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Bioacoustic monitoring of bird communities and behaviour in fragmented and regenerating tropical dry forests

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regenerating tropical dry forests

by

Kiirsti Colleen Owen

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July 27, 2020

Declaration of co-authorship/previous publication

I. *Co-authorship*

I hereby declare that this thesis incorporates material that is the results of joint research as follows:

I am the sole author of chapters 1 and 4, and the principal author of chapters 2 and 3. I completed chapters 2 and 3 under the supervision of Dan Mennill, who contributed to the experimental designs, data collection, analyses, figure creation, and writing. He also provided funding for this research. Four additional researchers contributed to the data collection and preparation of Chapter 2: Amanda Melin, Fernando Campos, Linda Fedigan, and Thomas Gillespie. For all chapters of this thesis, the primary ideas, design, analyses and interpretations were contributed by myself with input from my supervisor and co-authors.

I am aware of the University of Windsor Senate Policy on Authorship and I certify that I have properly acknowledged the contribution of other researchers to my thesis, and have obtained written permission from each of the co-authors to include the above materials in my thesis.

I certify that, with the above qualification, this thesis, and the research to which it refers, is the product of my own work.

II. *Previous publication*

This thesis includes one original paper that has been previously published in a peer-reviewed journal, as follows:

Owen, K. C., A. D. Melin, F. A. Campos, L. M. Fedigan, T. W. Gillespie, and D. J. Mennill. 2020. Bioacoustic analyses reveal that bird communities recover with forest succession in tropical dry forests. *Avian Conservation and Ecology* 15.

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Abstract

Vocalizations provide important information about how animals respond to changes in their environments. In this thesis, I use bioacoustic tools to investigate how birds respond to habitat restoration and fragmentation in tropical dry forests of Costa Rica. Tropical dry forests are one of the most imperilled ecosystems in the world, and my study site, the Área de Conservación Guanacaste, is home to the largest remaining stand of this unique ecosystem. In both chapters of this thesis, I collected recordings of bird vocalizations to study avian responses to changes in the Área de Conservación Guanacaste. In my first data chapter, I used passive acoustic monitoring and point-count surveys to measure bird community responses to tropical dry forest regeneration. I found that bird diversity and abundance increase as regenerating forests increase in age. I also found that bird communities become more similar to primary forests with increasing forest succession. My research provides evidence that restoration efforts in northwestern Costa Rica are encouraging the recovery of bird communities. In my second data chapter, I used bioacoustic monitoring to study female and male vocal behaviours in Rufous-and-white Wrens living in fragmented mature forests, with a focus on the influence of neighbours, time of day, and time of year. I found that female wrens changed song types more often with more neighbours, however, I did not observe any effect of neighbours on vocal behaviours in male wrens or on duetting behaviours in both sexes. I found changes in vocal behaviour of female and male wrens in response to time of day and time of year corroborating previous findings. My research contributes to two areas of bird conservation research that require more attention: long-term monitoring of bird communities in changing landscapes, and behavioural monitoring of birds for conservation purposes. Understanding how birds respond to changes in their environment can help guide conservation practices to ensure the continued persistence of healthy bird communities and populations.

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Chapter 1: General Introduction

Introduction

Bioacoustic monitoring of birds is a vital tool in ornithological, behavioural, and conservation research. In this thesis, I take advantage of different bioacoustic methods – including focal recordings, passive acoustic monitoring, and point counts – to study bird ecology and behaviour. In particular, I use bioacoustic monitoring to investigate how bird communities and bird behaviours are influenced by forest recovery and conspecific neighbours. In this General Introduction, I review background topics that are important to the two data chapters that comprise the heart of this thesis, including the importance of monitoring habitat change on wildlife, and why bioacoustic methods are an ideal approach for monitoring birds. My two data chapters arise from field research in one of Earth's most imperilled ecosystems, the tropical dry forest (Janzen 1988a), and therefore, I provide background information on the unique features of tropical dry forests in general, and in my study site in northwestern Costa Rica. I conclude by summarizing the goals of the two data chapters.

