INTRASPECIFIC VARIATION OF BRAIN ANATOMY AND DRUMMING ACTIVITY IN RUFFED GROUSE (*BONASA UMBELLUS*)

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ABSTRACT

The ruffed grouse is a widespread species, but much of its basic biology has remained understudied. I examined individual variation in two understudied aspects of their biology: brain anatomy and drumming behaviour. Individual grouse varied greatly in neuron numbers, sizes and volumes of two brains regions, so much so that sampling <6 individuals could yield inaccurate measurements. Drumming activity was also highly variable both among and within individual males. Multivariate models indicated that daily drumming activity occurred during the same week across years and hourly drumming activity peaks one hour before sunrise and one hour before sunset. Warmer temperatures also were associated with increased drumming activity, but this was a relatively weak effect compared with day of the year and time of day. Overall, these results improve our understanding of individual variation in ruffed grouse and has important implications for future studies of ruffed grouse.

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LIST OF ABBREVIATIONS

AICc – Akaike information criterion
ANCOVA – analysis of covariance
CI - confidence interval
CE - coefficient of error
Est. - estimate
F - f statistics
GAMM- generalized additive mixed model
n.a. - numerical aperture
NM - nucleus magnocellularis
nRt- nucleus rotundus
OLS- ordinary least squares
P - p-value
PFA- paraformaldehyde
POM - medial preoptic nucleus
RMA- reduced major axis
ROB- rest of brain
RUGR - ruffed grouse
S.E. - standard error
SMA- standard major axis

CHAPTER ONE: GENERAL INTRODUCTION

The ruffed grouse (*Bonasa umbellus*) is an important and widespread gamebird species in North America. Ranging from Central Alaska to Northern Georgia, the species has an expansive range with similar life history throughout (Rusch et al., 2020). The range of the ruffed grouse largely coincides with temperate forests, specifically mixed conifer woodlands featuring aspen (*Populus sp.*) and strictly aspen dominated forests (Rusch et al., 2020). Within these forests, ruffed grouse consume a variety of plant material including leaves from trees and shrubs, fruits, and insects (Bump et al., 1947; Servello & Kirkpatrick, 1987). During the winter, ruffed grouse are particularly reliant on woody material and buds. In the northern extremes of their range, buds and catkins from aspen, willow (*Salix sp.*), and birch trees (*Betula sp.*) provide a particularly important overwinter food source (Servello & Kirkpatrick, 1987; Svoboda & Gullion, 1972). The ruffed grouse not only relies on a variety of plants in mixed early successional woodlands, it is also an important food source for many terrestrial predators, making it an excellent indicator species of ecosystem health and community composition (Hansen et al., 2011; Niemi et al., 1997).

Because the ruffed grouse is an indicator of ecosystem health and a heavily hunted gamebird, much of the research about this species has focused on management (Berner & Gysel, 1969; Bump et al., 1947; Pollentier et al., 2021) and population ecology (Atwater & Schnell, 1989; Rusch et al., 2020). As a result of this applied focus, many basic questions about ruffed grouse biology remain unanswered or were addressed only recently. For example, male ruffed grouse

use a unique non-vocal courtship display known as “drumming” that has been known to scientists for over 100 years, but many aspects of this behaviour have only recently been investigated. Drumming consists of a male standing on an elevated structure, such as a rock, root, or log, and rapidly beating the wings in quick succession 40-50 times over a period of 8-11 seconds (Aubin, 1972; Hjorth, 1970). Even though the first study of drumming behaviour was published nearly a century ago (Bent, 1932), the first bioacoustic analysis of the drumming display was completed just within the last ten years (Garcia et al., 2012). Garcia et al. (2012) showed that most of the energy of the display is concentrated at frequencies below 100 Hz. Further, the number of wingbeats per display and speed varies more among males than within males, which might confer individual specific information to conspecifics. A subsequent study in the same population examined the behavioural responses of actively drumming males to playbacks of unfamiliar males (O'Neil et al. 2018). Overall, males responded to playback of an unfamiliar drummer by either approaching the speaker or drumming less often, but at a faster rate. Most recently, Déaux et al. (2020) demonstrated that the speed of the drumming display is more variable among individuals than within and showed that other factors such as the number of displays produced, body size, and temperatures influence the speed of the display. More specifically, cooler nighttime low temperatures reduced the speed of the drum for all grouse and smaller males had faster drumming displays that were more variable in speed than larger males. In combination, these recent studies demonstrate that drumming behaviour varies among and within males and that temperature and the behaviour of other males can influence the speed of the display.

Another important behaviour of ruffed grouse is winter snow burrowing; grouse dive into soft snow and submerge themselves to retain body heat (Rusch et al., 2020). Snow burrowing provides a way for grouse to maintain body temperature and reduce energy expenditure when snow levels are high (>15cm) and the use of these winter refugia is related to reduced stress (Gullion, 1970; Shipley et al., 2019). Snow burrowing is also associated with differential winter survival. Shipley et al. (2020) showed that over winter survival is linked to choice of winter refuge and that differences in snow burrow depth and switching refuge type when snow levels were low are the main drivers of overwinter survival. Thus, individual variation is not only important for the breeding season (discussed above), but also survival through harsh winters.

Even the population genetics of ruffed grouse have been ignored until recently. Ruffed grouse have a large distribution spanning almost all of Canada and a large swathe of the USA with multiple putative subspecies (Rusch et al., 2020), yet only two studies have examined their population genetics and gene flow (Honeycutt et al., 2019; Jensen et al., 2019). The first of these studies, Jensen et al. (2019), showed that in western populations of ruffed grouse, lack of aspen forest or other suitable habitat has led to significant genetic differences among geographic regions and the creation of isolated and distinct populations. Building upon these results, Honeycutt et al. (2019) showed that for a much larger part of the range, 3-4 genetically distinct groups were present that partially corresponded with the ranges of the subspecies. Whether all 14 designated subspecies are genetically distinct remains to be explored.

While ruffed grouse studies addressing basic biological questions have increased in frequency over the last decade, much remains to be understood about this widespread species. Better

understanding of ruffed grouse biology has the potential to improve management in areas where populations have faced decline, such as the Southern Appalachians (Devers et al., 2007), as well as provide insights into how and why the ruffed grouse differ from closely related species. In my thesis, I will focus on individual variation at two levels in the ruffed grouse: neuroanatomical and behavioural.

Only four studies have examined intraspecific variation in ruffed grouse neuroanatomy (Corfield et al., 2013; Krilrow and Iwaniuk, 2015; Corfield et al. ,2016; Cunha et al., 2020). Although these studies revealed new insights into grouse neurochemistry, seasonal variation in the brain, and allometric scaling, they all used relatively small sample sizes. How much variation is present among ruffed grouse and what factors might be responsible for that inter-individual variation is therefore largely unknown. In Chapter 2, I quantify how much intraspecific variation is present in the volume, neuron number, neuron density and neuron size within two brain regions across a large sample of ruffed grouse brains. Specifically, I examine the amount of individual variation in nucleus magnocellularis (NM) and nucleus rotundus (nRt) to address this question. These two sensory brain regions sampled are involved in processing auditory and visual stimuli, respectively. Variation in sensory brain region sizes can provide insights into the sensory abilities of a species (Iwaniuk and Wylie 2020). However, most comparative studies rely on small intraspecific sample sizes (Gutiérrez-Ibáñez et al., 2011;Yopak et al., 2019). This is of concern because intraspecific variation of brain anatomy is common in wild birds. For example, in the black-capped chickadee (*Poecile atricapillus*), populations that faced harsher winter conditions have more hippocampal neurons in the hippocampus (Pravosudov & Clayton, 2002). Despite ample evidence that the hippocampus, song system, and other regions vary seasonally,

between sexes, and among environments (Vigiletti-Panzica et al.,1986; Brenowitz, 2004; Roth & Pravosudov, 2009), the extent to which sensory regions of the brain vary across individuals remains untested in wild birds. Sensory brain regions process stimuli from the environment and the anatomy of these regions have the potential to vary in size based on experience, sex, and environmental variables in a similar fashion to the hippocampus. I selected NM because it is one of the first brain regions to receive information from the cochlear nerve and plays a key role in sound localization (Koppl, 2015), which is an important sensory signal for ruffed grouse to detect conspecifics during the breeding season and avoid predation. Specifically, NM is involved in processing differences in timing of sounds reaching the left and right ears (Carr & Konishi, 1990). Previous studies suggest that the size and number of neurons in NM reflects how accurately a species can localize a sound (Kubke et al., 2004; Gutierrez-Ibanez et al., 2011). Intraspecific variation in NM size could therefore suggest that sound localization capabilities vary among grouse. The second region, nRt, is a thalamic nucleus within the tectofugal pathway responsible for processing colour, luminance and looming stimuli (Wang et al., 1993). nRt is often used as a control region in seasonal comparisons of the song system (Nottebohm, 1980; Riters et al., 2000). The amount of intraspecific variation in nRt size, neuron numbers or neuron sizes has not, however, been adequately examined. If the anatomy of nRt varies greatly across individuals, that would not only potentially indicate individual differences in vision, it could also be problematic for studies that use this region as a control in studies of other brain regions.

To address the degree of neuroanatomical variation among ruffed grouse, I completed a suite of neuroanatomical measurements of two brain regions across a relatively large sample (n = 19). I first tested the extent to which brain region volume, neuron numbers, neuron density, and neuron

size are a product of overall brain size (i.e., allometry). Then, using a bootstrapping approach (Manly, 2006), I estimated the minimum sample sizes needed to approximate that of the entire population sampled across the four measurements (volume, neuron number, neuron density, neuron size) for both brain regions. The results from this study provide much needed information on how much intraspecific, anatomical variation occurs within sensory regions of a wild species that will guide future studies of both intraspecific and interspecific variation in brain anatomy.

In Chapter 3, I shift the focus from brain anatomy to individual variation in drumming activity. As described above, drumming is a wingbeating display that generates a low frequency sound that can travel far distances (Garcia et al., 2012; Gullion, 1984). While the definitive function of drumming remains unknown, the display likely plays a role in attracting female grouse and advertising the location of a drumming male to other males (Atwater & Schnell, 1989). Because ruffed grouse produce few sounds and are difficult to locate visually, counting the number of drumming males has provided a practical way to census ruffed grouse abundance (Jones, 2005) and density (Petraborg et al., 1953) for the last 60 years. Despite the widespread use of these drumming surveys, basic information on how much and when males drum is lacking. The few initial studies on drumming activity (Gullion, 1966; Archibald, 1976; Zimmerman & Gutiérrez, 2007) have incomplete data for only a few individuals and for a single season. Thus, the extent to which there is variation within and among males or across hours, days, or years is largely unknown. Addressing this major knowledge gap could therefore have significant implications for how drumming surveys are executed. Similarly, detection rates during drumming surveys increase when temperature increases and weather conditions are favourable (Gullion, 1966; Zimmerman & Gutiérrez, 2007), but it is unclear how temperature and other variables influence drumming activity. The goal of Chapter 3 was therefore to determine which factors influence

drumming activity throughout the breeding season. To do this, I collected drumming activity data across multiple males and seasons and used generalized additive mixed models (GAMMs), I to test how temporal (e.g., time of day and day of year) and environmental variables (e.g., temperature and precipitation) predict drumming activity. My findings reveal new insights into ruffed grouse drumming behaviour as well as significant implications for how drumming surveys are executed.

Although these two chapters differ greatly in the data being analyzed, together, they add to our knowledge of ruffed grouse biology, especially individual variation. This iconic inhabitant of early successional and mixed forests has been managed with little understanding of its anatomy, behaviour, genetics, or physiology. Both the neuroanatomical and behavioural questions asked in this thesis will increase our general understanding of the ruffed grouse as well as improve the accuracy of sampling techniques, albeit in two disparate fields.

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CHAPTER TWO: INTRASPECIFIC VARIATION IN THE QUANTITATIVE NEUROANATOMY OF SENSORY REGIONS OF THE AVIAN BRAIN

Introduction

Sensory systems allow organisms to perceive environmental stimuli. In many cases, enhanced sensory perception is associated with the enlargement of sensory brain regions that process the sensory information related to the behaviour. A prime example is the expansion of somatosensory regions of the brain in the star-nosed mole (*Condylura cristata*) that allow rapid, accurate, and sensitive detection of prey using fleshy projections from the nose (Catania, 1999). Due to this relationship between sensory acuity/sensitivity and neuroanatomy, the relative size of sensory regions in the brain can reveal a species' reliance on a particular sensory modality (Iwaniuk and Wylie 2020). In birds, this approach has been used extensively to better understand the evolution of differences in sensory acuity and sensitivity across species (Iwaniuk et al. 2006; Iwaniuk and Wylie 2020; Wylie et al., 2015; Wylie et al., 2009).

Despite the importance of comparative studies in understanding the evolution of sensory systems (Wylie et al., 2015), many of these studies rely on low sample sizes (often n = 1) to represent a species (Corfield et al., 2011; Iwaniuk et al., 2006). This is problematic because brain regions do vary in absolute and relative size within species in birds across multiple contexts. For example, the medial preoptic nucleus (POM), is significantly larger in male Japanese quail (*Coturnix japonica*) compared to females due to its role in male sexual behaviour (Viglietti-Panzica et al., 1986). Similarly, brain regions involved in song production differ in volume, neuron numbers and soma size between seasons and sexes in the majority of songbird species studied to date (Brenowitz, 2004; MacDougall-Shackleton et al., 2001). Indeed, environmental conditions and

season can affect intraspecific brain region size. Food caching mountain chickadees (*Poecile gambeli*) even vary in the size, neurogenesis rates, neuron numbers, and soma sizes within the hippocampus among populations living at different levels of environmental harshness (Freas et al., 2012; Roth & Pravosudov, 2009). Thus, there is ample evidence to suggest that the volume, neuron numbers, and soma sizes of brain regions vary intraspecifically in birds. However, most of the brain regions studied at the intraspecific level are located within the telencephalon (e.g., hippocampus), which typically exhibits more plasticity than non-telencephalic regions of the brain (Alvarez-Buylla, 1992; Nottebohm, 1981). The extent to which brain regions outside of the telencephalon exhibit similar levels of intraspecific variation remains largely unknown.

Some of the intraspecific variation observed in the size of brain regions is thought to arise from allometry (Herculano-Houzel et al., 2014). Across species, larger brains typically have larger brain regions with more neurons (Herculano-Houzel et al., 2014), but a recent study in laboratory mice (*Mus musculus*) did not show the same pattern intraspecifically. Instead, neuron number and other neuronal cell types did not vary with brain region mass, meaning that individuals with larger brains did not necessarily have more neurons than smaller brained individuals (Herculano-Houzel et al., 2015). However, this study used males of a single strain of lab reared mice and only quantified numbers in large, dissectible brain regions, so whether the same pattern applies to smaller, more functionally specific brain region and/or in wild species remains unknown. Quantifying intraspecific variation in sensory region size, neuron numbers, neuron sizes and neuronal density in a wild species is important for the interpretation of comparative studies that often rely on small sample sizes. For example, if low sample sizes are used for each species, variation in neuron number or density may not reflect species level variation. Small intraspecific

sample sizes, especially in wild species, could therefore decrease the reliability of interspecific comparisons because a few individuals may not be representative of the species.

