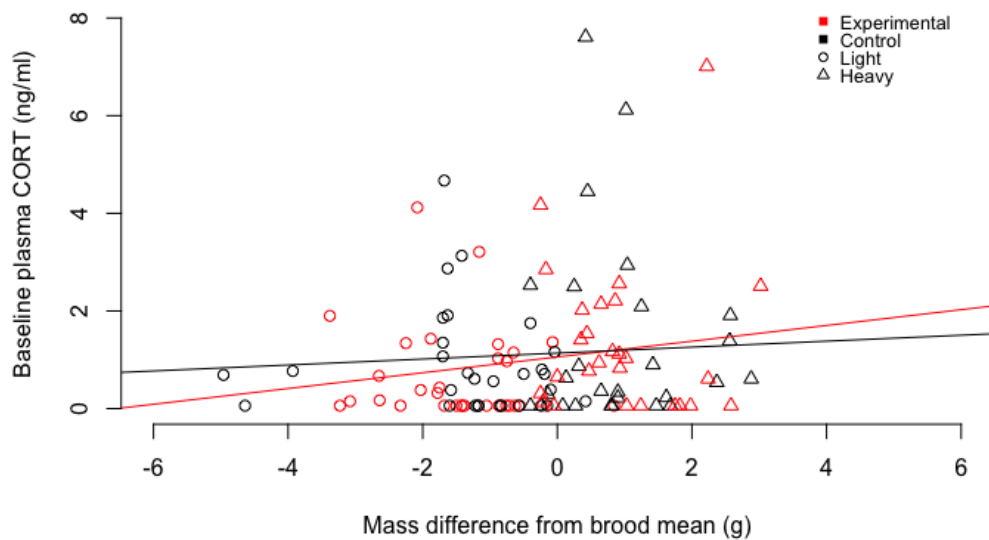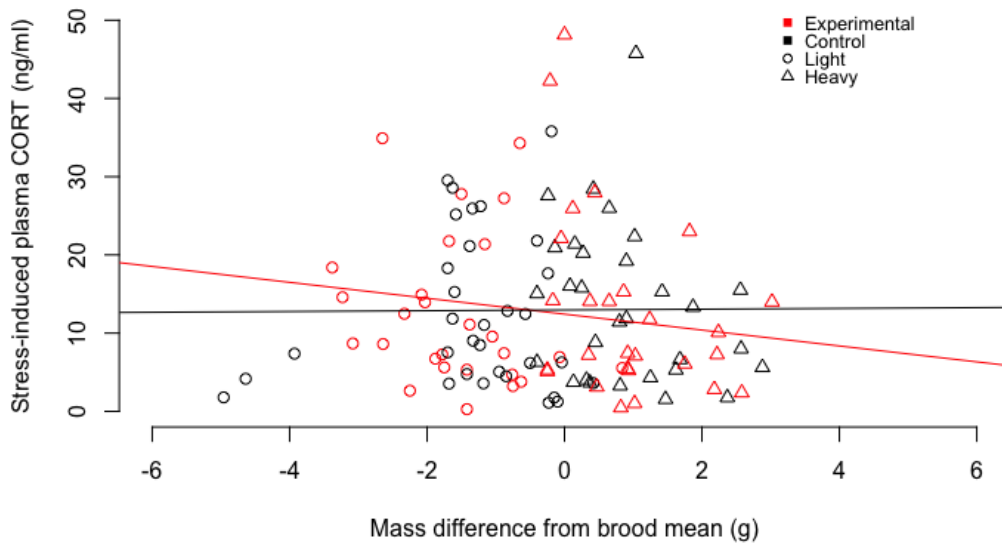
Figure 2.6. Feather CORT concentrations (ng/g) in relation to mass difference (g) from the brood mean (i.e. nestling size relative to other nestmates) for the heaviest and lightest Tree Swallow nestlings in experimental and control treatments on day 14 post-hatch. The x-axis is a continuum where negative values represent nestlings that are lighter relative to the brood mean, positive values represent nestlings that are heavier relative to the brood mean, and 0-values represent nestlings that are similar in mass to the brood mean.