Habitat change and wildlife

Anthropogenic habitat change has significant effects on wildlife and biodiversity (Brooks et al. 2002, Fahrig 2003, Fischer and Lindenmayer 2007). Habitat change can occur in the form of habitat degradation, fragmentation, or complete loss, all of which generally have negative impacts on wildlife (Fahrig 2003). As these forms of habitat change increase, habitats become disconnected, populations decline, culture erodes, and species face extinction (Fischer and Lindenmayer 2007, Laiolo 2010). Some forms of habitat change, including habitat restoration or creation, are intended to benefit wildlife by increasing the amount of suitable habitat or

reconnecting fragmented areas (Crouzeilles et al. 2016). Understanding how different types of habitat change influence wildlife can only be done through effective monitoring regimes.

Monitoring wildlife may involve measuring changes in the diversity and composition of entire communities or it may involve examining differences in individual animals' behaviours. In a recent study on the impacts of different agricultural methods on avian biodiversity, for example, birds exhibited long-term shifts in community composition in agricultural areas (Hendershot et al. 2020). Another example involved monitoring bird communities in Peruvian mountains where high-elevation species are facing reduced ranges and abundances due to climate change (Freeman et al. 2018). Monitoring may also focus on a single species' response to change. In a recent study of Hawai'i 'Amakihi (*Chlorodrepanis viren*), for example, habitat fragmentation and introduced disease lead to reduced song complexity (Pang-Ching, et al. 2018). In Savannah Sparrows (*Passerculus sandwichensis*) living near energy infrastructure, researchers found that acoustic properties of birds' songs were affected by different types and levels of noise pollution (Warrington et al. 2018).

Effective monitoring is also needed to understand whether conservation efforts are actually achieving the intended benefits for wildlife. For example, recent monitoring of passive restoration efforts in Brazilian Pampa grasslands found no difference in species richness or composition between restored and native grasslands, suggesting that passive restoration efforts successfully provided habitat for bird communities (da Silva et al. 2019). Unfortunately, monitoring programs are often limited to short time scales or, worse, not possible due to funding limitations (Lindenmayer 2020). As we move into the "Decade of Ecosystem Restoration" (United Nations 2019), researchers are emphasizing the importance of monitoring as a vital

component of any restoration project, and demanding that more attention and funding be put towards understanding the effectiveness of restoration efforts (Lindenmayer 2020).

Bioacoustic approaches for monitoring birds

Bioacoustic technologies provide tools that can help answer many questions related to wildlife ecology, behaviour, and evolution (Gibb et al. 2019). In birds, vocal communication plays a key role in many life stages including migration, reproduction, and territory defence (Catchpole and Slater 2008). The highly vocal nature of birds makes them a compelling study system for bioacoustic studies (Lewis et al. 2020). Bioacoustic methods have been used to study diverse topics in ornithology, including behavioural ecology, animal culture, biodiversity, habitat use, species recovery, and animal movement (Laiolo 2010, Shonfield and Bayne 2017, Teixeira et al. 2019, Lewis et al. 2020). In a conservation context, changes in avian vocalizations can indicate changes in behaviours or culture, which may impact population viability (Laiolo et al. 2008, Laiolo 2010). Therefore, by recording and analyzing bird vocalizations, researchers may be able to understand anthropogenic impacts on populations before it becomes more obvious through population decline.

Bioacoustic methods include focal recordings that rely on handheld microphones used to sample vocalizing animals or communities (e.g. Haselmayer and Quinn 2002, Hick et al. 2015, Demko and Mennill 2019), or passive recording using autonomous recording units placed strategically to record vocalizing animals or communities (e.g. Blumstein et al. 2011, Wilson and Bayne 2018). Passive acoustic monitoring through autonomous recording units is increasingly being used to collect information on bird communities, and to provide estimates of diversity and abundance (Shonfield and Bayne 2017, Darras et al. 2018). In a meta-analysis of passive

acoustic monitoring methods in comparison to traditional bird counts by humans, the acoustic recorders performed as well or better in most cases (Shonfield and Bayne 2017). Autonomous recording units allow researchers to collect large amounts of data in an efficient and cost-effective manner (Darras et al. 2018). An additional benefit of both focal and passive recording is the ability to permanently store data and review them as many times as necessary (Shonfield and Bayne 2017).