Here, I quantify the volumes, neuron numbers, neuron sizes and neuron densities of two sensory regions in the brains of wild caught ruffed grouse (*Bonasa umbellus*). I not only test if larger brained grouse have larger brain regions with more and larger neurons (i.e., intraspecific allometry), I also test the extent to which sample size affects estimates of the mean volume, neuron number, neuronal density, and neuron size of a brain region. The sample size required for a given level of accuracy depends on the relationship between sample size and estimation accuracy. A variety of methods can be used to characterize the relationship between sample size and mean estimates (Allgoewer & Mayer, 2017; Charan & Kantharia, 2013). Bootstrapping is a special kind of Monte Carlo simulation that assumes the summary statistics gathered from a sample can be used to accurately represent the population mean and is appropriate to use with small sample sizes (Manly, 2006). It uses resampling with replacement to estimate sample means and confidence intervals across a range of possible sample sizes (Efron, 1979; Manly, 2006) and can be used to estimate appropriate sample sizes (Bros & Cowell, 1987; DePatta Pillar, 1998; Hamilton & Collings, 1991; Kritzer et al., 2001). We used bootstrapping to estimate variation across different sample sizes of our stereological data and the minimum sample sizes needed to approximate the population level means for each individual measurement.

Material and Methods

Specimens

We obtained ruffed grouse (n = 19) from hunters in Alberta or collected in the field using mirror traps (Gullion, 1965). In total, this sample consisted of 8 fall males, 5 spring males, and 6 fall females. No specimens of spring females were collected. Although some methods have proven moderately effective for trapping female ruffed grouse during the breeding season (Maxson, 1977), they are extremely difficult to trap (Bump et al., 1947; Atwater and Schnell, 1989), and as a result, we have yet to collect any breeding season females. All of the donated specimens were decapitated, and immersion fixed in 4% paraformaldehyde (PFA) in 0.1M phosphate buffer. Specimens collected during the breeding season using mirror traps (n=5), were euthanized with an intraperitoneal injection of sodium pentobarbital and the entire head immediately immersion fixed in 4% PFA. All of these procedures adhere to the Canadian Council for Animal Care Guidelines and were approved by the University of Lethbridge Animal Welfare Committee. Specimens were collected under research and collection permits issued by the Alberta Department of Environment and Sustainable Resource Development.

Following two weeks of fixation in PFA, the brains were extracted and cryoprotected in 30% sucrose in 0.1 M of phosphate buffered saline (PBS) until they sank. Brains were then embedded in gelatin and sectioned on a freezing stage microtome at a thickness of 40 μm in the coronal plane. Every second section (1:2 series) was mounted onto gelatinized microscope slides. The sections were then dehydrated through a graded ethanol series, cleared in Hemo-D, stained with thionin for Nissl substance, and coverslipped with Permount histological mounting medium (SP15-500, Fisher Scientific).

Brain regions

We selected two sensory nuclei involved in two different pathways (auditory and visual) for the quantitative measurements. The visual brain region measured was nucleus rotundus (nRt), which is a well-defined thalamic nucleus within the tectofugal pathway (Iwaniuk et al., 2010). nRt was measured for 19 ruffed grouse. To define nRt borders, we referred to the chick brain atlas and previous publications (Iwaniuk et al., 2010; Krilow & Iwaniuk, 2015; Puelles, 2018). nRt is easily distinguished from surrounding regions by its large, darkly stained cells with relatively low cell density, enabling neurons to be effectively counted and soma sizes measured (Figure 2.1). All nRt measurements included nucleus triangularis, a small nucleus dorsal to nRt. Nucleus triangularis, similar to nRt, is part of the thalamic relay centre of the tectofugal pathway (Iwaniuk et al., 2010). nRt is largely responsible for processing colour, luminance, motion, and depth of motion (Wylie et al., 2009). This means nRt mainly processes stimulus properties of visual information, not position (Wylie et al., 2009). In contrast, nucleus triangularis is thought to process stimulus position within the visual field or where the stimulus is located (Hellmann & Güntürkün, 2001). Despite this difference in function, the border between nRt and nucleus triangularis is not always discernable in Nissl-stained sections (Iwaniuk et al., 2010), so triangularis was included in our measurements of nRt.

The auditory region measured was nucleus magnocellularis (NM), a cochlear nucleus in the brainstem (Köppl, 2015). For this brain region, only 18 specimens were sampled because one specimen had poor cellular staining that prevented accurate estimates of neuron numbers and sizes. NM, along with two other nuclei, form the beginning of the ascending auditory pathway in

the avian brain (Köppl, 2015). Respectively, nucleus angularis (NA) and NM process the amplitude and temporal differences between left and right ears (Köppl, 2015). For this reason, NM is often referred to as the beginning of the auditory time or interaural time difference (ITD) pathway. The boundaries for NM were defined using the chick brain atlas and descriptions in previous studies (Corfield et al., 2016 Wylie, & Iwaniuk, 2016; Puelles, 2018). Briefly, at the rostral end NM is a small, irregularly shaped clump of large cells located dorsomedial to another cochlear nucleus, nucleus laminaris (Köppl, 2015). Moving caudally, the NM region flattens and expands to form a horizontal slab of cells (Takahashi & Konishi, 1988). Finally, the caudal pole of NM is located at the pontomedullary junction and is oval in shape. NM, similar to nRt, is relatively simple in cytoarchitecture with a single neuronal type specialized for processing temporal information from the cochlear nerve and receiving input from the superior olive (Kubke & Carr, 2005).

Stereological measurements

All measurements described below relied upon unbiased stereology and were conducted bilaterally. Bilateral measurements were important because environmental factors and experience can cause asymmetries in soma size and volume between left and right brain regions (Güntürkün, 1997; Manns, 1999; Mehlhorn et al., 2010). However, paired t-tests revealed no significant differences between the hemispheres for any of our quantitative measurements ($p= 0.25$-0.75), so we combined left and right values for all subsequent analyses.

Volumes were estimated using the Cavalieri method as implemented in Stereo Investigator (Gundersen et al., 1999) on a Zeiss Axio Imager 2 microscope (Carl Zeiss, Microbrightfield Inc., Colchester, VT, USA; Microimaging GmBH, Germany). nRt volumes were estimated using a 2.5x objective (n.a. = 0.075) with a grid size of 150 μm. Due to the smaller size of the brain region, NM volumes were estimated using a 10x objective (n.a.= 0.05) and a grid size of 100 μm. Both NM and nRt volumes were measured for every mounted section. The coefficients of error (CE) of all volumetric measurements for NM were less than or equal to 0.05, and less than or equal to 0.005 for nRt.

Neurons were counted using the optical fractionator method as implemented in Stereo Investigator (Gundersen et al., 1999) on the same microscope. Neurons were counted bilaterally for both regions in every second section (i.e., distance of 160 μm between sections). This meant 24-28 sections were used for nRt cell counts, and 15-18 sections for NM cell counts. The count from each hemisphere was summed to yield the total number of neurons per brain region. Both neuronal types measured in this study were easily identifiable and distinguishable from glial cells. nRt neurons are large, round and well stained. NM neurons are also large with a distinctive, rounded shape. Neurons were counted if they contained a nucleus and an intact cellular membrane. Section thickness was measured at each counting site as the distance between the first particle becoming visible and the last particle coming out of focus (West et al., 1991). To account for "lost caps" effects (Hedreen, 1998) when counting neurons for NM, a guard zone of 7 μm was implemented along with a 7 μm dissector height, 200x200μm grid size, and a counting frame of 100x100 μm. nRt neurons were counted without a guard zone and with a dissector

height of 15 μm, a grid size of 400x400 μm, and a counting frame of 60x60 μm. At least 250 cells were counted per hemisphere, for a total of at least 500 cells in total counted per specimen sampled. The coefficient of error for all cell counts was equal to or below 0.08.

Soma size was measured using the nucleator probe (Gundersen, 1988) with four rays as implemented in StereoInvestigator with a 20x objective (n.a. = 0.5). Soma size measurements were conducted on the same microscope used for the volumetric and cell count measurements. Cells were selected randomly and throughout the medio-lateral and rostro-caudal extent of each brain region. As mentioned above, both nRt and NM have large, well stained neurons that are easily differentiated from glial and other non-neuronal cells. nRt neurons were selected based on following criteria: all cells contained a nucleus, cells were not overlaying each other, four clear edges of the cell were membrane visible, and cells contained an intact cellular membrane. NM neurons were selected if they contained a visible nucleus, an intact cellular membrane, and clear edges that could be easily differentiated from other surrounding neurons. For each brain region and specimen, 150 neurons in total were measured, with 75 from each hemisphere. All cell size measurements had a CE equal to or below 0.004 for each specimen.

Statistical analyses

Linear regression analyses were conducted to determine if nRt and NM volume, neuron number, and soma size increased with brain size. Total brain size was defined as total brain size minus the region of interest (NM or nRt). Thus, any mention of total brain size in this study is in reference to total brain size minus nRt or NM. Within each brain region, we also determined the scaling relationship between: volume and neuron number; soma size and volume; soma size with neuron

number; neuronal density and volume; and neuronal density and soma size using bivariate linear regressions. This was done to determine how NM and nRt size vary with neuron number, soma size, and neuronal density.

The lmodel2 package (Legendre, 2018) in R (R Development Core Team, 2017) was used to produce two bivariate linear regression models: ordinary least squares (OLS) and standard major axis (SMA). Prior to analysis, normality was determined using the interquartile range (IQR) of the distribution. The use of both OLS and SMA regression types was necessary because they assign measurement error differently along the regression line (Smith, 2009). SMA assumes that measurement error is present for both the explanatory and dependent variables, which results in a symmetrical regression (Smith, 2009). OLS only assumes no measurement error is present in the explanatory variables (Kilmer, 2017). Due to the potential for measurement error in all the intraspecific allometric data measured and the asymmetrical nature of the scaling relationship of total brain size with nRt and NM size, we include both SMA and OLS regression types (Smith, 2009). The ggplot2 package was used to visualize all linear regressions (Wickham, 2016).

Sex and season clearly influence brain region size in birds (MacDougall-Shackleton et al., 2001; Viglietti-Panzica et al., 1986). Thus, analyses of covariance (ANCOVAs) were necessary to test for differences in allometric scaling among three groups that vary by sex and season: fall female (n=6), spring male (n=5), and fall male (n=8). Where significant group effects were detected, we used Tukey-Kramer HSD tests to identify pairwise differences. All data used in the linear regressions and ANCOVA were log transformed prior to analysis.

We also used bootstrapped Monte Carlo simulations to estimate the expected accuracy of sample means across sample sizes. The simulations were performed with the Poptools plug-in for Excel (Hood, 2009). For each measurement, we began by resampling measurements with replacement into 19 vectors (or 18 vectors in the case of NM). Each vector had a different length, such that vector one had one row, vector two had two rows, and so on up to vector 19 (or 18). We then took the mean of each vector. This process was repeated for 100,000 iterations to generate distributions of sample means for each sample size from 1 to 19. We calculated grand means as well as 90% and 95 % confidence intervals (CIs) for each variable, for each sample size.

While larger samples are expected to yield higher accuracy, they can be more difficult to acquire due to logistics, so it is often desirable to identify a sample size that is large enough to meet a minimally acceptable level of accuracy. Here, we defined the minimum acceptable sample size as the sample size at which 90% of measurements were within 10 or 20% of the total sample mean. Both minimum sample size thresholds were applied to nRt and NM volume, neuron number, soma size, and neuronal density measurements. From this, we could determine minimum sample size estimates at two levels of accuracy and compare the estimates generated for each measurement type.

Results

Nucleus rotundus

All coefficient of variation (CV) values of our stereological measurements were under 20%, but varied in magnitude across our measurements (Table 2.1). More specifically, the number of neurons was the most variable and soma size the least variable of the three measurements.

Linear regression analyses showed that nRt volume, neuron number, soma sizes, and neuronal density were not significantly associated with brain size (Table 2.2; Fig. 2 A-D). However, nRt volume increased significantly with neuron number (p= 0.004, Table 2.2, Fig. 2 G) and the slope of this regression line had a confidence interval encompassing 1, indicating a near isometric relationship. Neither soma size nor neuronal density varied with nRt volume (Table 2.2). nRt soma size did, however, significantly decrease (p= 0.007) with neuron density (Table 2, Fig. 2H). No other significant scaling relationships were found.

The ANCOVAs yielded no significant relationship between volume and total brain size (F= 1.51, d.f. = 1,13, p = 0.24), group differences (F= 0.19, d.f. = 2,13, p = 0.83), or interaction effects (F= 0.13, d.f. = 2,13, p = 0.88). Similarly, no significant relationships were found between neuron number and total brain size (F= 1.78, d.f. = 1,12, p = 0.21), group differences (F= 2.19, d.f. = 2,12, p = 0.16), or interactions (F= 1.11, d.f. = 2,12, p = 0.36). No significant relationships were found between soma size and total brain size (F= 2.80, d.f. = 1,13, p = 0.12), group differences (F= 1.10, d.f. = 2,13, p = 0.36), or interactions (F= 3.04, d.f. = 2,13, p = 0.08). Last, neuronal density showed a similar trend to other measurements with no significant relationship between neuronal density and total brain volume (F= 0.26, d.f. = 1,13, p = 0.62), or

group differences (F= 1.72, d.f. = 2,13, p = 0.22), and no interactions (F= 0.21, d.f. = 2,13, p = 0.82). Thus, nRt anatomy does not scale significantly with total brain size even when factors such as sex and season of capture are considered.

The two significant allometric scaling relationships, nRt volume and neuron number and soma size and neuronal density, showed a similar lack of sex and season effects. Volume and neuron number were significantly correlated with one another as also highlighted by the linear regression models (F= 11.76, d.f. = 1,13, p = 0.004), but no group differences were found (F= 1.64, d.f. = 1,13, p = 0.23) and no interaction effects were present (F= 0.96, d.f. = 1,13, p = 0.41). Similarly, soma size was significantly correlated with neuronal density (F= 7.30, d.f. = 1,13, p = 0.02) but no significant group effects were present (F= 0.06, d.f. = 1,13, p = 0.95) and no interaction effects were present (F= 7.30, d.f. = 1,13, p = 0.02). Thus, intraspecific allometric relationships within nRt are not significantly different based on sex or season of capture.

The Monte Carlo simulations revealed the same trend for all measurement types: as sample size increases, the confidence interval for the estimate of the population mean asymptotes with diminishing marginal returns (Fig 2.3 A-H). Minimum sample size estimates yielded varying results based on the measurement type and the accuracy of the threshold used. The thresholds at which 90% of the resampled values were within ±10 and 20% of the estimated population mean are shown in Table 2.4. When the minimal sample size estimates were calculated where 90% of the measurements were within 10% of the estimated population mean, only nRt soma size (n = 10) and volume (n = 16) could be estimated using sample sizes smaller than 19 (Table 2.4).