Figure 2.7. Boxplot of phytohemagglutinin (PHA) swelling responses (mm difference in wing-web thickness before and after PHA injection) for Tree Swallow nestlings in experimental and control treatments on day 14 post-hatch. Boxes represent interquartile ranges of swelling values and the dark horizontal lines in the centre of boxes indicate the median. Whiskers represent the spread of swelling values and circles represent outliers based on interquartile ranges.
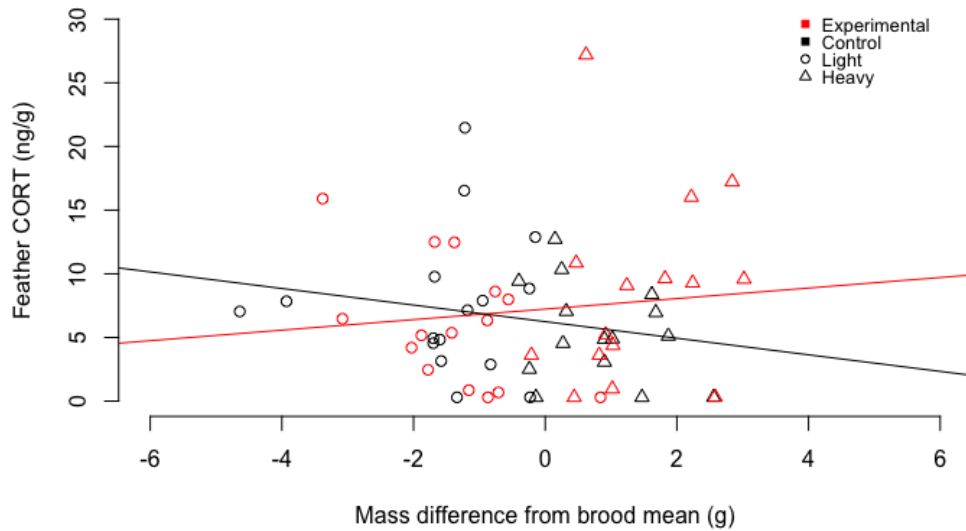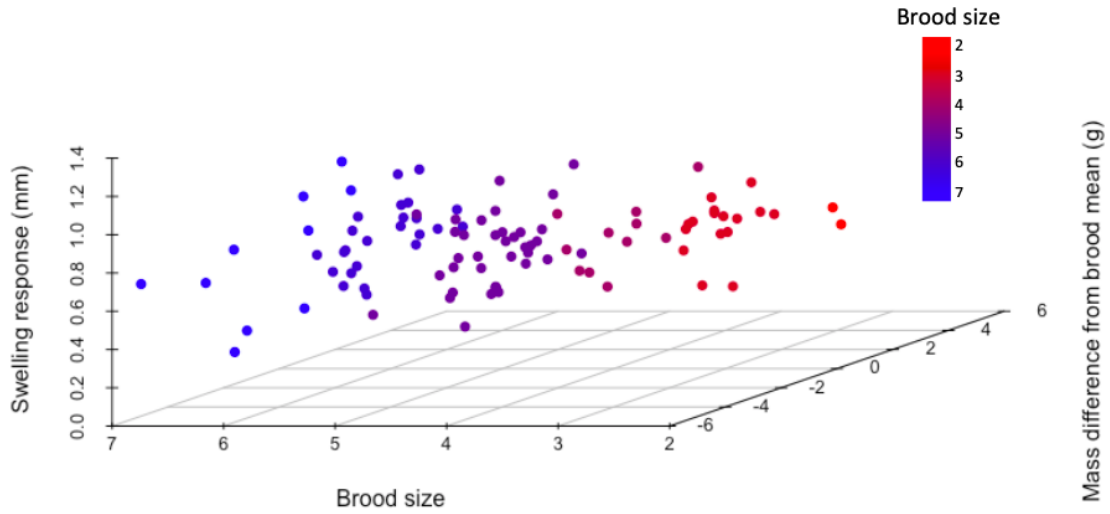
Figure 2.8. Phytohemagglutinin (PHA) swelling responses (mm difference in wing-web thickness before and after PHA injection) in relation to mass difference (g) from the brood mean (i.e. nestling size relative to other nestmates) and brood size for Tree Swallow nestlings on day 14 post-hatch. 'Mass difference (g) from the brood mean' is a continuum where negative values represent nestlings that are lighter relative to the brood mean, positive values represent nestlings that are heavier relative to the brood mean, and 0-values represent nestlings that are similar in mass to the brood mean.
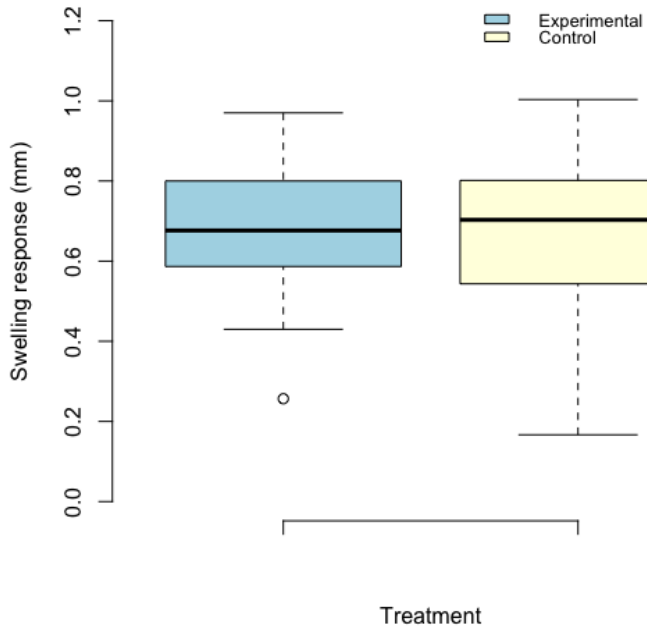
Figure 2.9. Phytohemagglutinin (PHA) swelling responses (difference in wing-web thickness (mm) before and after PHA injection) for Tree Swallow nestlings on day 14 post-hatch in relation to (A) baseline plasma CORT concentrations (ng/ml) and, (B) feather CORT concentrations (ng/g).