Bioacoustic methods can be used to monitor animal vocal behaviours in a conservation context (Teixeira et al. 2019). There is evidence that vocal behaviours can provide early indications of conservation problems (Laiolo 2010). For example, Dupont's Lark (*Chersophilus duponti*) living in fragmented habitats of Spain exhibited a decline in song repertoire size with decreasing habitat patch size, male density, and male dispersal distance (Laiolo and Tella 2007). This example reveals that bioacoustic monitoring of focal species can provide valuable insight into wildlife ecology, and can detect demographic and behavioural changes in response to habitat change or other anthropogenic disturbances.

Dry forests and the Área de Conservación Guanacaste

One of the most fragmented and restricted types of forests in the tropics are tropical dry forests (Janzen 1988b). Dry forests are areas where mean annual temperature is between 24 - 28°C and mean annual precipitation varies typically between 1500-3500 mm (Echeverri et al. 2019). Tropical dry forests differ from other tropical forests by the presence of pronounced wet and dry seasons. The majority of precipitation in tropical dry forests falls during a six to eight-month period of each year, with the remaining four to six months experiencing little or no rainfall (Janzen 1988a).

In Mesoamerica, tropical dry forests are the most threatened forest type with less than 2% of dry forest still intact (Janzen 1988a). The primary threats to these forests are fragmentation, anthropogenic disturbances, and climate change (Miles et al. 2006). Tropical dry forests have been disproportionately disturbed compared to wet forests and rainforests due to their suitability for human habitation, agriculture, and ranching (Murphy and Lugo 1986). In particular, tropical dry forests are easy to convert to agricultural land using fire during the dry season (Allen 2001). Fire removes deciduous vegetation, which accounts for much of the lower to mid-canopy species, and damages mature evergreen trees, which make up the upper canopy of mature stands. Continued burning discourages regrowth of evergreen tree species resulting in grasses and other deciduous low vegetation (Janzen 1986). Despite heavy exploitation and degradation of tropical dry forest, this ecosystem has received less attention among conservation biologists than other tropical forest types due to lower overall species diversity (Janzen 1988a).

Diverse wildlife exists in the tropical dry forests of Costa Rica, including over 250 species of birds (Janzen 1986). Overall, bird species diversity in dry forests is lower than that of rainforests (Stotz et al. 1996), including fewer migratory birds (Janzen 1988a). This could be in part due to the timing of dry season, with the hottest, driest, and most resource-limited time of year occurring at roughly the same time that many birds migrate through or settle into overwintering territories (Janzen 1986). Despite having lower bird diversity than rainforests, tropical dry forests are highly biodiverse, and many species of tropical dry forests exhibit unique adaptations, life histories, and behaviours not seen in rainforests (Janzen 1986, Graham et al. 2017). This is in part due to the extreme seasonality of tropical dry forests, which requires many species to develop special coping strategies. For example, many animals move into mature forest patches that retain their leaves and moisture during the dry season to escape the extreme heat and drought (Janzen 1986). Wildlife move into these refugia during the dry season when resources

are limited and then return to deciduous areas once the rains arrive (Janzen 1986). For these reasons, it is important to protect species diversity in dry forests and conserve these unique life histories and behaviours. Given their adaptations to deal with extreme climatic events, wildlife within dry forests may be better able to cope with climate change, which will likely result in increased numbers of extreme weather events and more severe climatic conditions (Meehl et al. 2002). However, a very recent publication suggests that the opposite could be true, and that tropical dry forests may be at higher risk of negative impacts from climate change than wetter forests (Aguirre-Gutiérrez et al. 2020).