Because neuron number and neuronal density required minimal sample sizes larger than 19, it can be assumed that small sample sizes do not provide accurate estimates of population averages.

For the second criteria, where 90% of measurements were within 20% of the sample mean, all measurement types had a minimum sample size estimate (Table 2.4). However, these minimum sample size estimates varied greatly by measurement type. Soma size (n = 3) and volume (n = 4) had the lowest minimum sample size estimates (Table 2.4). The minimal sample size estimates for neuron number and neuronal density were much higher (Table 2.4), suggesting that neuron number is more variable measurement intraspecifically than the other measurements.

Nucleus magnocellularis

As with the nRt measurements, all CV's for the NM measurements were under 20%, but varied in magnitude across the measurement types (Table 2.1). Again, the number of neurons was the most variable and soma size the least variable measurement collected.

Linear regression analyses did not yield significant relationships between total brain size and NM volume, neuron number, soma size, or neuronal density (Table 2.3). There was a significant scaling relationship between NM neuronal density and volume, where neuronal density increased at a similar rate to volume (Table 2.3). However, this relationship is weak as signified by the low r^2 value (Table 2.3). The general lack of strong allometric relationships for any of the NM measurements is supported by the scatterplots (Fig. 2.4 A-D) and relatively low correlation coefficients (Table 2.3).

Two-way ANCOVAs showed NM volume did not vary significantly by sex-season groups (F= 0.80, d.f. = 2,12, p = 0.47) or total brain size (F= 0.16, d.f. = 1,12, p= 0.70) and no significant interaction was found (F= 1.64, d.f. = 2,12, p=0.23). Neuron number did not vary significantly by group (F= 2.18, d.f. = 2,12, p = 0.16) or total brain size (F= 1.77, d.f. = 2,12, p = 0.21) and no significant interaction was found (F= 1.10, d.f. = 2,12, p = 0.36). Similarly, soma size also did not vary significantly by group (F= 0.33, d.f. = 2,12, p = 0.73), total brain size (F= 0.89, d.f. = 1,12, p = 0.37) and no significant interaction was found (F= 0.11, d.f. = 2,12, p = 0.90). Neuronal density, however, did vary significantly by group (F= 6.50, d.f. = 2,12, p = 0.01), but did not vary significantly with total brain size (F= 1.03, d.f. = 1,12, p = 0.33) nor was any significant interaction found (F= 0.72, d.f. = 2,12, p = 0.51). Post hoc Tukey HSD revealed the group difference was due to different neuronal densities between fall males and fall females. However, the percent difference between the two groups was only 3%.

The Monte Carlo simulations revealed the same trend as nRt: for all NM measurements, the upper and lower bounds of the CIs were large at small sample sizes and decreased with increasing sample sizes (Fig 2.5). To achieve a distribution where 90% of measurements were within 10% of the estimated population mean, samples sizes larger than 18 were needed for volume, neuron number, and neuronal density (Table 2.4). Only soma size could be estimated with a sample size <19 (n=16).

In contrast, for a distribution in which 90% of measurements were within 20% of the population mean, samples sizes <19 were applicable across all four measurements (Table 2.4). Soma size had the lowest minimum sample size estimate of all the measurements (n=4), consistent with the

results for nRt (Table 2.4). The NM volume minimum sample size required a larger sample size to reach the criterion than nRt (Table 2.4). NM volume, neuron number, and neuronal density all yielded similar minimum sample size estimates (Table 2.4). This suggests that for NM, all three of these measurements have similar accuracy. Soma size required the lowest minimum sample sizes for both nRt and NM minimum sample size estimation at both criteria (Table 2.4). In summary, soma size appears to be the least variable measurement intraspecifically due to the low minimum sample size estimates (Table 2.4).

Discussion

Overall, neither nRt nor NM exhibited strong allometric relationships with brain size. Additionally, sex and season did not significantly affect the majority of our quantitative measurements. The Monte Carlo simulations indicate that small sample sizes yielded large confidence intervals, but reasonable estimates can be made through sampling 3-10 individuals. Together this suggests that the effect of allometry on sensory region size, neuron numbers, soma sizes, and neuron densities might be relatively weak at an intraspecific level, corroborating the conclusions of Herculano-Houzel et al. (2015). Moreover, minimal sample size estimates suggest that researchers should use caution when sampling less than three individuals of a species when conducting soma size and five individuals when using volume, neuron number, or neuronal density measurements.

While the findings presented here provide some valuable insights, we note that our quantitative measurements differ from that of previously published data on ruffed grouse for both NM and nRt, with nRt estimates being smaller in the current study and NM slightly larger (Corfield et al.,

2016; Iwaniuk et al., 2010; Krilow & Iwaniuk, 2015). Some of these differences are due to sample size as both Iwaniuk et al. (2010) and Corfield et al. (2016) used much smaller sample sizes (n= 2 and n=3, respectively) than the total number of individuals in the current study. Krilow and Iwaniuk (2015) had a higher coefficient of error (CE) and sampled a different set of specimens, as well as relied upon a different observer for the measurements themselves. Each of these factors could have contributed to difference in values betwen Krilow and Iwaniuk (2015) and the current study and highlight how variable quantitative measurements can be across studies of the same species. The differences between the measurements used in the current study and previous studies does not, however, discount the lack of intraspecific allometry of the brain regions measured.

Intraspecific allometry of brain size has been studied quite extensively (Bennett et al., 1969; Ebinger & Röhrs, 1995; Gonda et al., 2010), but relatively few studies have been conducted on the intraspecific allometry of individual brain regions (Axelrod et al., 2018, Ebinger, 1995; Gonda et al., 2009; Kruska, 1996). In laboratory mice, total brain size did not influence neuron number and neuronal density across multiple brain regions (Herculano-Houzel et al., 2015). The mouse study, however, examined only large, dissectible brain regions in a domesticated, lab-bred species, which might not reflect intraspecific variation in wild species for two reasons. First, allometric patterns, or lack thereof, of large, dissectible brain regions might not reflect intraspecific scaling of smaller brain regions that are more functionally specialized, such as the sensory regions studied here and in a variety of other studies (Gutiérrez-Ibáñez et al., 2009; Iwaniuk et al., 2006). Second, domesticated and captive bred species often have smaller brains and brain region sizes compared to their wild counterparts (Kruska, 1988, 1996). Thus, findings

derived from lab populations might not apply to wild populations. Despite these concerns, we show the same pattern as Herculano-Houzel et al. (2015): the volume, neuron number, soma size, and neuronal density of both brain regions did not vary significantly with total brain size. This suggests that intraspecific allometry is not a significant source of intraspecific variation in smaller, functionally specific brain regions.

Although our sex-season groups did not differ significantly across most measurements, NM neuronal density did differ between fall males and fall females. The number of individuals for each sex-season group was small (n = 5-8) and there were no females collected during the spring (see Materials and Methods). It is possible that if a larger sample with more individuals from both sexes and all seasons of capture, the effects of sex and season might differ across all measurements. Without the benefit of additional samples, it is therefore difficult to interpret the difference in NM neuronal density and lack of effects across all measurements. In the current study, we show that the effect of intraspecific allometry is rather weak in two sensory brain regions of a wild gamebird species. Our findings corroborate with those in captive mammals which show a similar lack of intraspecific allometry (Herculano-Houzel et al., 2015). Specifically, intraspecific allometry in non-telencephalic sensory brain regions has never been conducted in a wild avian species. The findings presented here suggest that intraspecific allometry is not a potential source of bias in comparative studies that have small intraspecific sample sizes when studying sensory brain regions. However, the current study only examined intraspecific scaling in two sensory brain regions. It is possible that due to variability of function, other sensory brain regions may exhibit significant intraspecific allometry that could potentially bias estimates. Therefore, it is imperative that future studies of intraspecific neuroanatomical

variation determine if significant allometric relationships exist that could potentially bias the findings of comparative studies.

Estimates of minimal sample size

Many comparative studies focus on the precision and accuracy of stereological measurements and measure only one or a few individuals from a species (Corfield et al., 2011; Herculano-Houzel et al., 2007; Herculano-Houzel & Kaas, 2011; Iwaniuk et al., 2006). While focusing on the precision and accuracy of measurement parameters can increase data reliability for the few individuals sampled, the effect that sample size may have on mean values has remained largely uncertain. Using our bootstrapping approach, we show that small sample sizes may provide poor estimates of species or population level variation for all measurements: volumes, neuron numbers, neuronal density, and soma sizes.

Bootstrapping allows for small samples to be resampled with replacement to determine population level summary statistics and distribution. In a traditional bootstrap, only the original data points would be resampled to achieve the estimated population mean. In the current study, we used a Monte Carlo simulation to generate an estimated population mean and CI bounds for each sample size. Although this type of analysis is often used to determine sampling accuracy, there are some caveats with the method and considerations for future studies. First, some authors take issue with using Monte Carlo simulations in minimal sample size estimates (Bros & Cowell, 1987). This is because Monte Carlo simulates data based on the summary statistics provided (e.g. sample mean) for each category (sample size) to build a normal distribution for the data as opposed to a bootstrap, which would simply resample without replacement to generate

population level data (Bros & Cowell, 1987). In the context of the current study, this does not pose a significant issue. If measurements from 100,000 different ruffed grouse were used to build the population sample, the values would be expected to be slightly different for each individual grouse and more variable than a sample population formed from resampling without replacement like a bootstrap would. Therefore, using a Monte Carlo simulated data based on summary statistics is more reflective of the randomness seen in wild populations. Second, because every simulation run is generating slightly different data points, there is inherent variation in the combination of data points that are generated for each simulation. While this may be problematic for other studies, this is not a source of concern in the current study because each Monte Carlo simulation was set to 100,000 iterations. While variation in the values will occur between simulations, the large sample population generated makes it unlikely to affect the overall distribution. Finally, some authors urge caution when using bootstrapped means with a sample size of less than 20 (Schenker, 1985). However, as stated above, we did not rely solely on the original sample values to generate the estimated population mean. This means that despite the smaller sample size (n= 19) there is more variation in our estimate than would be expected with a traditional bootstrap. Since the goal of the Monte Carlo approach was to simulate the variability of neuroanatomical measurements, our estimates are likely more reflective of real-life population level values than population estimates that use resampling with replacement alone.

Confidence interval decreased in breadth with increasing sample size across all of our measurements for both brain regions (Figs. 2.3 and 2.5). However, the rate at which this occurred and the minimum sample sizes needed to achieve a distribution of 90% of measurements within 10 or 20% of the total sample mean varied among the different measurements. For both NM and

nRt, neuron number and neuronal density had the highest minimal sample size estimates. In fact, there was so much variation in the number of neurons within both regions that sampling all 19 individuals was not sufficient to meet our criteria. This suggests that neuron number is the most variable measurement among individuals, regardless of which brain region is examined. Conversely, soma size consistently required the lowest minimal sample size (Table 2.4). The reason for this could likely be attributed to the repeated measurements of individual soma, which are averaged to generate soma size for a specimen, whereas neuron number, neuronal density, and volume are all based on a single measurement. Volume was in between these two extremes requiring lower sample sizes than neuron number and higher than soma size (Table 2.4). Together this suggests that studies focusing on volumetrics or soma size could potentially use fewer specimens than those focusing on neuron numbers (Corfield et al., 2011; Cunningham et al., 2013), without sacrificing accuracy of their results. However, studies that rely on small sample sizes to examine neuron numbers or neuronal density may be inaccurate (Azevedo et al., 2009), especially if the individuals sampled represent extremes within the intraspecific distribution.

While a sample size greater than three or four is considered small, it is difficult to obtain a sample size of that magnitude for some species. Those that are critically endangered may never have sample sizes larger than one or two individuals for a neuroanatomical study. While it may seem that little information can be gleaned from studying a single or two individuals of a species, broad inferences about how avian sensory systems adapt to handle different environmental difficulties can be made by comparing these individuals to those of other species. For example, the critically endangered kakapo (*Strigops habroptilus*) was the focus of a comparative study of

the visual system (Corfield et al., 2011). The study revealed that several brain regions involved in visual processing, such as nRt, were reduced in size compared to diurnal species, while others were similar in size to diurnal species. This suggests a unique evolution of the kakapo visual system, but is based on the examination of a single kakapo specimen (Corfield et al., 2011). Thus, it is possible to use low sample sizes to determine broad patterns of anatomical variation between species, without overestimating the power of the sample size. However, larger intraspecific sample sizes will result in more solid conclusions when conducting comparative neuroanatomical studies because larger sample sizes lead to more statistically robust results (Button et al., 2013).

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Table 2.1. Descriptive statistics for stereological estimates of volume, neuron number, soma size, and neuronal density of the sensory brain regions sampled nucleus rotundus (nRt) and nucleus magnocellularis (NM). Including number of grouse sampled (n), mean, standard deviation (SD), and coefficient of variation (CV).

Brain region	**Volume (mm^3)**				**Neuron number (# neurons)**			**Soma size (μm^2)**			**Neuronal density (# of neurons/volume)**		
	n	mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV
nRt	19	6.121	0.738	12.06	99,994	19,148	19.15	552.30	53.70	9.72	16342.85	2482.84	15.19
NM	18	0.647	0.109	16.80	33,329	5,698	17.10	504.87	60.48	11.98	52239.11	9024.59	17.27

Table 2.2. Results of bivariate linear regressions (OLS and SMA) between nucleus rotundus (nRt) and total brain size as well as allometric relationships between nRt measurements. Including F-values, degrees of freedom (df), p-values, and coefficient of determination (r^2) for each regression conducted. Significant p-values are in bold.

Independent variable	**Dependent variable**	***F***	**df**	**p- value**	**r^2**
Brain – nRt	nRt volume	1.88	1,17	0.19	0.09
Brain – nRt	nRt neuron number	0.20	1,17	0.66	0.01
Brain – nRt	nRt soma size	2.24	1,17	0.15	0.11
Brain – nRt	nRt neuronal density	0.26	1,17	0.62	0.02
nRt volume	nRt neuron number	10.98	1,17	**<0.01**	0.39
nRt volume	nRt soma size	0.36	1,17	0.56	0.02
nRt neuron number	nRt soma size	2.73	1,17	0.12	0.14
nRt soma size	nRt neuronal density	9.43	1,17	**<0.01**	0.36
nRt volume	nRt neuronal density	0.01	1,17	0.92	<0.01

Table 2.3. Results of bivariate linear regressions (OLS and SMA) between nucleus magnocellularis (NM) and total brain size as well as allometric relationships between NM measurements. Including F-values, degrees of freedom (df), p-values, and coefficient of determination (r^2) for each regression conducted. Significant p-values are in bold.