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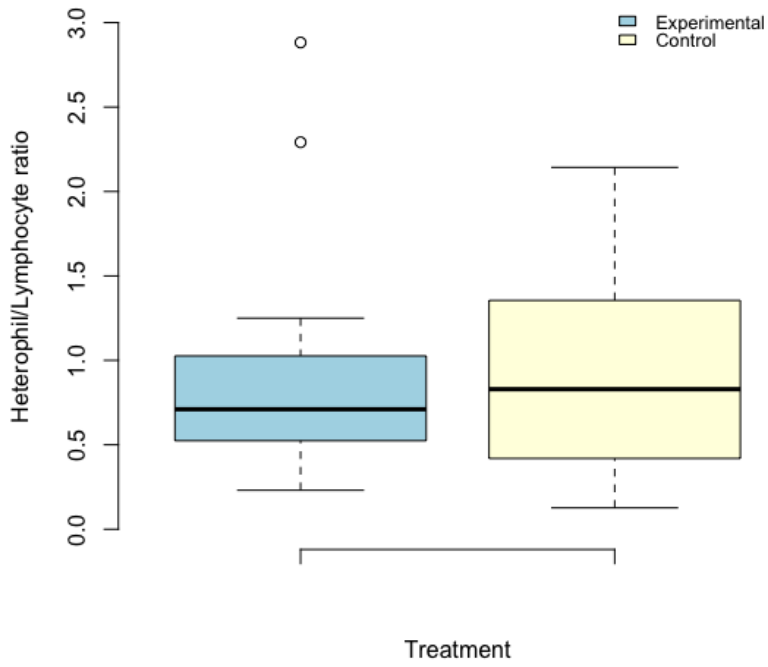
Figure 2.10. Boxplot of heterophil/lymphocyte (H/L) ratios for Tree Swallow nestlings in experimental and control treatments on day 13 post-hatch. Boxes represent interquartile ranges of H/L ratio values and the dark horizontal lines in the centre of boxes indicate the median. Whiskers represent the spread of H/L ratio values and circles represent outliers based on interquartile ranges.