The Área de Conservación Guanacaste is the site of a long-term restoration effort to regrow tropical dry forests to late successional states (Janzen and Hallawachs 2020). This ambitious endeavour began with the creation of Parque Nacional Santa Rosa in 1966, and was followed by further land procurements, and eventually, in the 1980s, the beginning of active and passive restoration efforts. Today the large conservation area represents a mosaic of forests at different stages of the regeneration process, and is an important site for scientific research (Janzen and Hallawachs 2020).

I conducted field research in the dry forests of the Área de Conservación Guanacaste in northwestern Costa Rica (10°51'N, 85°36'W; Figure 1.1). I collected data for Chapter 2 in Sectors Santa Rosa, Santa Elena, and Pocosol, and data for Chapter 3 in Sector Santa Rosa. These sections of the Área de Conservación Guanacaste are mostly regenerating tropical dry forest surrounding patches of primary mature forest. Heavy rains of the wet season arrive abruptly sometime in April to June, but most often in mid-May (Campos 2018). With the onset of the wet season, the ecosystem quickly responds to the influx of water after a long period of

extreme drought; many bird species begin nesting, trees and shrubs begin to leaf out, and insects start hatching in large numbers with the first rains (Janzen 1986).

Thesis goals

In this thesis my goal is to improve our understanding of how birds in tropical dry forests are influenced by habitat changes and conspecific neighbours. I use bioacoustic methods to answer questions related to birds at the community-level by investigating diversity and abundance measures in Chapter 2, and at the species-level by looking at behavioural differences in Chapter 3.

In Chapter 2, I examine how bird communities respond to several decades of tropical dry forest regeneration using passive acoustic monitoring and point-count surveys. I test the hypothesis that as forests increase in maturity, bird species richness, diversity, and abundance will also increase, and that community composition of regenerating forests will become more similar to that of undisturbed forests. My investigation centres on recordings of communities at many sites at different stages of recovery, and includes recordings collected in both the dry and wet seasons to address whether avian biodiversity changes seasonally.

In Chapter 3, I focus on a single species, the Rufous-and-white Wren (*Thryophilus rufalbus*), that lives year-round in mature fragments of tropical dry forests of Costa Rica. I use a 17-year dataset to test the hypothesis that vocal behaviours of male and female wrens are influenced by the number of neighbours with which they share their fragmented habitat. Specifically, I test if independent song rate, duet responsiveness, repertoire use, and song-type switching rate changes with the number of neighbours for both male and female wrens. My aim

is to increase our understanding of how the vocal behaviour of a tropical songbird is influenced

by the presence of conspecifics in fragmented forest habitat.