Independent variable	**Dependent variable**	***F***	**df**	**p-value**	**r^2**
Brain – NM	NM volume	0.15	1,16	0.71	0.01
Brain – NM	NM neuron number	1.53	1,16	0.23	0.09
Brain – NM	NM soma size	1.10	1,16	0.31	0.06
Brain – NM	NM neuronal density	0.62	1,16	0.44	0.04
NM volume	NM neuron number	3.62	1,16	0.08	0.19
NM volume	NM soma size	2.57	1,16	0.13	0.14
NM neuron number	NM soma size	0.08	1,16	0.89	<0.01
NM soma size	NM neuronal density	1.70	1,16	0.21	0.04
NM volume	NM neuronal density	5.61	1,16	**0.03**	0.21

Table 2.4. Minimum sample size estimates using nucleus rotundus (nRt) and nucleus magnocellularis (NM) volume, neuron number, soma size, and neuronal density measurements collected using unbiased stereology. The first two rows for both brain regions show the number of individuals needed so that 90% of values were within 10% of the population mean. While the second row demonstrates the number of individuals needed to have 90% of values within 20% of the mean.

Measurement type	**nRt**		**NM**	
	90% of values within 10% of mean	90% of values within 20% of mean	90% of values within 10% of mean	90% of values within 20% of mean
Volume (mm^3)	n = 16	n = 4	n > 18	n = 7
Neuron number (# of neurons)	n > 19	n = 10	n >18	n = 8
Soma size (**μ**m^2)	n = 10	n = 3	n =16	n = 4
Neuronal density (# of neurons/volume)	n > 19	n = 6	n > 18	n = 9

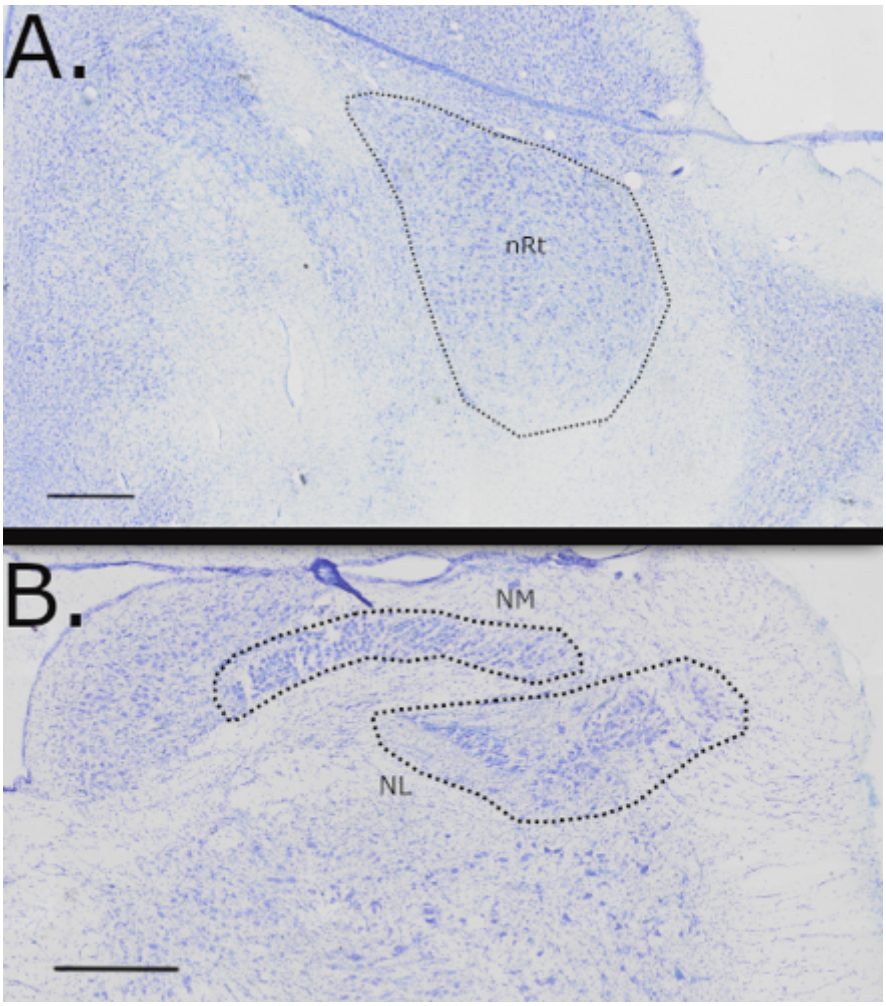

Figure 2.1. Coronal sections of **(A)** nucleus rotundus (nRt) and **(B)** nucleus mangnocellularis (NM) of a ruffed grouse (*Bonasa umbellus*). The dotted black line denotes the borders of these regions in the section. Scale bar represents 500 µm in both brain regions.

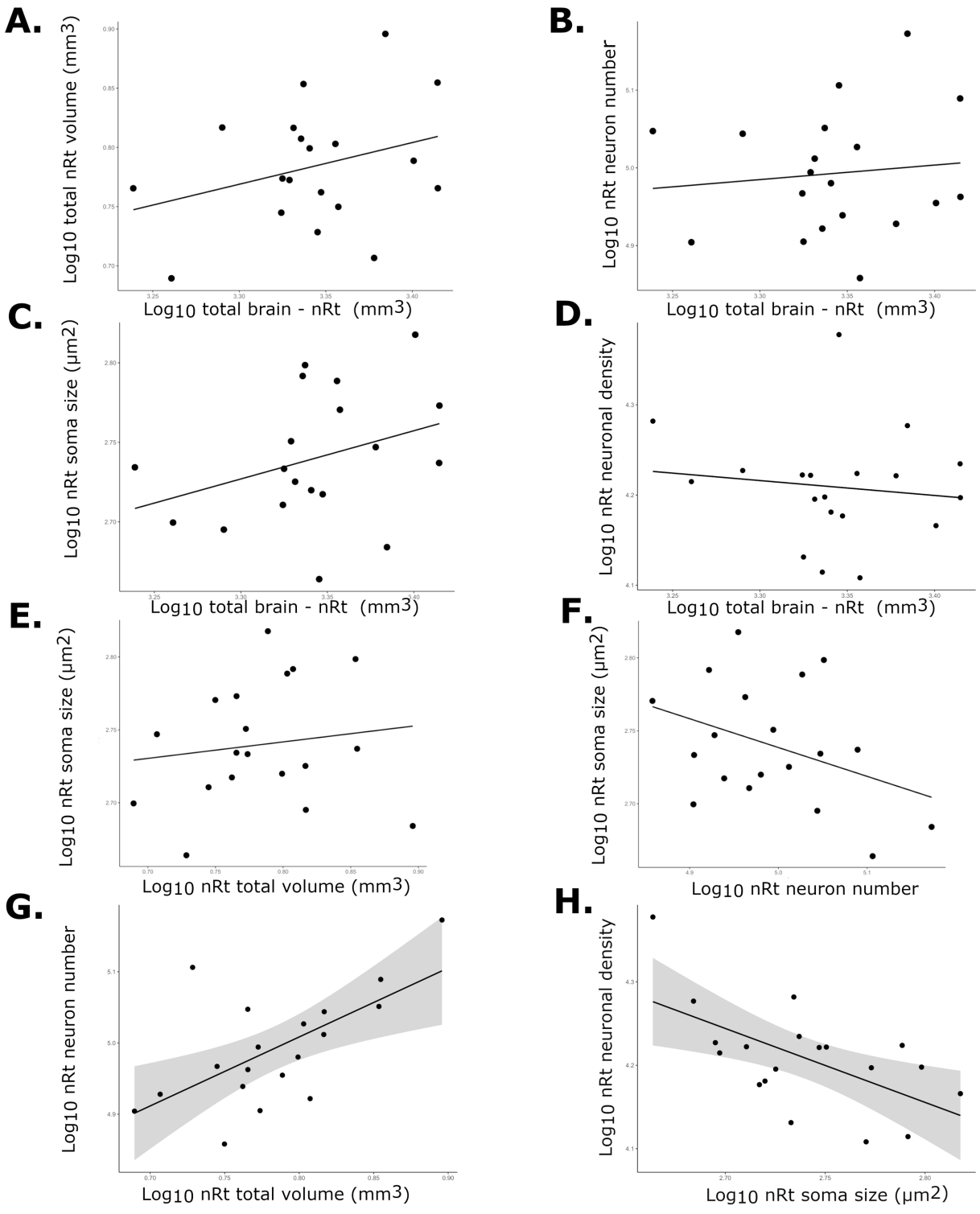

Figure 2.2. Scatterplots of log-transformed volume, neuron number, soma size and neuronal density with total brain size - nRt **(A-D)**. Scaling relationships between nRt measurements **(E-H)**. The significant scaling relationships between volume and neuron number **(G)** and soma size and neuronal density **(H)** include a shaded 95% CI

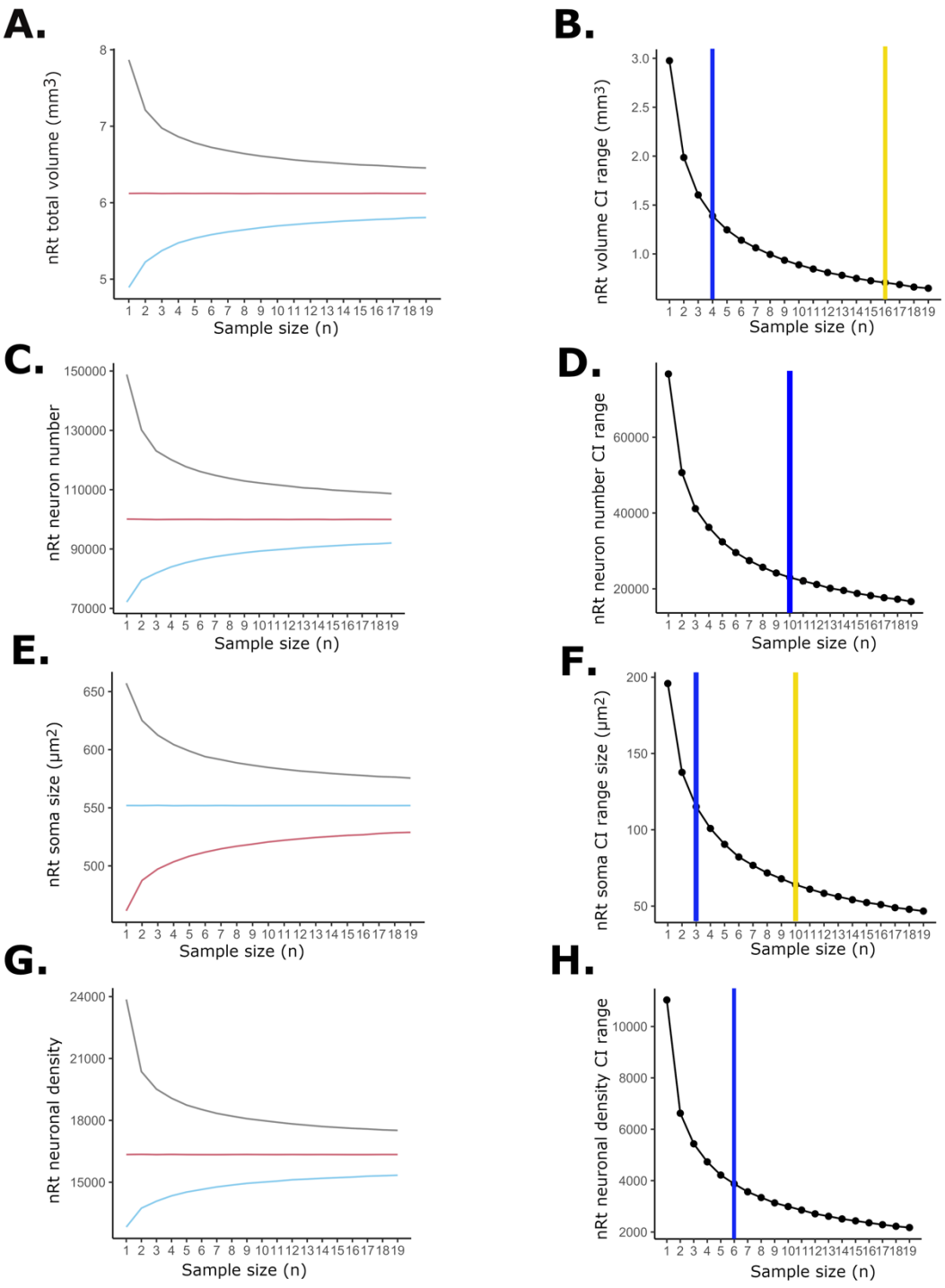

Figure 2.3. nRt Monte Carlo simulations set at a 95% confidence interval that asymptotes the sample population mean by increasing sample size **(A, C, E,G)**. Minimal sample size estimates where the blue line is the smallest sample size where 90% of measurements were within 20% of the population mean and the yellow line is the smallest sample size where 90% of measurements were within 10% of the mean **(B, D,F,H)**.

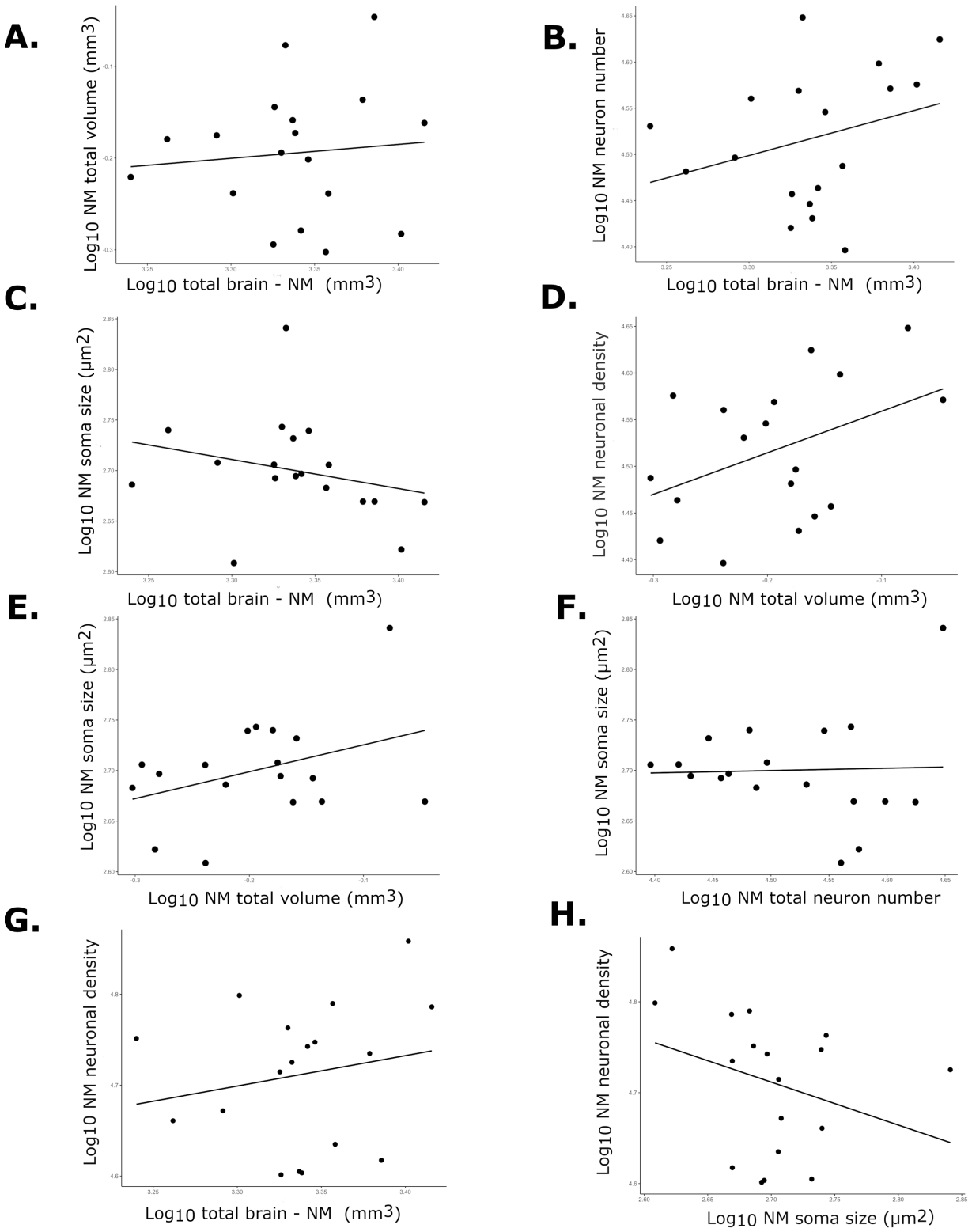

Figure 2.4. Scatterplots of log-transformed volume, neuron number, soma size and neuronal density with total brain size - NM **(A-D)**. Scaling relationships between NM measurements **(E-H)**.