Figure 2.11. Boxplot of baseline plasma CORT concentrations (ng/ml) for Tree Swallow nestlings on day 13 post-hatch in 2018 and 2019. Boxes represent interquartile ranges of baseline plasma CORT values and the dark horizontal lines in the centre of boxes indicate the median. Whiskers represent the spread of baseline plasma CORT values and circles represent outliers based on interquartile ranges.
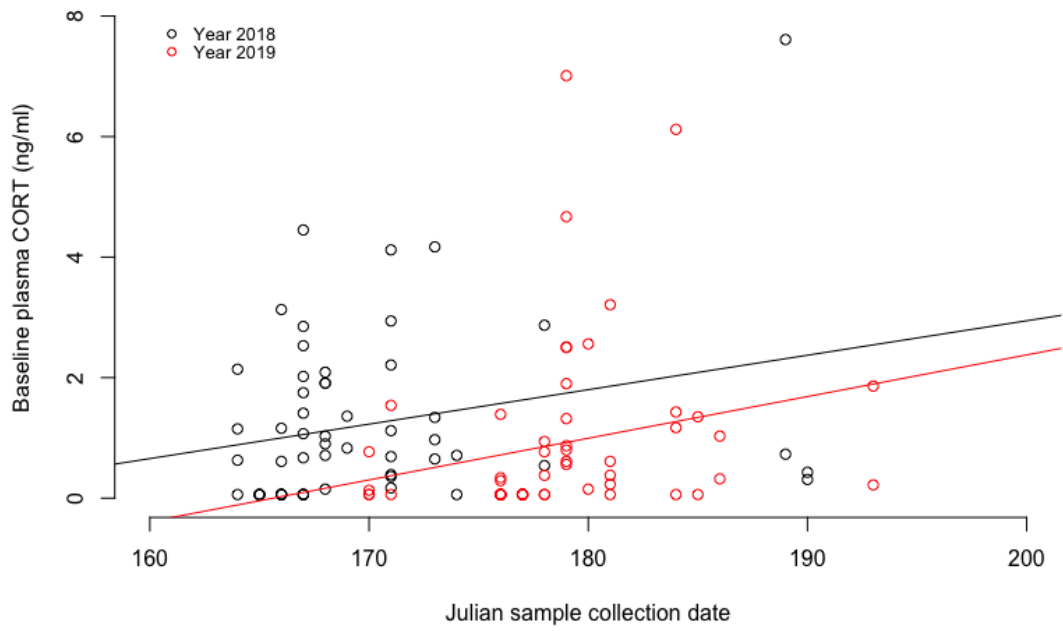
Figure 2.12. Baseline plasma CORT concentrations (ng/ml) in relation to the Julian date of sample collection for Tree Swallow nestlings on day 13 post-hatch in 2018 and 2019.
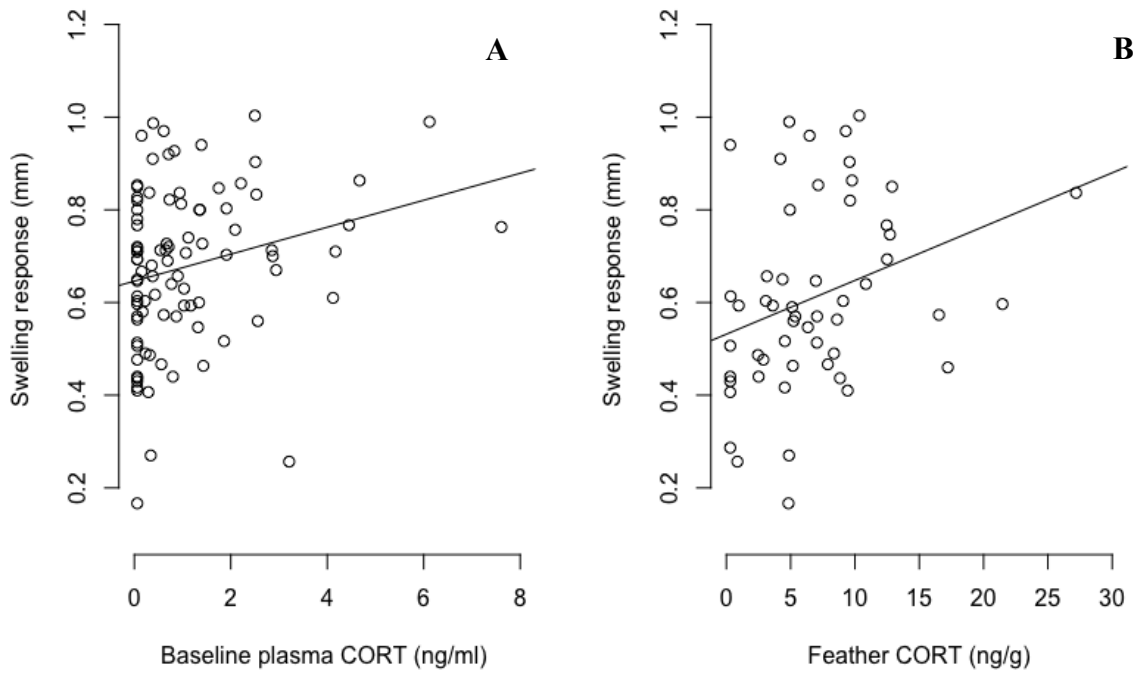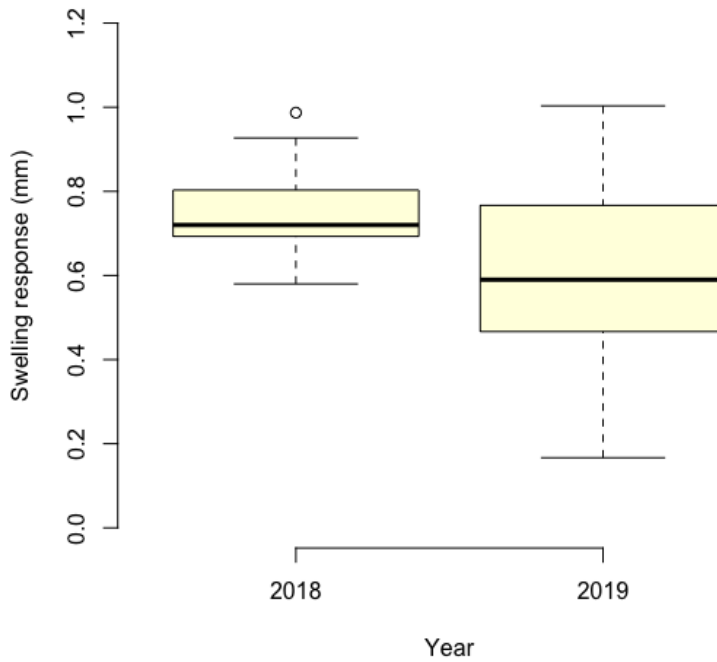
Figure 2.13. Boxplot of phytohemagglutinin (PHA) swelling responses (mm difference in wing-web thickness before and after PHA