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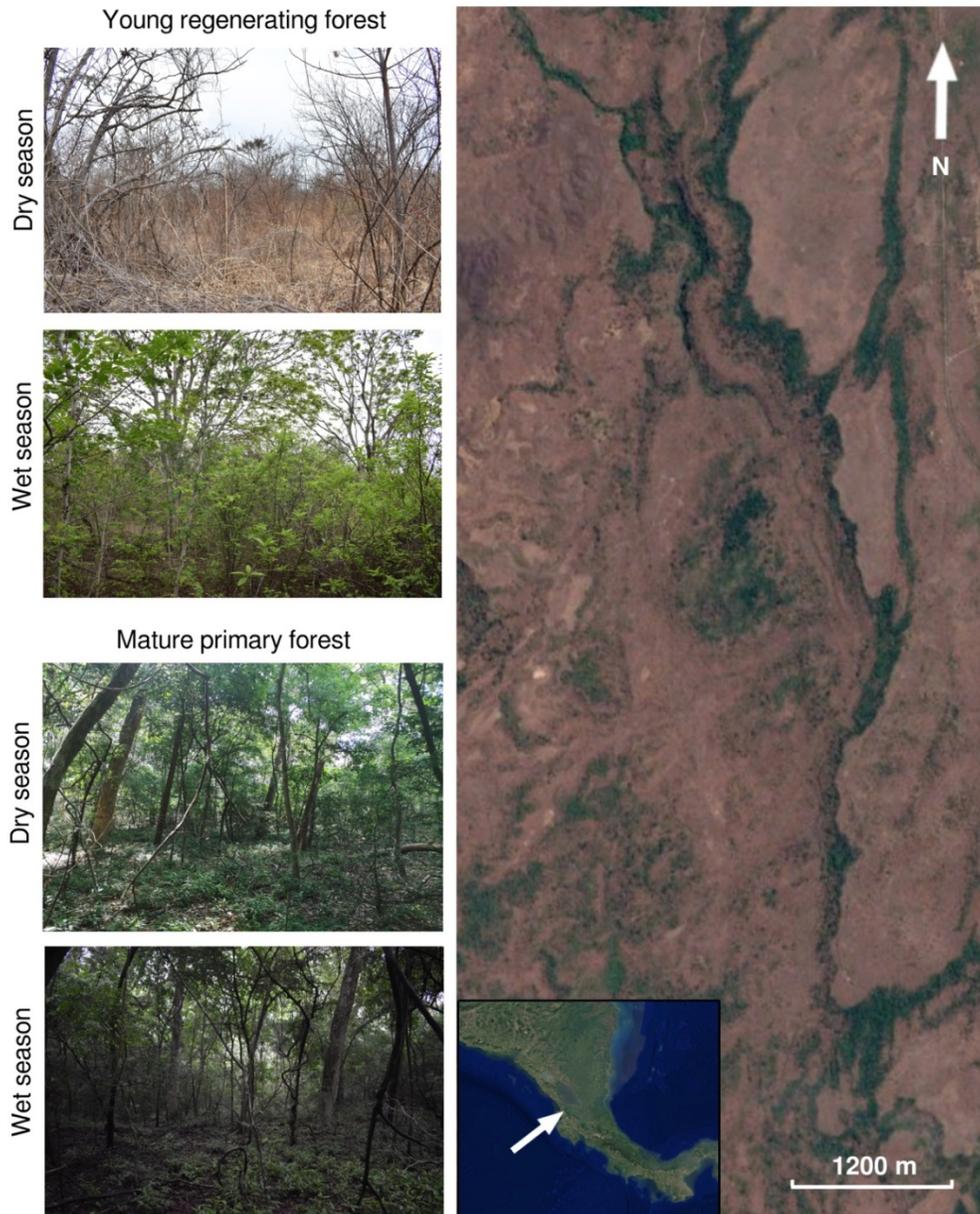
Figures

Figure 1.1. Photos from the Área de Conservación Guanacaste (left), showing a young regenerating forest (top left) and a mature primary forest (bottom left) in both dry and wet seasons, next to a map of Sector Santa Rosa (right) during a dry season, showing a clear delineation of mature primary forests patches (dark green) surrounded by regenerating forests (brown; Google Earth historical imagery from 2013). Arrow in inset map shows the location of the study area within Central America.

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Chapter 2: Evaluating effects of forest regeneration on bird communities in a tropical dry forest using a bioacoustic approach

Chapter Summary

With expanding anthropogenic disturbances to forests around the world, forest restoration is increasingly important for bird conservation. Restoration monitoring is critical for understanding how birds respond to forest regeneration and for assessing the effectiveness of restoration efforts. Using bioacoustic monitoring, we recorded bird communities during both dry and wet seasons at 62 sites along a chronosequence of tropical dry forests in the Área de Conservación Guanacaste in Costa Rica. Tropical dry forests rank among the globe's most imperilled ecosystems, adding special urgency to their restoration and accompanying restoration monitoring. We found that bird species diversity, richness, and abundance increase with measures of forest maturity. Our results show that bird communities in regenerating areas become more similar to those of undisturbed areas as forests mature. This suggests that bird communities are recovering to pre-disturbed conditions in regenerating sites, and that maturing tropical dry forests are home to an increasingly diverse and abundant community of birds. We conducted an additional assessment, by sampling 30 locations using point-counts that were originally surveyed 23 years ago. We found that species richness and abundance were similar across this 23-year interval, although bird community composition changed because several forest-specialist species were only detected in the later period. Our research reveals that the regenerating tropical dry forests of northwestern Costa Rica have recovered species richness and abundance levels and are currently undergoing a succession in community composition towards that of a primary tropical dry forest. Our study shows bird communities recovering in a nearly century-old chronosequence of regenerating forests.