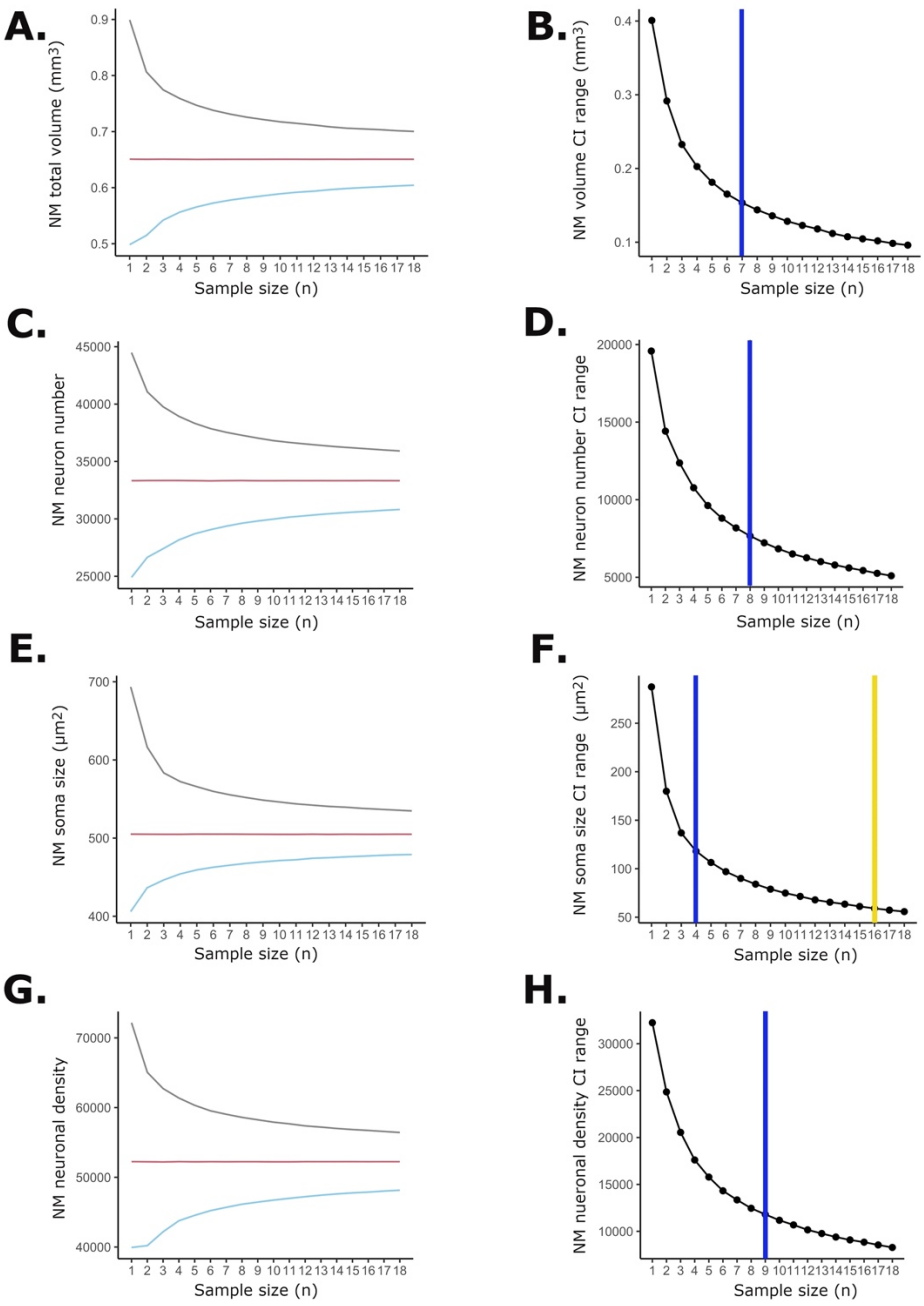

Figure 2.5. NM Monte Carlo simulations set at a 95% confidence interval that asymptotes the sample population mean by increasing sample size **(A, C, E,G)**. Minimal sample size estimates where the blue line is the smallest sample size where 90% of measurements were within 20% of the population mean and the yellow line is the smallest sample size where 90% of measurements were within 10% of the mean **(B, D,F,H)**.

CHAPTER THREE: INFLUENCE OF TEMPORAL AND ENVIRONMENTAL FACTORS ON THE FREQUENCY OF DAILY AND HOURLY DRUMMING

Introduction

Population ecology of the ruffed grouse (*Bonasa umbellus*) has been of interest to researchers for nearly a century due its widespread distribution and popularity as a gamebird (Bent, 1932; Leopold, 1931). The ruffed grouse can be found in early successional forests characterized by thick understory and a canopy comprised of aspen (*Populus sp.*) or birch (*Betula sp.*) mixed with conifer species (Berner & Gysel, 1969). Within these habitats, ruffed grouse are often difficult to locate due to their cryptic colouration and rare vocalizations. For over 60 years, springtime drumming surveys have therefore provided a practical way to census ruffed grouse (Petraborg et al., 1953). Drumming is a courtship display produced by males that is comprised of a series of rapid wing beats while standing on a log, root, or rock (Garcia et al., 2012). The drumming display provides a useful signal for surveying ruffed grouse because males typically remain within a specific territory when drumming (Gullion, 1967) and the ratio of male to female ruffed grouse in the spring is assumed to be near 1:1 (Bump et al., 1947; Gullion & Marshall, 1968). Thus, population trends (Ammann & Ryel, 1963; Dorney & Kabat, 1960; Jones et al., 2005) and grouse density (Petraborg et al., 1953) can be estimated by counting the number of drumming males heard within the spring breeding season. Surveys are typically conducted over several weeks and begin 30-45 minutes before or at sunrise and continue until 3-5 hours after sunrise (Felix-Locher & Campa, 2010; Hansen et al., 2011; Jones et al., 2005; Kouffeld et al., 2013; Zimmerman & Gutiérrez, 2008). During these surveys, a variety of counting methods can be used ranging from line transects listening for grouse or roadside surveys with designated survey points (Atwater & Schnell, 1989; Gullion, 1966; Petraborg et al., 1953).

Despite the widespread and long-term use of drumming surveys, several aspects of ruffed grouse drumming behaviour remain poorly understood. For example, only a single study quantified drumming activity throughout the day (Archibald, 1976) and while this study provided important data on hourly and daily drumming activity, it has several limitations. First, Archibald (1976) examined a relatively small number of drumming males (n=7), all of which were observed for only a single breeding season. This is problematic because drumming behaviour varies among individuals (Gullion, 1966) and likely across years. Second, although Archibald (1976) used a radiotelemetry system to track hourly drumming activity, data were often missing for individual males. To combat this, drumming activity was averaged over 15-minute periods and when the mean drumming frequency was greater than 0.5 (i.e., more than half a drum per 15 min), it was recorded as an active period. This method of measuring hourly drumming activity did not quantify the number of drums per hour of activity among males and instead noted the frequency of occurrence. To improve the accuracy of drumming surveys and learn more about ruffed grouse drumming behaviour, hourly drumming activity needs to be quantified using a larger sample size, across two or more years, and the times when peak drumming activity occurs throughout the day must be determined accurately. An analysis that quantifies how much ruffed grouse drum throughout the day while accounting for variation in drumming patterns among individuals will provide valuable information on when surveys should occur based on hourly activity.

Previous studies of annual drumming detection by surveyors suggest that drumming activity is influenced by temperature and date (Archibald, 1976; Gullion, 1966; Zimmerman & Gutiérrez, 2007). Even during peak dates of the breeding season, drumming activity was higher when

temperatures were within an ideal range of -3 to 2 °C, no precipitation, and low wind speeds as judged by the surveyor (Gullion 1966). More detailed analyses by Zimmerman and Gutierrez (2007) found that detection probability of drumming increased on mornings in which temperature increased more rapidly and the highest detection rates were on mornings where temperature at the start of the survey was relatively warm. However, these studies focused on whether drumming grouse are detected or not along transects rather than addressing how temperature influences the drumming activity of individual grouse throughout the day and across days. Further, no study has tested if precipitation influences drumming activity, even though some researchers have suggested precipitation influences drumming activity (Archibald, 1976; Gullion, 1966). Any assessment of variation in drumming activity throughout the day or across days should therefore also incorporate data on temperature and precipitation.

Here, we test the effects of time of day, date, temperature, and precipitation to drumming activity in multivariate models within and among males and across several years. Using autonomous audio recording units to quantify drumming activity throughout the day and across days over four years we amassed large amounts of highly precise measurements and avoid the issues of observer reliability in drumming surveys (Zimmerman & Gutiérrez, 2007). This comprehensive analysis will be important for refining drumming surveys further as well as improve our general understanding of drumming behaviour.

Material and Methods

Drumming activity of ruffed grouse was recorded during the breeding season (April and May) across four years on public lands near Buck Lake, Alberta, Canada (52.97°N, 114.77°W). Male ruffed grouse were located by listening for drumming while traveling along rural roads and trails within the study areas. Drumming structures were marked using a GPS unit (GSPMAP 64s, Garmin, Olathe, KS, USA) and latitude and longitude were recorded. Weatherproof acoustic recorders, Song Meters SM2 and SM2+ (Wildlife Acoustics, Maynard, MA, USA) recorded drumming activity across days at each site. Each Song Meter was placed within 10m of the drumming log in the afternoon when grouse are least likely to be occupying their drumming log. Male ruffed grouse typically occupy a single drumming log throughout the breeding season, and alternate logs are often located close to the primary log (Archibald, 1976; Gullion, 1967). Song Meters were positioned such that drumming activity at alternate logs could also be recorded. Batteries were changed every 3-4 days in the afternoon hours when males are generally not on their log (Iwaniuk, pers. obs.) to avoid disturbing the focal grouse during the active periods of the morning and evening.

Overall, we analyzed recordings from a total of 21 males across four years: five from 2013, four from 2014, six from 2015, and six from 2020 with 7,493 hours and 269 full days of drumming activity data. Drumming activity was scored using Audacity software (Audacity Team, 2020, Version 2.4.2). For each male, drumming displays were counted from 0:00-23:00h, a similar time frame to previous studies on the same population of ruffed grouse (Déaux et al., 2020). In cases where recordings were present for only part of a day, those hours were still counted and included.

Most drums begin with a quartet, a series of four low amplitude wing beats that occur before the drum itself (Fig. 3.1). Approximately 1/1500 drumming events will contain just a quartet. All displays that were counted had a visible waveform characteristic of a drumming display (Fig. 3.1) and could be heard in the recording. The total number of drums per hour and per day were tallied.

Data for precipitation (total mm/day) and daily minimum and maximum temperature were provided from the closest Environment Canada weather station in Rocky Mountain House (52.25°N, 114.77°W), a distance of approximately 60 km from the focal males. The daily temperature range was calculated by subtracting daily minimum from maximum temperature.

Analysis

Daily and hourly drumming activity is non-linear and environmental variables (e.g., temperature) can also follow a non-linear pattern daily and/or seasonally. To address non-linear patterns, generalized additive mixed models (GAMMs) were used to determine how time of day, date, and environmental factors predict drumming activity. All the GAMMS described below were fitted in R (R Core Development Team, 2017) using the mgcv package (Wood, 2006). Mixed models were used to account for random and fixed effects and smoothers accommodated non-independence between variables (Zuur et al., 2009).

The first GAMM modeled hourly activity (the number of drums counted per hour) as a function of time of day (0:00-23:00MDT) and Julian date using a quasipoisson log link function to

account for the overdispersion in the data set. Cubic regression splines were used to smooth time of day and cyclic splines were used for Julian date. Multicollinearity between Julian date and time of day was extremely low ($r = 0.02$). Year was included as a fixed effect and grouse ID as a random effect to account for inter-annual and individual variation respectively. For all smoothing functions of covariates, k was selected independently to optimize model fit and did not exceed 25 (Zuur et al., 2009).

Next, we constructed GAMMs that included our temperature variables to determine how Julian date and temperature influenced daily drumming activity. Hourly drumming activity was summed for each day to yield daily drumming activity totals. For these models, we omitted days with missing data. In total, 293 full days of daily drumming activity were included in the model. Three GAMMs were run using daily maximum, minimum, and temperature range as predictors of daily drumming activity. For all temperature models, the log link function and quasipoisson distribution were used to account for high variance in the number of drums counted per day and both Julian date and precipitation were included in all three models. For each of the three temperature models, multicollinearity between Julian date, precipitation, and temperature variables were checked and were low enough among variables to proceed with the model ($r \geq 0.60$). A cyclic spline was used for Julian date (Zuur et al., 2009) and cubic regression splines were used for each temperature variable and daily precipitation. Tensor products were included for the interactions between Julian date and minimum temperature and Julian date and precipitation to improve the fit of the model (Wood, 2006). Only one tensor product could be used per model. We selected Julian date and temperature because these variables were the most

significant predictors of daily drumming activity. For all three models, k was set between 15-25 for all smoothers to avoid overfitting (Granadeiro et al., 2004).

Results

Across the 21 males sampled, we tallied drumming activity across 4-35 days (average = 15 days per grouse) between April 12th and May 25th across four years. Daily drumming activity varied greatly among individuals. As shown in Fig. 3.2, daily drumming activity patterns vary among males across breeding seasons. For all of the days examined, the mean number of drumming displays performed per day was 151 and the highest was 405.