injection) for Tree Swallow nestlings on day 14 post-hatch in 2018 and 2019. Boxes represent interquartile ranges of PHA swelling values and the dark horizontal lines in the centre of boxes indicate the median. Whiskers represent the spread of PHA swelling values and circles represent outliers based on interquartile ranges.
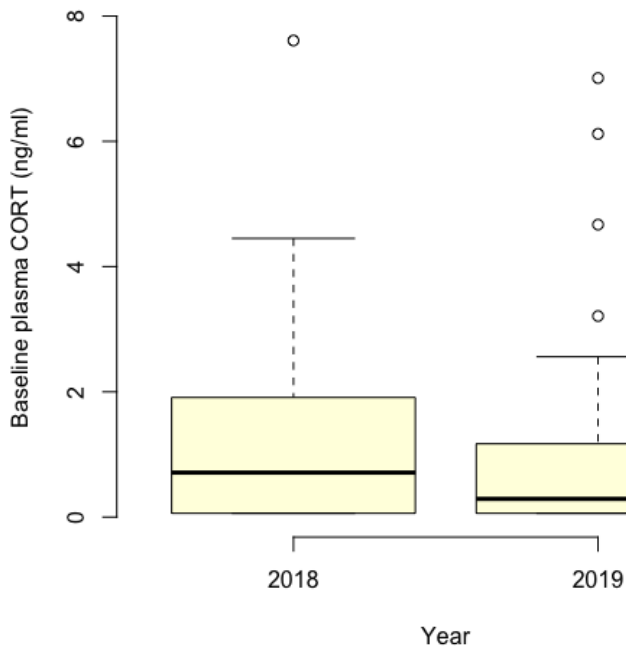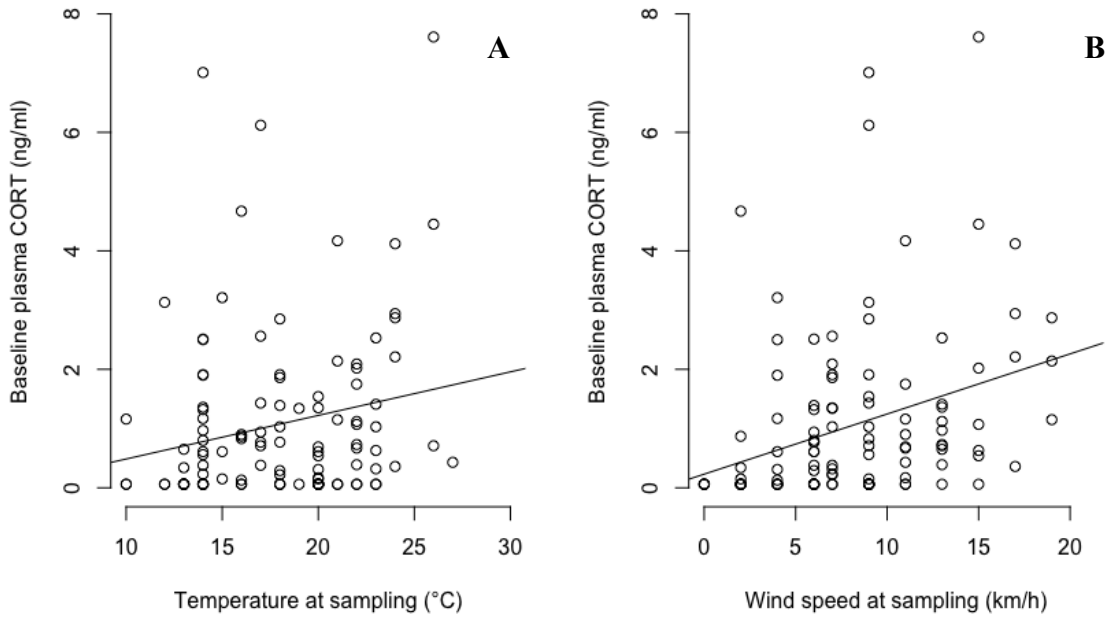
Figure 2.14. Baseline plasma CORT concentrations (ng/ml) for Tree Swallow nestlings on day 13 post-hatch in relation to (A) the temperature (°C) and (B) the wind speed (km/h) at the time of sampling.
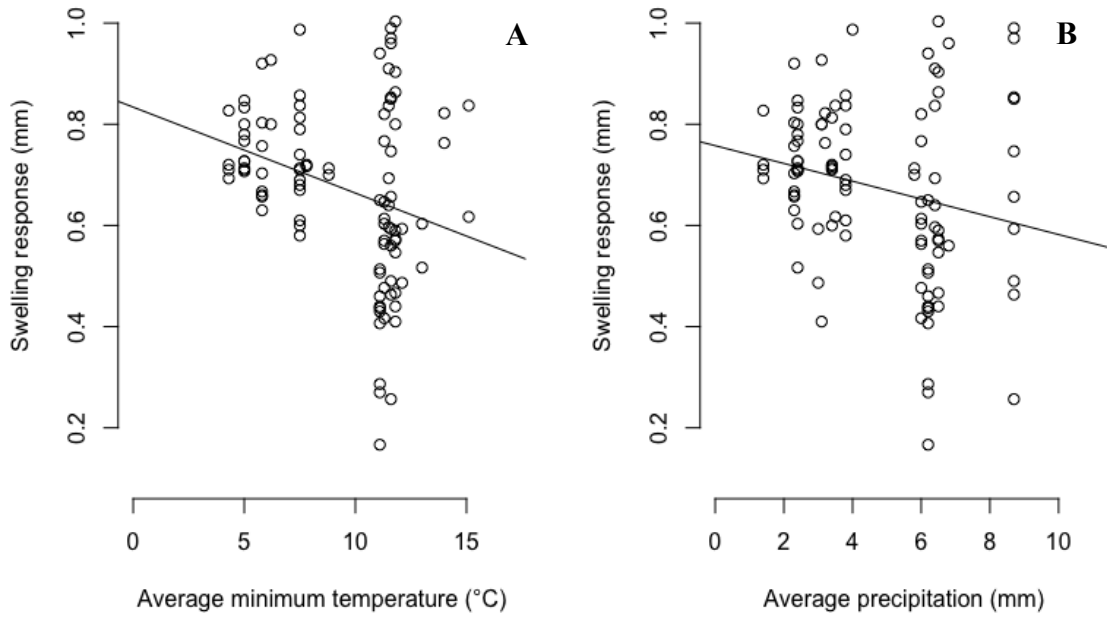
Figure 2.15. Phytohemagglutinin (PHA) swelling responses (mm difference in wing-web thickness before and after PHA injection) for Tree Swallow nestlings on day 14 post-hatch