Introduction

The impacts of environmental change on wildlife are well documented, with overwhelming evidence demonstrating that habitat loss and fragmentation have negative impacts on biodiversity (Brooks et al. 2002, Fahrig 2003). However, some forms of habitat change, such as habitat restoration or forest regeneration, can result in significant benefits for wildlife, especially in areas where some of the original forest has been removed (Rey-Benayas et al. 2009, Edwards et al. 2017). While restoration is emerging as an important approach to counteract the negative effects of habitat loss and degradation (Montoya et al. 2012), restoration monitoring is given less attention (Wortley et al. 2013). Without adequate monitoring of wildlife responses, we cannot assess whether restoration efforts are beneficial to wildlife (Block et al. 2001, DeLuca et al. 2010). Therefore, it is crucial that restoration is accompanied by wildlife monitoring to understanding whether restoration efforts are successful.

Previous investigations of habitat restoration and forest regeneration have shown that monitoring can help to guide good conservation practice (Wortley et al. 2013). For example, in northern Sweden, monitoring efforts revealed that bird diversity varied between two boreal forest restoration methods; sites with prescribed burns showed higher bird species richness and abundance, whereas sites with artificial gap creation showed no change (Versluijs et al. 2017). In the northwestern United States, monitoring efforts showed that an endangered butterfly species successfully established itself in a recovering forest, but indicated that larval resources were insufficient for long-term recovery (Schultz 2001). These examples highlight the importance of monitoring restoration programs to inform future conservation efforts.

In northwestern Costa Rica, an ambitious effort in ecological restoration began four decades ago, with the goal of protecting remaining patches of primary tropical dry forest and

restoring the land surrounding these areas to facilitate forest maturation (Allen 2001, Janzen and Hallwachs 2020). These forests have been subject to centuries of anthropogenic disturbance where most of the original forests were logged, burned, and cleared for ranching and agriculture (Moline 1999). Since the 1980s, the government and people of Costa Rica, with support from the international community, have endeavoured to regrow parcels of tropical dry forest, thereby reconnecting the remnant patches of primary mature forest (Allen 2001). This significant undertaking resulted in the creation of a new protected area called the Área de Conservación Guanacaste, which encompasses over 169,000 hectares (Janzen and Hallwachs 2020). Restoration has largely been through methods that promote natural regeneration, including fire suppression and cattle removal, although tree planting of locally-grown native species has also occurred (Moline 1999, Allen 2001). Today, the conservation area represents a mosaic of primary and secondary forests ranging in age from less than 1 year to over 400 years old (Janzen 1988, Janzen and Hallwachs 2020). The few remaining patches of primary forest in this mosaic are considered reference sites for restoration efforts in the tropical dry forest areas of the Guanacaste province (Janzen 1986). Recent research in the Área de Conservación Guanacaste suggests that secondary tropical dry forests have functional traits and vegetative structure similar to that of old growth forests within 100 years of successional regrowth (Buzzard et al. 2016). Vegetation features change with forest regeneration, with plant species richness and above-ground biomass increasing with forest age, and with maturing forests exhibiting different tree species composition (Buzzard et al. 2016). These results suggest that efforts to restore tropical dry forests in the Área de Conservación Guanacaste are promoting vegetation conditions similar to pre-disturbed states.

Tropical dry forests are considered to be one of the most imperilled tropical ecosystems in the world (Janzen 1988, Miles et al. 2006). These forests experience extreme seasonality with

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pronounced dry and wet seasons that each span approximately six months of the year (Janzen 1983a, Melin et al. 2014). Plant and animal species living in these forests have adapted to the extreme conditions of tropical dry forests, with some species being endemic to tropical dry forests, and others exhibiting unique life histories or behaviours not seen in nearby rainforests (Janzen 1986). In Guanacaste, annual rainfall varies between 800 and 2600 mm, with the overwhelming majority of rainfall occurring during the wet season (approximately May to November; Moline 1999). The end of the dry season is characterized by drought and high temperatures, and most trees drop their leaves to conserve water (Moline 1999). In the dry season, only late successional forests retain a closed canopy cover that exceeds 50% (Kalacska et al. 2005). For this reason, many animals move into patches of mature forest during the dry season where the microclimate is cooler and moister than surrounding forests (Janzen 1986). Even animals living within the mature tropical dry forests face reduced survival in the heat and desiccation of the late dry season, and this pressure is becoming more pronounced with global climate change (Woodworth et al. 2018).