Hourly drumming activity

In the hourly drumming model, time of day had a robust, significant effect on the number of drums performed per hour (Fig. 3.3A, $p < 0.01$). The daily drumming activity pattern consisted of a pre-dawn activity peak beginning roughly one hour before sunrise followed by a brief plateau at sunrise (Fig 3.3A). Sunrise times were adjusted for variation by year and are indicated by the yellow line in Fig. 3.3A. Drumming activity then steadily declined to a trough centred at approximately 13:30MDT when males are rarely, if ever, drumming. Next, drumming activity begins to increase around 15:00MDT until the activity peaks around 19:30MDT, just prior to sunset (Fig. 3.3A). The secondary evening peak was much smaller than the morning peak.

Julian date was also a significant predictor of hourly drumming activity. The number of drums produced per hour peaked between April 25th and May 6th, as shown in Fig 3.3B. In contrast to time of day and date, the fixed effect of year was not significant (Table 3.1). The variation within

days and across dates is therefore consistent across years. Last, the random effect of grouse ID was significant ($p < 0.01$), indicating that hourly drumming activity varies significantly among individual grouse. Based on the F-values, the effect of grouse ID is smaller than that of time of day (Table 3.1), suggesting that the variability from hour to hour is greater than individual variation in drumming activity among grouse.

Minimum temperature

The first temperature model tested if daily minimum temperature was a significant predictor of drumming activity. Julian date was a significant predictor of drumming activity in this model (Fig. 3.4A, Table 3.2) with a peak of daily drumming activity between April 25th and May 6th. Minimum temperature was not a significant predictor of daily drumming activity (Table 3.2). However, the smoother for minimum temperature shows that as minimum temperature increases, daily drumming activity also increased (Fig. 3.4B). Neither precipitation nor the tensor product between Julian date and minimum temperature were significant predictors of drumming activity (Table 3.2). The random effect of grouse ID was significant (Table 3.2), but the fixed effect of year was non-significant (Table 3.2). Thus, individual grouse vary in their daily drumming activity, but there are no differences across years. The same is also true of the two other temperature models described below.

Maximum temperature

Unlike minimum temperature, maximum temperature was a significant predictor of daily drumming activity (Table 3.2). Julian date was a significant predictor of daily drumming activity (Table 3.2) with peak drumming occurring between April 25th and May 6th (Fig. 3.5A) and as

maximum temperature increased, so did daily drumming activity (Fig. 3.5B). Examination of the curve in Fig. 3.4B reveals that daily maximum temperatures between 0 and 14°C do not increase drumming activity (Fig. 3.4B), but when daily maximum temperatures reaches 15°C, daily drumming activity increases sharply (Fig. 3.4B). Like the minimum temperature model, neither precipitation nor the tensor product between minimum temperature and Julian date were significant predictors of drumming activity (Table 3.2). The random effect of grouse ID was significant (Table 3.2), and the fixed effect of year was not significant (Table 3.2).

Temperature range

As with the previous models, Julian date was a significant predictor of drumming activity (Fig. 3.6A), with a peak between April 25th and May 6th (Fig. 3.6A). Temperature range was also a significant predictor of daily drumming activity (Table 3.2), but the relationship between daily drumming activity and temperature range was u-shaped rather than linear (Fig 3.6B). Thus, drumming activity increased when the daily temperature changes by less than 10°C or more than 20°C or when temperatures were constant throughout the day. Together, this suggests extreme changes in daily temperature and stable daily temperatures with little variability throughout the day both increase drumming activity. However, there were relatively few days with consistent temperatures compared to days with large increases in temperature, so it is likely that the relationship between large daily temperature changes is more robust than those with consistent temperatures (Fig. 3.6A). As in the other temperature models, neither precipitation nor the tensor product between temperature range and Julian date, were significant (Table 3.2). Similarly, the random effect of grouse ID was significant and the fixed effect of year was not (Table 3.2).

Overall, the maximum temperature model had the lowest Akaike information criteria (AICc) score (AICc = 417) than the minimum temperature (AICc = 420) and temperature range (AICc = 418) models. However, with differences in AICc scores < 3 among the models, the maximum temperature and temperature range models are essentially equivocal.

Discussion

Based on our analyses, the peak of drumming activity occurs one hour before sunrise, with a second, lower peak one hour before sunset. Despite high variation among individual grouse, the hourly drumming peaks before sunrise and sunset were consistent and did not vary significantly across years with peak dates between April 25th to May 6th. Daily drumming activity also peaked at approximately the same time each year, with May 1st-3rd constituting the peak of daily drumming activity among years. Precipitation was not a significant predictor of daily drumming activity, but both maximum temperature and daily temperature range affected daily drumming activity, albeit not in a linear fashion. Together, these findings suggest that individual grouse vary in drumming activity and drumming activity is modulated by temperature, but clear and robust patterns of hourly and daily activity exist.

Only in the last ten years has research emerged that showed drumming displays vary significantly both within males and among males in the speed and number of drumbeats (Garcia et al., 2012). In addition, males vary markedly in their response to playbacks of drumming displays (O'Neil et al., 2018) and increase their drumming speed during the first 1-2 hours that they are drumming each day (Déaux et al., 2020). This so-called “warm-up” period in the morning was revealed through GAMMs that included significant random effects of individual,

like the current study and also linked this variation to colder low temperatures prior to drumming (Tables 3.1 and 3.2). Building upon these results, I show that daily drumming activity is also variable among individuals and covaries with temperature.

Many factors contribute to inter-individual variation in daily and hourly drumming activity. During the pre-laying period when males are drumming, female grouse have expanded home ranges and cover far more ground compared to the incubation period (Maxson, 1978). In contrast, drumming males remain within their activity centres during the breeding season and even when using a secondary drumming log, will not travel far from their primary log (Rusch et al., 2020). Brander (1967) showed that female grouse move between the activity centres of multiple drumming males, but females will not always mate and are thought to mate with multiple males. When a female approaches a log, males cease drumming and engage in a tail and ruff fanning display (Hjorth, 1970) and mating does not occur on the log (Berkley, 2014). Movement of drumming males off the log to pursue females that enter their activity centre likely contributes to the variability of drumming activity among and even within males. The threat of other males intruding upon the activity centre also creates variation in drumming activity as a male would need to leave the log to defend against an intruder. Predation risk or perceived predation risk influences drumming activity as well. Typically, if a larger animal (e.g., human, deer, moose, coyote, fox) gets too close to a drumming platform, the male will cease drumming and either walk or fly away. In addition, anthropogenic noise, such as road noise from large vehicles (e.g., semi-trucks) or noise produced by off-road vehicles often causes a male to stop drumming, a phenomenon that could clearly be observed in the Song Meter data. Many of the grouse recorded for this study had drumming logs that were located close to roads or off-road

tracks, and so intermittent anthropogenic noise was a contributing factor to period of drumming inactivity. Due to the random occurrence of anthropogenic noise at the study site, it is difficult to test how the presence or type of noise affects drumming activity but should be investigated in the future.

The fixed effect of year was not significant in any of the GAMMs (Tables 3.1 and 3.2). This is despite variation in winter snowpack, temperatures, precipitation, and other environmental factors among years. In addition, the dates when drumming activity was highest were similar across all models (Figs. 3.3-3.6A). Specifically, drumming activity increases markedly starting on April 25th, reaching a maximum on May 1st, followed by a gradual decline (Figs. 3.3-3.6A) and this is repeatable across four years of data. While it is unclear why drumming activity appears to decline in May, the shift in activity may be related to female reproductive physiology. At the peak of the breeding season, a brief pair bond forms and copulation occurs (Rusch et al., 2020). Within 3-7 days of copulation, the hen begins constructing a nest on the ground, and will often rebuild the nest if the initial nest site is destroyed (Maxson, 1977; Balzer,1995). During the nest building phase, hens are no longer receptive to mating and unlikely to visit drumming males. In the context of the Buck Lake population, the drumming peak, from April 25th to May 1st is likely when the majority of copulations occur and the decline following the peak of drumming activity would then correspond to the nest building period. In the future, the role of female reproductive cycles on drumming activity should be assessed, as it is likely a source of significant variation in drumming among males.

Regardless of the reason for the decrease in drumming activity in early May, all daily drumming models do suggest that day of the year is a robust predictor of drumming activity and this is demonstrated by the large effect size of this variable (Table 3.2). In fact, date appears to play a greater role than temperature in dictating drumming activity. With date corresponding to drumming patterns among males, surveys should focus on the peak days of drumming activity, but these need to be determined for each population as there may be regional variation in drumming activity patterns due to environmental factors and ecological factors such as predator abundance, which would result in variable drumming activity patterns throughout the species' range.

While the effect of date was strong, precipitation was not a significant predictor of drumming activity in any of our GAMMs (Table 3.2). A caveat of this result is that it could be an effect of the precipitation data available. The majority of days for which we had recordings, no precipitation was recorded and when precipitation did occur, it averaged 1mm. In addition to the general lack of precipitation across years, the closest weather station to the field site is approximately 60 km away, so an effective test of the effects of precipitation could be compromised by distance. Due to these issues with the precipitation data associated with our field site, it would be premature to conclude that precipitation does not affect drumming activity. One potential way to address the effects of precipitation would be to collect drumming activity data in a region that experiences more daily rainfall on average during the breeding season and if possible, precipitation measurements close to the drumming logs. For example, populations of grouse living in The Cascades mountain range have been understudied and likely experience

more rain during the springtime season (Wing, 1944) and would therefore be better suited for an effective test of the effects of precipitation.

In contrast to precipitation, maximum daily temperature, specifically higher than 15°C, was a significant predictor of drumming activity (Figs. 3.5B). This supports the findings of (Gullion, 1966) who showed that years with warmer (2-3°C) temperatures had more actively drumming males. In addition, large increases in daily temperature were a significant predictor of drumming activity (Fig. 3.6B, Table 3.2). With the model strongly supporting large temperature changes (greater than 15°C in one day) as a factor that increases daily drumming activity, which corroborates with the findings of previous studies. Zimmerman and Gutiérrez (2007) found that large increases in temperature on warmer mornings increased the probability of detecting drumming grouse. The effects of both temperature range and maximum temperature are relatively strong, which suggests that warm daily temperatures or days with large increases in temperature have more drummers active throughout the day (Table 3.2). To further test if warm temperatures promote drumming activity among males, temperature changes specific to the drumming centre should be recorded. In addition, the influence of microclimate on drumming activity has yet to be assessed and could provide new insights into how canopy cover, wind, humidity, and other variables relate to drumming behaviour.

In terms of when grouse are drumming, our hourly drumming activity model revealed largely similar patterns of activity to that of Archibald (1976). That is, drumming activity consistently peaks at approximately 04:30MDT and declines to a trough at 13:00MDT (Figure 3.3A). Two novel findings were revealed by the GAMMs. First, an evening peak of drumming activity

throughout the breeding season. Second, both peaks of hourly drumming activity occurred one hour prior to changes in light cycle (i.e. sunrise and sunset). Although an evening peak is present, this peak has less drumming activity overall compared to the morning drumming peak (Fig. 3.3A). As shown in Fig. 3.7, evening and morning peaks in drumming are present across multiple weeks' worth of activity for two grouse, despite being from different breeding seasons. This reinforces the idea that peaks in hourly drumming activity occur at similar times of day across individuals and years, including an evening peak. There are a multitude of factors that may contribute to this second peak of activity, but again female grouse movements could be a contributing factor. Hens are more mobile in the evening activity peak than the morning during the pre-incubation period (Maxson, 1978), so males could enhance their chances of breeding success by drumming in the evening. While this suggests that evening drumming could be linked in part to pre-incubation movements of female grouse, many other factors, such as predation, likely influence evening drumming activity. Predator presence during the period immediately following sunset could be linked to the decrease in hourly drumming after sunset, but future research will have to examine what variables influence the presence of an evening peak and how much drumming occurs within that secondary activity peak.

Conclusions

Based on the effects of time of day and date on drumming activity, we propose several recommendations for drumming surveys. First, surveys should be conducted during peak days of the breeding season to maximize the probability of detecting a drumming male. Second, due to high inter-individual variability in drumming activity, survey routes or transects should be visited more than once to account for the presence of grouse that may not have drummed during the first survey. Third, surveys should begin 2-3 hours before sunrise to capture the peak of morning drumming activity. Currently, survey start times begin 30 minutes before or at sunrise (Gullion, 1966; Jones et al., 2005; Zimmerman & Gutiérrez, 2008). Because the peak of hourly drumming activity occurs one hour before sunrise, drumming surveys that begin at or slightly after sunrise will miss the peak of daily activity. These are all feasible considerations that will improve the accuracy of a long-standing survey method that is necessary to maintain healthy populations of ruffed grouse.

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Table 3.1. Summary of GAMM relating the probability of male ruffed grouse hourly drumming activity to smoothed effects of time of day and Julian date. Tensor products (ti), random, and fixed effects are included with smoothers (s) for each model. Estimate and standard error (S.E.) are provided for the intercept and fixed effects of the model. Estimated degrees of freedom (EDF), F-values (*F*), and p-values are provided for smoothed effects.

Parameter	**Est.**	**S.E.**	**EDF**	***F***	**p-value**
Intercept	-53.577	48.62			
Fixed effect: year	0.027	0.024			
s(time of day)			16.34	168.58	**<0.01**
s(Julian date)			17.36	4.76	**<0.01**
ti(time of day and Julian date)			92.09	4.82	**<0.01**
s(random effect: grouse id)			18.42	15.87	**<0.01**

Table 3.2. Summary of GAMMs relating (**A)** the probability of male ruffed grouse daily drumming activity to smoothed effects of minimum temperature, Julian date, and precipitation. **(B)** the probability of daily drumming activity to smoothed effects of maximum temperature, Julian date, and precipitation. **(C)** daily drumming activity related to the smoothed effects of daily temperature range, Julian date, and precipitation**.** Tensor products, random, and fixed effects are included beneath smoothers for each model. Estimate and standard error (S.E.) are provided for the intercept and fixed effects of each model. Estimated degrees of freedom (EDF), F, and p-values are provided for smoothed effects.