in relation to the (A) average daily minimum temperatures (°C) and (B) average daily precipitation (mm) over 13 days prior to sampling.
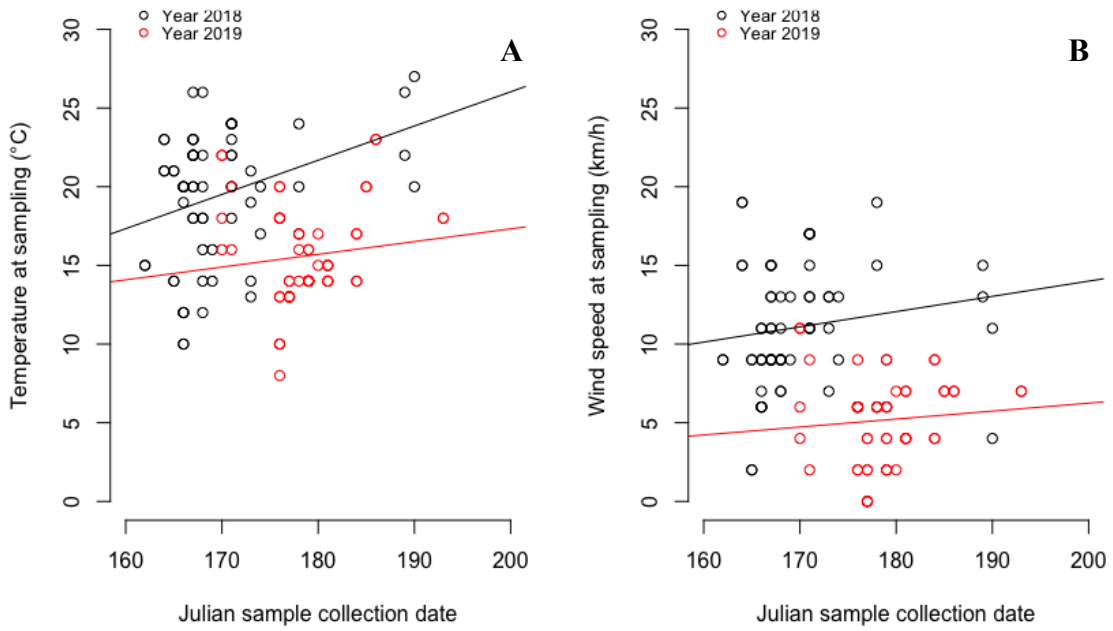
Figure 2.16. (A) Temperature (°C) and (B) wind speed (km/h) at the time of sampling in relation to the Julian date of sample collection in 2018 and 2019.
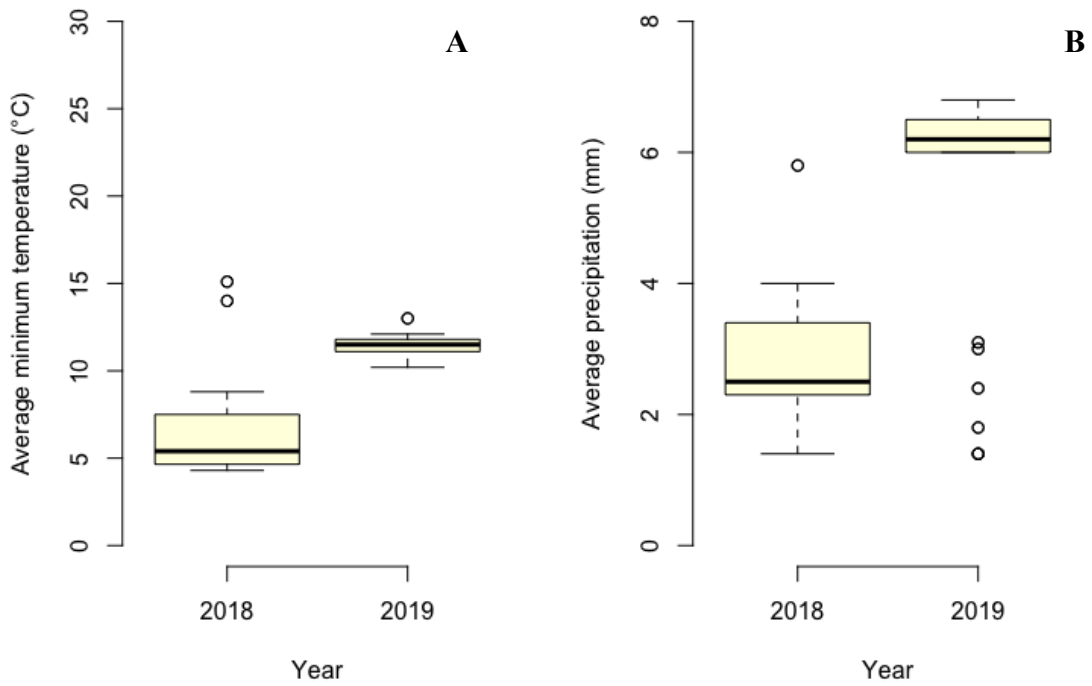
Figure 2.17. Boxplot of the (A) average daily minimum temperatures (°C), and (B) average daily precipitation (mm) over the 13 days prior to sampling in 2018 and 2019. Boxes represent interquartile ranges of temperature and precipitation values and the dark horizontal lines in the centre of boxes indicate the median. Whiskers represent the spread of temperature and precipitation values and circles represent outliers based on interquartile ranges.