More generally, a meta-analysis on tropical bird community recovery in regenerating forests found encouraging results; species richness and abundance can recover relatively quickly (within decades), although full recovery of community composition can take centuries (Dunn 2004). Most studies of bird community responses to environmental changes have used traditional methods for collecting information on avian biodiversity (e.g. point-count surveys or spot mapping; e.g. Pejchar et al. 2018, Frishkoff and Karp 2019, Roels et al. 2019, Santillán et al. 2020). Bioacoustic monitoring is an emerging tool for studying animals on the basis of their vocalizations, and has proven to be very effective for tracking bird communities (Burivalova et al. 2019, Teixeira et al. 2019). Bioacoustic surveys can increase the amount of data collected while creating a permanent record of these data and using fewer resources (Shonfield and Bayne

2017, Teixeira et al. 2019). Furthermore, there is evidence to suggest that passive acoustic monitoring performs similarly to traditional survey methods (Darras et al. 2018a). By using a bioacoustic approach to study changes in bird communities in recovering neotropical forests, we stand to develop a deeper understanding of the consequences of conservation actions on avian biodiversity.

We used passive acoustic monitoring to study bird communities in the regenerating forests of the Área de Conservación Guanacaste. We tested the hypothesis that bird communities change with the successional state of the forest (Catterall et al. 2012, Frishkoff and Karp 2019). We predicted that as forests increase in maturity and complexity, bird species diversity and bird abundance would change to resemble diversity and abundance features found in primary forests (Latja et al. 2016). We also predicted that different assemblages of birds would exist in forests of different ages, and that these assemblages would continue to become more similar to those found in primary forests over time. Given that the tropical dry forest ecosystem is defined by a dramatic transition between the dry and wet season, we were also interested in quantifying bird communities in both dry and wet seasons. We predicted that season would influence bird diversity, abundance, and composition, which would be reflected by differences in these variables between dry and wet seasons.

Methods

Study sites

We monitored bird diversity and abundance using acoustic recordings that we collected between April and July of 2018 and 2019 in the Área de Conservación Guanacaste in northwestern Costa Rica (10°52'N, 85°36'W; Figure 2.1). These recordings coincided with the end of the dry season

and the beginning of the wet season (typically mid-May), a time period when most birds in this ecosystem initiate breeding. We chose 62 sites within the Área de Conservación Guanacaste to collect recordings (Figure 2.1; Appendix 2.1). The regenerating sites in our study area have been subject to continued anthropogenic disturbance in the form of ranching and farming for over four centuries, and each site only began the regeneration process when it was given protected status at different times in the past century (Allen 2001, Janzen and Hallwachs 2020). Staff of the Área de Conservación Guanacaste continue to use passive methods, such as fire suppression, cattle exclusion, and prohibition of logging, to promote natural regeneration of these forests (Allen 2001, Janzen and Hallwachs 2020). In both 2018 and 2019, we sampled bird communities in 13 sites that were part of a previous vegetation study by Buzzard et al. (2016). We sampled 49 additional sites within Sector Santa Rosa over those same two years (n = 31 sites in 2018; n = 18 sites in 2019; details below). Within the tropical dry forests in the Área de Conservación Guanacaste, these sites comprise a chronosequence – a collection of forest sites that represent the different successional stages (Chazdon et al. 2007, Quesada et al. 2009). When long-term data do not exist to evaluate restoration success, a chronosequence can be used in place of temporal data (Chazdon et al. 2007). Our chronosequence includes sites at different stages of the regeneration process, and also primary forest sites that we considered to be mature reference sites. Information on the age of all 62 sites was provided by the scientific director of the Área de Conservación Guanacaste, R. Blanco. We considered all sites with no disturbance in the last 100 years to be reference sites (i.e. mature primary forest), whereas all of the regenerating sites were deforested within the last 100 years (range: 5 – 70 years; R. Blanco, pers. comm.). Although the mature sites have never been cleared, and trees that are many hundreds of years old are commonplace in those sites, mahogany trees were selectively removed in the 1940s (Janzen 1983b).