Parameter	**Est.**	**S.E.**	**EDF**	**F**	**p-value**
A. Minimum temperature model					
Intercept	4.961	0.059			
s(Minimum temperature)			1.00	1.96	0.16
s(Julian date)			8.27	18.11	**<0.01**
t*i*(Minimum temperature and Julian date)			7.65	0.18	0.16
Random effect: Grouse ID			14.46	3.17	**<0.01**
B. Maximum temperature model					
Intercept	4.954	0.059			
s(Maximum temperature)			2.597	3.84	**0.01**
s(Julian date)			7.440	18.51	**<0.01**
ti(Maximum temperature and Julian date)			6.226	0.00	0.63
Random effect: Grouse ID			1.453	3.18	**<0.01**
C. Temperature range model					
Intercept	4.956	0.062			
s(Temperature range)			2.552	3.06	**0.02**
s(Julian date)			8.908	16.03	**<0.01**
ti(Temperature range and Julian date)			4.667	0.00	0.75
Random effect: Grouse ID			1.488	2.57	**<0.01**

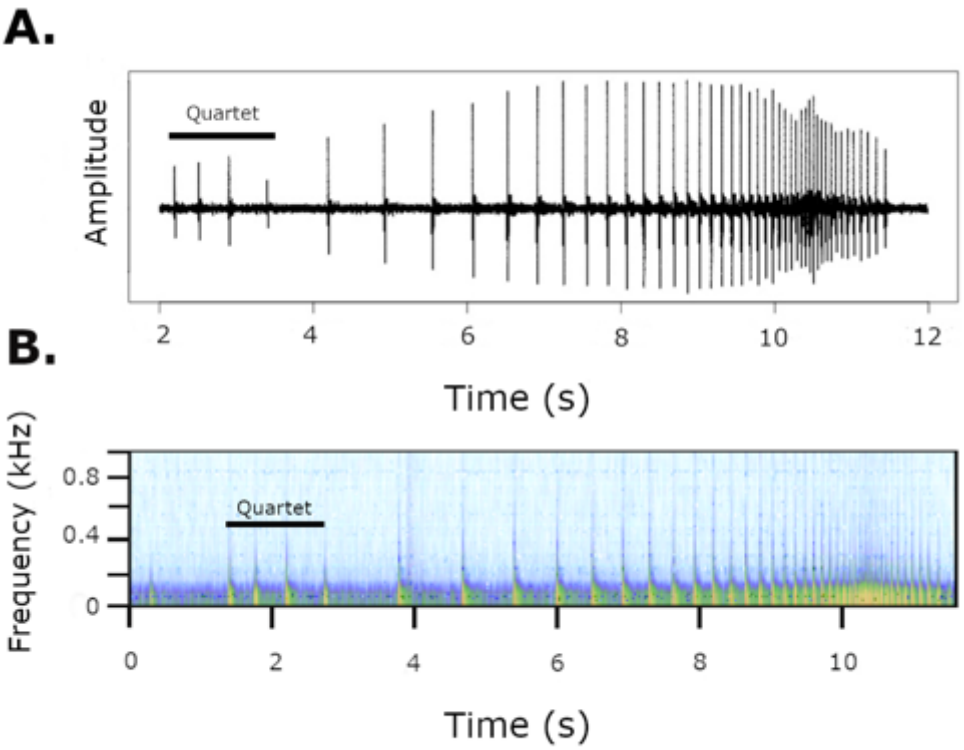

Figure 3.1. Spectral components of the drumming display of the ruffed grouse. Spectrogram **(A)** and waveform **(B)** highlighting the frequency (kHz) and amplitude of the display over time (s). Shows the quartet that precedes the drum along with the complete drumming display.

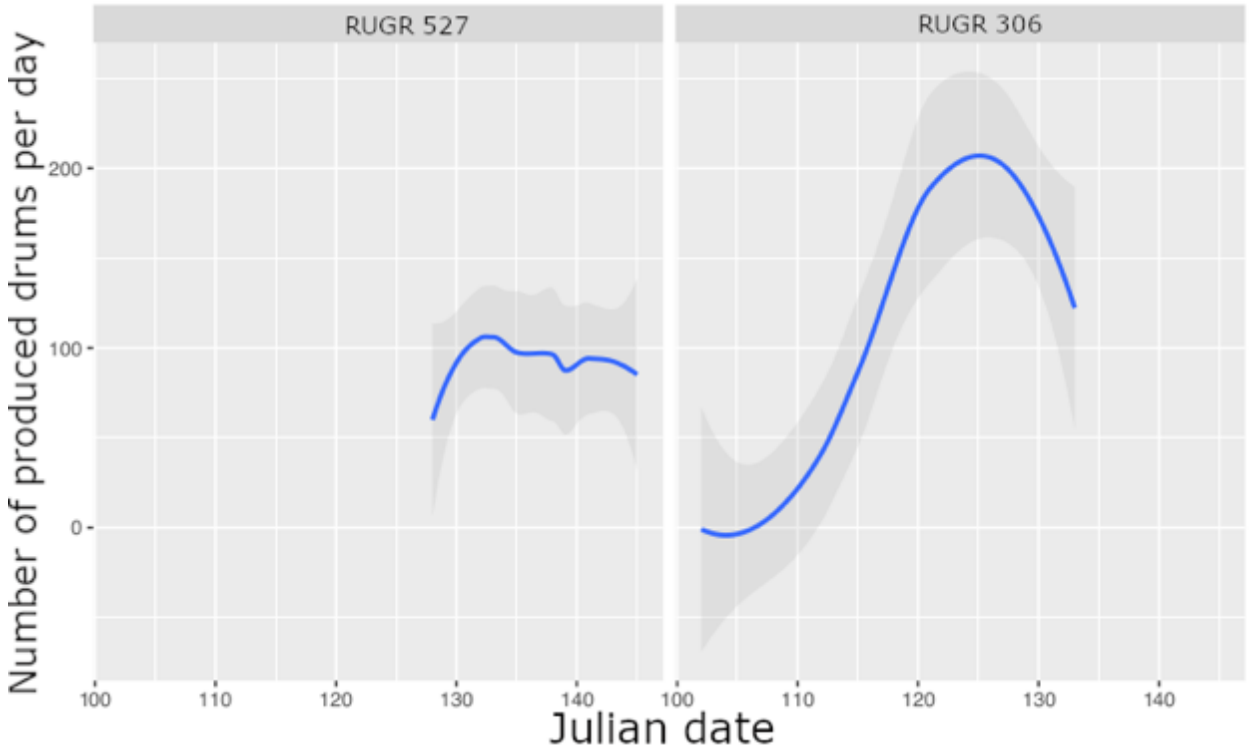

Figure 3.2. Variation in daily drumming activity between two breeding males from the 2015 and 2020 seasons. Daily drumming totals plotted against day of the year (Julian date). Drumming activity trends for two grouse, RUGR 527 and 306. Line shows that daily drumming activity varied daily and between individuals that were active during different seasons. Shaded area around the line of fit represents a 95% confidence interval.

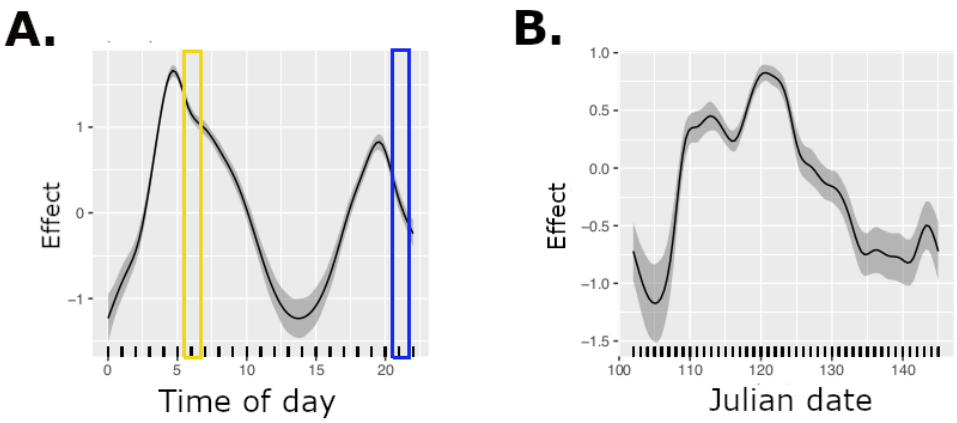

Figure 3.3. GAMM showing the partial effects of **(A)** time of day (0:00-22:00 hours) and **(B)** day of the year (Julian date)on hourly drumming activity (number of drums per hour) of 21 male ruffed grouse recorded at Buck Lake, AB across four breeding seasons. Gray shading around the lines of best fit represents 95% confidence intervals. The model incorporated time of day and Julian date as predictors of drumming activity while accounting for the fixed effect of year and the random effect of grouse ID. The yellow lines in panel **(A)** represent sunrise times (05:32-06:48 MDT) among years and months sampled, while the blue lines represent sunset times (20:33- 21:43 MDT). Panel **(B)** demonstrates how hourly drumming activity varies by day of the year, with an activity peak centered around May 1st.

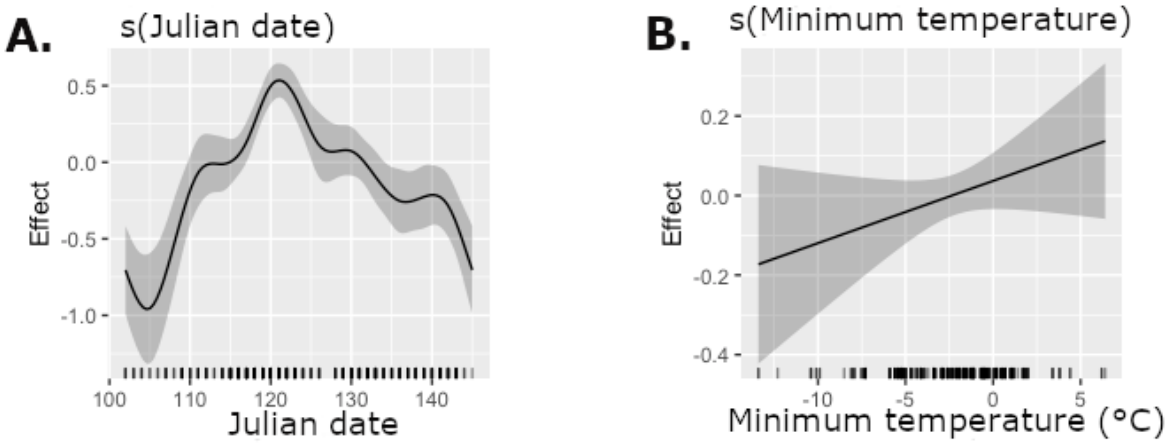

Fig. 3.4. GAMM showing the partial effects of **(A)** day of the year, **(B)** minimum temperature (C°), on daily drumming activity (sum of drums performed per day) of 21 male ruffed grouse recorded at Buck Lake, AB across four breeding seasons. Gray shading around the lines of best fit represents 95% confidence intervals. The model incorporated Julian date, minimum temperature, and precipitation as predictors of drumming activity while accounting for the fixed effect of year and the random effect of grouse ID. Panel **(A)** shows a general peak of daily activity centered around May1st. Panel **(B)** demonstrates the non-significant relationship between drumming activity and minimum temperature (linear increase).

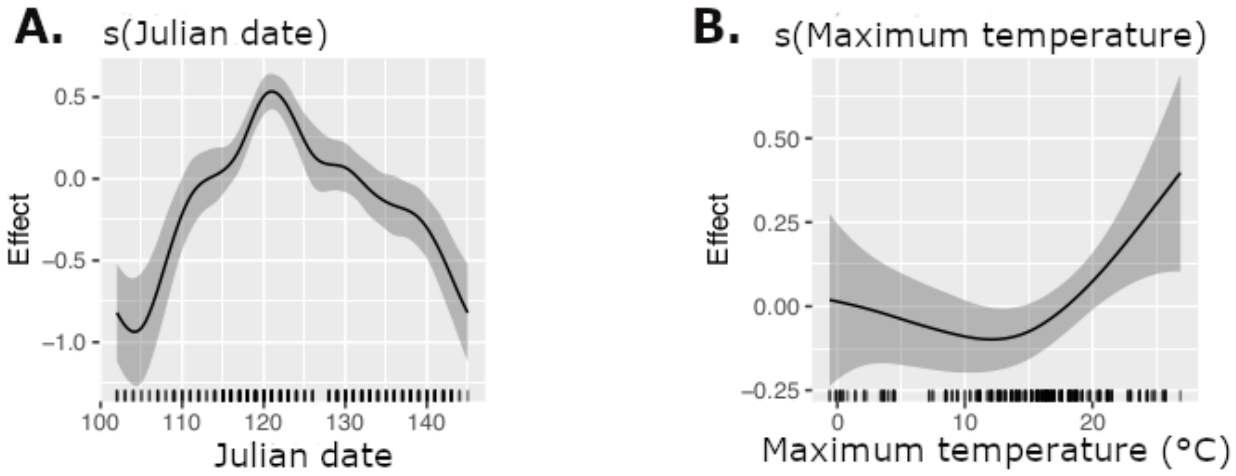

Fig. 3.5. GAMM showing the partial effects of **(A)** day of the year, **(B)** maximum temperature (C°) (sum of drums performed per day) of 21 male ruffed grouse recorded at Buck Lake, AB across four breeding seasons. Gray shading around the lines of best fit represents 95% confidence intervals. The model incorporated Julian date, maximum temperature, and precipitation as predictors of drumming activity while accounting for the fixed effect of year and the random effect of grouse ID. Panel **(A)** shows a general peak of daily activity centered around May1st. Panel **(B)** demonstrates the significant partial effect of maximum temperature on daily drumming activity with the highest drumming activity at temperatures greater than 15 C°.

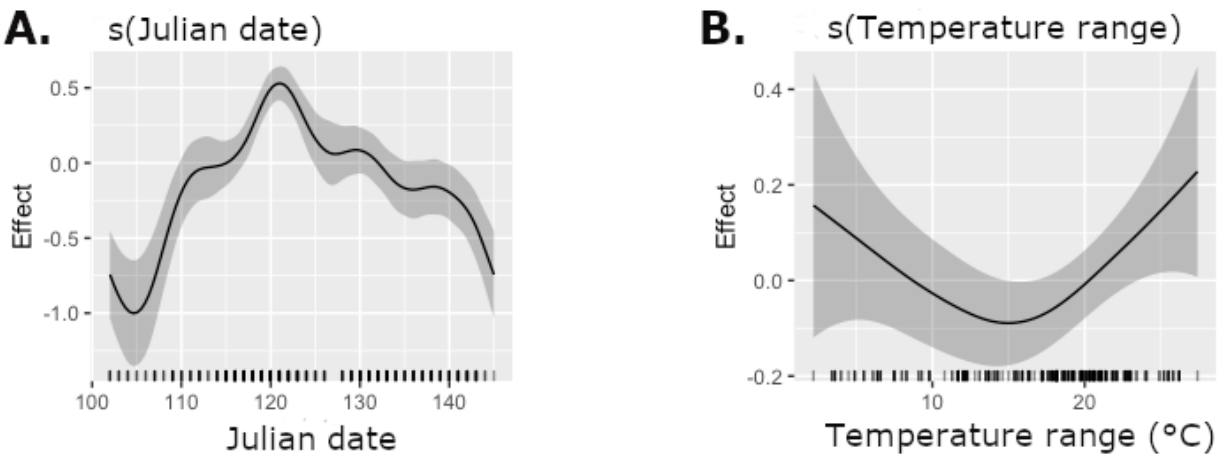

Fig 3.6. GAMM showing the partial effects of **(A)** day of the year, **(B)** Daily temperature range (C°) on daily drumming activity (sum of drums performed per day) of 21 male ruffed grouse recorded at Buck Lake, AB across four breeding seasons. Gray shading around the lines of best fit represents 95% confidence intervals. The model incorporated Julian date, temperature range, and precipitation as predictors of drumming activity while accounting for the fixed effect of year and the random effect of grouse ID. Panel **(A)** shows a general peak of daily activity centered around May1st. Panel **(B)** demonstrates the significant relationship between drumming activity and temperature range, forming a u-shaped curve.