CHAPTER 3 : GENERAL DISCUSSION

In my study, noise exposure appeared to alter nestling stress physiology but not in a straightforward way. Specifically, for nestlings potentially experiencing stress associated with competition for resources, noise exposure may have altered the HPA axis in ways that lowered baseline CORT levels and sensitized responses to acute stressors. My results showed that even at low levels, noise may push nestlings already experiencing stress (e.g. sibling competition for resources) closer to a threshold above which these combined stressors result in physiological effects. This study adds to a small but growing body of evidence that exposure to noise during development affects nestling stress physiology, and highlights that these effects may vary depending on developmental conditions.

Implications of developmental stress for populations breeding in noise

The results of my study provide some perspective from which to consider the effects of noise on stress physiology and how chronic stress fits into the broader framework of noise effects on avian populations. Many mechanisms have been proposed to explain why birds nesting in noise experience reduced reproductive success, including the inability to assess mate quality, altered settlement patterns, reduced parental quality, and disruptions to parent-offspring communication (Barber et al. 2010, Halfwerk et al. 2011, Halfwerk and Slabbekoorn 2013, Shannon et al. 2016, Rosa and Koper 2018). Of these, noise-induced disruptions to parent-offspring communication appear to have the most support. Disruptions to parent-offspring communication have been shown to directly reduce reproductive success in some studies (Schroeder et al. 2012, but see Leonard et al. 2015, Injaian 2018b) or result in chronic physiological stress, which, in turn, may reduce reproductive success (Kight and Swaddle 2011, Mulhollen et al. 2018, Kleist et al. 2018).

That noise induces chronic stress in passerine nestlings is supported by recent studies (Kleist et al. 2018, Injaian et al. 2018a,b, 2019, Heathcote 2019). Chronically stressed nestlings in noise have been found to exhibit reduced body condition (Injaian et al. 2018a), feather development (Kleist et al. 2018), and growth (Injaian et al. 2018b,

Kleist et al. 2018), increased oxidative stress (Injaian et al. 2018b), and delayed fledging (Injaian et al. 2018b) in Tree Swallows and in three other species of cavity nesting birds (Injaian et al. 2018a,b, Kleist et al. 2018). These effects may in part, be attributed to disruptions to parent-offspring communication (Kleist et al. 2018), however, in Tree Swallows, it appears that negative fitness consequences are direct effects of chronic stress on nestlings (Injaian et al. 2019).

My results suggest that noise has context-dependent effects on nestlings, which is consistent with a large body of evidence suggesting that effects of chronic stressors on individual stress physiology are context-dependent (Dickens and Romero 2013). While overall, noise, at least at the levels presented in my study, does not negatively affect nestling growth (Leonard and Horn 2008) or immune function, my results suggest that effects of noise on nestling physiology and fitness may be greater in populations experiencing multiple environmental stressors, such as inclement weather, unpredictable food sources and more intense competition for resources, for example (Davies et al. 2017, Zollinger et al. 2019).

Nestlings experiencing chronic developmental stress that survive to adulthood may experience reduced song complexity (MacDougall-Shackleton et al. 2009), hypersensitivity (Spencer et al. 2009), and shifts from territorial and parental behaviour (e.g. incubation and feeding effort) to self-maintenance (Wingfield and Sapolsky 2003), all of which may negatively affect reproductive success. Therefore, noise not only has the potential to induce chronic developmental stress, but it may also have long-term, and potentially population-level implications for populations living in noisy areas.

Given the potential effects of noise on nestling stress physiology, conservation efforts should be targeted towards mitigating the effects of noise during this sensitive life stage (McGregor et al. 2013). A variety of noise mitigation strategies exist. Physical barriers such as noise-reducing walls along-side roads for example, are commonly recommended as a method to insulate surrounding areas from noise (Slabbekoorn and Ripmeester 2008). However, there are some limitations to this method. Physical barriers often do not extend a long distance and noise-reducing walls may restrict animal movement (Shannon et al. 2016). They also may be costly and so industries are unlikely

to adopt these measures to reduce the impacts of industrial noise, such as from oil and gas extraction (Shannon et al. 2016). Introducing noise-reducing road surfaces is another physical way to reduce noise associated with roads, however, similarly to physical barriers, these surfaces may not extend long enough distances to entirely mitigate noise effects and can be expensive (Slabbekoorn and Ripmeester 2008). Where permanent structures are not feasible, transient noise-reducing strategies may be more effective. Transient strategies include establishing noise-free areas around biological communities and restrictions on speed limits during sensitive periods of breeding (Zollinger et al. 2019). While these strategies have the potential to reduce reproductive failure associated with noise exposure, there is a need to evaluate the relative benefits of these methods and how they should be employed to most effectively reduce effects of noise (Shannon et al. 2016).

Future directions

Monitoring CORT levels has been proposed as a way to assess the effects of anthropogenic disturbance on the fitness and success of individuals and populations before population-level changes (e.g. declines) can be identified (Dantzer et al. 2014). However, my results suggest that the way in which CORT responds to chronic stressors such as noise, and interacts with the environment to affect fitness, is complex and context-dependent (Dickens and Romero 2013). Future studies should aim to document events of altered stress physiology in noise and how these effects vary with other environmental stressors (e.g. shortage of resources or inclement weather) in a range of species. This will provide a better understanding of what external factors make individuals and populations more vulnerable to ambient noise and how noise interacts with an individual's environment to affect their physiology. Chronic stress from noise has been associated with increases and decreases in both baseline and stress-induced CORT in developing birds (Injaian et al. 2020). In fact, an analysis of field and laboratory studies of species across taxa revealed that there is no consensus endocrine response to chronic stress (Dickens and Romero 2013). So rather than searching for evidence of elevated stress hormones in response to noise, it is more important to understand how an individual's physiological response to noise exposure varies with factors such as

geographic location, developmental conditions, and the presence of additional environmental stressors (Injaian et al. 2020).

In response to anthropogenic disturbances, alterations to stress physiology may be beneficial or costly (Boonstra 2013), and little is known about how permanent these alterations are (Bonier et al. 2009a). More research should be geared towards understanding the long-term effects of altered stress physiology in noise on future behaviour (e.g. in foraging, territory defense, and reproduction) and physiology (e.g. in immunity, growth, and metabolism) across species (Breuner et al. 2013, Dantzer et al. 2014). Such information will elucidate the impacts of noise at the population level (Kight and Swaddle 2011). Furthermore, urban environments are complex and noise represents only one factor that can affect developing animals. While studies using playbacks to isolate noise from other factors are valuable in disentangling the effects of noise from confounding factors, more research is needed to understand the relative influence of other sensory stimuli associated with human development such as light, chemical pollution, and vibration (Crino et al. 2013, Flores et al. 2019). This will allow us to understand how all factors associated with human development interact with one another to alter stress physiology in developing animals.

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