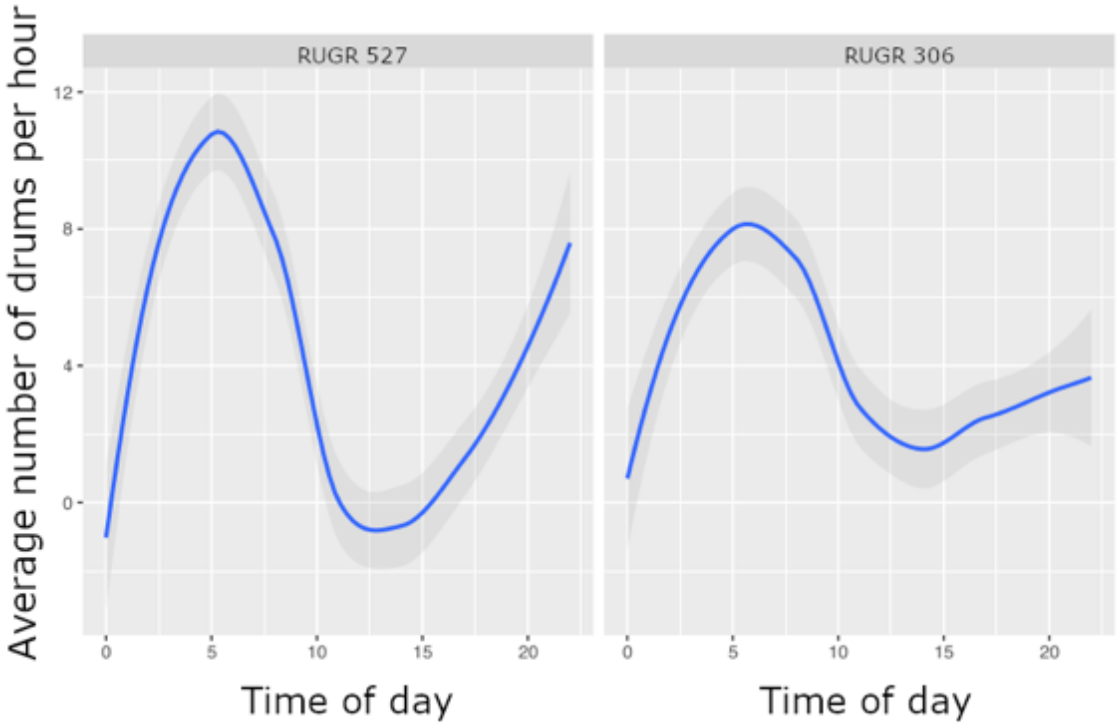

Fig. 3.7. Variation in hourly drumming activity between breeding males from the 2015 and 2013 seasons. Hourly drumming totals were plotted against time of day for 15 days. Drumming activity trends were included for two grouse, RUGR 527 and 306. Shaded area around the line of fit represents a 95% confidence interval.

CHAPTER FOUR: GENERAL DISCUSSION

Overall, this thesis has highlighted several aspects of individual variation among ruffed grouse (*Bonasa umbellus*). In chapter 2, I examined intraspecific allometry of two sensory brain regions nucleus magnocellularis (NM) and nucleus rotundus (nRt) and estimated the minimum number of individuals needed to accurately estimate the volume, neuron numbers, neuron sizes, and neuron density of the same two brain regions. Most of the measurements of NM and nRt did not vary significantly with overall brain size (Figs. 2.2 and 2.4) suggesting that the effect of intraspecific allometry is rather weak and corroborating similar findings in mammals (Herculano-Houzel et al., 2015). The bootstrap analysis revealed small sample sizes result in poor estimates of population averages across all neuroanatomical measurements (Figs. 2.3 and 2.5) and that some measurements are more variable than others. In fact, for most accurate estimates of the population mean, at least 10 individuals need to be sampled (Table 2.4). From these analyses I concluded that when extremely small ($n < 3$) sample sizes are used in interspecific studies, researchers should use caution in interpreting their results because they may not reflect population averages.

In chapter 3, I examined drumming activity throughout the day and the breeding season and tested if temperature and precipitation affected drumming activity. Hourly drumming activity was most strongly predicted by time of day and day of the year (Fig. 3.3, Table 3.1). Drumming activity is highest around one hour before sunrise, with a second, lower peak in drumming activity one hour before sunset (Fig. 3.3). In addition, daily maximum temperature and daily temperature range were significant predictors of daily drumming activity, while precipitation and minimum temperature were not (Fig. 3.5 and 3.6, Table 3.2). The random effect of grouse ID and

day of the year have a more significant overall influence on daily drumming than temperature variables (Table 3.2). Together, the statistical models indicated that daily and hourly drumming activity are mainly predicted by time of day and date, with temperature having modulatory effects. Despite the influence of date, time, and temperature, there are high amounts of variation among grouse in both hourly and daily drumming activity (Tables 3.1 and 3.2). This suggests that participation in drumming activity is also influenced by other, more individual specific factors as well. These findings not only improve our knowledge of ruffed grouse basic biology, they are also important for future studies in comparative neuroanatomy and the application of drumming surveys as a method of censusing ruffed grouse. In the next two sections, I discuss these implications in greater detail.

Individual variation and avian sensory brain regions: allometric scaling and sampling

As shown in Chapter 2, volume, neuron number, soma size, and neuronal density of both nRt and NM do not scale with total brain size among ruffed grouse. In fact, the scatterplots indicated that no clear relationship was even discernable (Figs. 2.2 and 2.4), highlighting the weak allometric effects. Different sensory brain regions process different aspects of sensory information, so it is possible that allometric scaling relationships vary across sensory systems and brain regions within sensory pathways. To determine differences of intraspecific allometric scaling among sensory brain regions, measurements should be conducted on brain regions that vary greatly in size and function. Not only would this provide useful information on how different sensory brain regions scale with total brain size, but it would also enable an estimation of variability of measurements within and across brain regions to help inform comparative studies and brain-behaviour relationships within species.

Although interspecific variation is often considered to be greater than intraspecific variation (Herculano-Houzel et al., 2014), my nRt and NM measurements demonstrate that the size, neuron numbers and neuron sizes of brain regions may be more variable at the intraspecific level than what is often assumed. Using Monte Carlo simulations, I showed that relatively large ($n > 19$) sample sizes may be needed to accurately estimate neuron number, neuronal density, or volume of a brain region. That said, the minimal sample size estimates varied across measurements, reflecting an additional source of variation: the measurements themselves. More specifically, neuron numbers appear to be more variable than other quantitative measurements, which could pose significant problems for recent studies focused on neuron numbers and neuron density differences across species (Herculano-Houzel & Kaas, 2011; Jardim-Messeder et al., 2017). Volumes were less variable and are still prone to error from small sample sizes, but that error is likely less than that of neuron numbers. As discussed in Chapter 2, soma size was the least variable, but this is a product of averaging soma size across 150 neurons per specimen whereas only a single measurement can be obtained per specimen of volume, neuron numbers or neuron density. Regardless of the variation among the measurements, I recommend using caution when small intraspecific sample sizes ($n < 3$) are used for any measurement and this needs to be discussed more explicitly in comparative studies.

Provided multiple specimens can be obtained of a species, one way to initially assess the degree of intraspecific variation of a brain region would be to determine if an allometric scaling relationship exists between the brain region and total brain size. If a significant scaling relationship is found, and the size of the sensory brain region varies with brain size, this could

indicate a lower degree of intraspecific variation or at least variation that can be accounted for with allometric scaling. In addition, bootstrapping could be used to estimate how representative measurements of sensory brain regions are of sample population averages, in a similar fashion to that presented in this thesis. There are multiple applications for this approach in interspecific comparative studies, particularly those that are aiming to determine differences in sensory brain region size in species where the number of specimens available may be limited. When studies are conducted that focus on sensory specialization of a rare or elusive species where the total number of individuals remaining in the wild is known (e.g., less than 100,000 individuals), simulations could be conducted that simulate as many data points as there are individuals remaining in the population. This would provide a means of taking intraspecific variation into account and more accurate test of whether a given species is truly different from others.

Watching the sunrise and feeling the heat: How time of day and temperature influence drumming activity in the ruffed grouse

Researchers have had an interest in documenting the behaviour of the ruffed grouse for over a century (Torrey & Knight, 1919). However, the large majority of research on this species has focused on the population ecology in an effort to understand what can be done to increase grouse numbers for hunting (Rusch et al., 2020). Because many ruffed grouse populations require active management, surveys must be done to determine grouse abundance. One way to census ruffed grouse populations is to conduct springtime drumming surveys, which count the number of non-vocal drumming displays made by male grouse at a survey stop or transect (Atwater & Schnell, 1989). Drumming displays of male ruffed grouse are audible to humans and have provided a way to census ruffed grouse for many years (Gullion, 1966; Jones et al., 2005; Petraborg et al.,

1953). In Chapter 3, I tested how drumming activity varies temporally and with temperature and precipitation to determine if time of day, date, and/or weather conditions are potentially impacting the accuracy of drumming surveys as they currently run.

Some authors suggested that precipitation might influence drumming activity (Archibald, 1976; Gullion, 1966), but precipitation was not a significant predictor of drumming activity in any of the models (Figs. 3.3-3.5). As discussed previously (Chapter 3), the lack of a significant effect of precipitation could be attributed to the weather data available and I recommend additional testing in regions with higher precipitation levels and/or more site-specific weather data. Regardless of whether or how precipitation affects drumming activity, it is important to note that it may also be too difficult for an observer to detect drumming grouse when it is raining. The sound of rainfall can cause interference for a surveyor and limit the propagation of the drumming sound, even though it is at a low frequency. Similarly, wind likely influences drum detectability, but there was insufficient wind data available to include in my models and it can often be specific to microclimate. Daily wind cycles could even play a role in modulating the time of day effects. The transmission distance of drumming displays would be greater during at times when wind speed is typically low, such as before sunrise. Future studies should consider the possible influence of wind on drumming activity, but through the use of locally deployed weather stations at or near drumming structures to ensure that the data is specific to each male being recorded.

In contrast to precipitation, temperature was a significant predictor of daily drumming activity. Specifically, maximum temperature and daily temperature range were significant predictors in our models (Figs. 3.5 and 3.6), while low minimum temperatures did not significantly influence

drumming activity (Fig. 3.3), which was unexpected. Following colder nighttime temperatures, ruffed grouse tend to drum slower and their "warm-up period", the time it takes for them to achieve their daily average speed, is longer (Déaux et al., 2020). The flip side of this relationship is that warmer nighttime temperatures are associated with increased drumming speed and a shorter warm-up period. Our data further shows that drumming activity increases with warmer maximum temperatures (Table 3.2). Thus, warm weather is associated with both the speed of the drumming display throughout the important morning peak of activity and the number of displays produced.

Temperature range was also a significant predictor, providing further evidence that temperature can be an important modulator of drumming behaviour. Given that rapid increases in temperature were also associated with increased detectability of drumming males (Zimmerman et al., 2007), an investigation of the effects of small scale (e.g., minute, and hourly) changes in temperature on drumming activity would shed new insights on the relationship between temperature and/or microclimate and drumming behaviour. Again, the deployment of miniature weather stations near drumming logs, as I described above for recording precipitation levels, would enable recording gradual changes in temperature within and across days in relation to drumming behaviour.

It is tempting to invoke a metabolic explanation for the effects of temperature on both drumming activity and speed (Déaux et al., 2020). However, it remains unclear how much energy is expended in drumming or how temperature might influence metabolic expenditure during the breeding season. Future studies should examine energy expenditure and metabolic rate in grouse

throughout the breeding season to compare with weather conditions, as this may influence the number and "quality" of displays produced.

Finally, the multivariate models revealed a high amount of variation in drumming activity among and within individuals (Table 3.1). As shown in Figure 3.7, males might share similar patterns of activity throughout the day, but very greatly in the number of drumming displays produced. When tallying drumming activity, it was also clear that males stop drumming at somewhat random intervals. As discussed in Chapter 3, males likely cease drumming for a variety of reasons. Individual variation in when and how much males are drumming has implications for drumming surveys above and beyond ensuring that survey start early enough and are done during the seasonal activity peak. Because daily, and especially hourly, drumming activity patterns vary among males and across days for the same individual, drumming surveys are unlikely to capture actual abundance of drumming males if only conducted once and with short listening periods. To provide more accurate estimates of grouse abundance, each survey route should be completed at least two times and if possible, one of these surveys could be done during the evening period. This might result in more accurate estimates of grouse abundance because surveys during both morning and evening activity peaks could mean that males that were not drumming in the morning, due to another conspecific, a predator, or other event, would then be counted during the evening survey.

An additional factor that might contribute to variation in drumming behaviour among males is habitat selection. Although ruffed grouse typically prefer aspen stands for the location of drumming activities, some males appear to prefer conifer stands. Even when population densities

are low, some grouse choose to establish themselves in conifer and mixed conifer forest over early successional forests dominated by young aspen (Zimmerman et al., 2009). The only comparison of drumming activity and visual display rates between conifer and aspen dominated habitat types yielded ambiguous results (Berkeley & Gutiérrez, 2017). However, Berkeley & Gutierrez (2017) used video rather than audio recording to tally drumming activity over short periods of time, which is unlikely to accurately reflect drumming activity. It is crucial to further determine how habitat composition affects drumming activity with the rapid maturation of eastern early successional forests and the influence of climate change on temperate forests (Rusch et al., 2020). Audio recordings of drumming activity throughout the breeding season from grouse drumming in different habitat types would provide a more accurate way to determine differences in drumming activity among males. With the wide distribution of the ruffed grouse (Rusch et al., 2020), it is especially important to consider the pressures faced by males with respect to habitat selection and other factors examined here, such as the influence of environmental and temporal variables. In the current study, we used audio recordings of drumming activity to find temporal and environmental predictors of daily drumming activity. Building from this method of quantifying drumming activity, the influence of other environmental and ecological factors on drumming activity could be assessed more accurately.

Conclusions

The ruffed grouse is a species that may be somewhat uniform in appearance, but as this thesis shows, they vary markedly in neuroanatomy and behaviour across individuals. While basic biological questions have long been overlooked in the long-studied ruffed grouse, I add to a growing number of researchers addressing basic biological questions that reveal new insights

into the anatomy and behaviour of this species. In addressing these basic biological questions, I also provide useful considerations of how to incorporate intraspecific variation into neuroanatomical studies as well as drumming surveys, a long-standing method to census and monitor ruffed grouse populations.

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