Vegetation measurements

Vegetation measurements came from two different datasets. In 2016 and 2017, vegetation transect surveys were completed in Sector Santa Rosa as part of a concurrent research project (Orkin et al. 2019). We included 49 of these survey sites to collect acoustic recordings of birds, choosing sites that were at least 150 m apart and that together created a representative sample of different aged forests (from 5 to more than 100 years old). Vegetation data from these surveys included total basal area, tree species richness, and tree abundance. In 2010, Buzzard et al. (2016) collected vegetation data at 13 sites, which represented different ages of tropical dry forests ranging from 20 years to >100 years. From these vegetation data, we looked at basal area, tree species richness, and tree abundance.

Canopy height and canopy cover data were not included in either of the vegetation datasets, yet canopy height and canopy cover are recognized to be important for birds (Matlock and Edwards 2006). Therefore, in 2019, we collected canopy height and cover measurements at n = 31 sites that were recorded that year. We measured canopy height based on a single representative tree using a clinometer (Matlock and Edwards 2006). We collected canopy cover measurements using wide-angle photography at each site during both the dry and wet seasons. We obtained a measurement of percent cover using Gap Light Analyzer imaging software (Frazer et al. 1999, Kalacska et al. 2005) and we calculated the percent cover across four photos per site to obtain a single estimate for each site.

Bird community measurements

We collected recordings using autonomous recording units (models Song Meter SM1 and SM2+; these models use the same microphones and hardware; Wildlife Acoustics Inc., Maynard, MA).

We used six recorders and moved them to different recording sites on subsequent days. Each recorder had an equally likely chance of being used at regenerating sites and reference sites. We used one recorder per site and recorded multiple sites per day. Recorders were placed at a height of approximately 1.5 meter from the ground. All 62 sites were recorded in the same location (Appendix 2.1) at two different time periods: in the dry season, and then again shortly after the onset of heavy rains (the first sustained rainfall was May 16 in 2018 and May 13 in 2019). Resampling sites during both dry and wet seasons allowed for a comparison of bird vocal activity at different sites during both seasons. We recorded sites continuously for at least one day, and we left recorders in place at sites for an additional day of recording if heavy rain or wind interrupted the recording during the dawn chorus. All recordings are archived in the Mennill Sound Analysis Laboratory.

Bird community recording analyses

We evaluated vocal activity from 150 recordings (62 sites, each recorded in the dry and wet seasons, some in both years; see Appendix 2.1). We analyzed 10-minute samples from 0600 to 0610 h, which coincides with a peak in avian vocal activity. Other studies, including studies in this ecosystem, have found that bird activity peaks around sunrise or in the hour following sunrise (Robbins 1981, Mennill and Vehrencamp 2005, Baldo and Mennill 2011, Koloff and Mennill 2013, Demko and Mennill 2019). Sunrise in Sector Santa Rosa from April to July occurs at approximately 0515 h. Field recordings were listened to and manually scanned by a single skilled observer (KCO) who visualized recordings as stereo sound spectrograms in Audacity (v2.2, Audacity Team 2018). To aid in species identification, we used online resources, such as Xeno-Canto (http://www.xeno-canto.org), the Macaulay Library (http://macaulay.ibrary.org), as well as a library of recordings that our research team has developed over the last 17 years of

working in this environment. We ascribed all vocalizations to species whenever possible. In many cases it was obvious that there were multiple individuals of a single species within a recording due to overlapping songs or the position of the vocalization relative to the stereo microphones; we conservatively calculated abundance using the position of birds relative to the microphones and overlapping songs as indicators of multiple individuals (Pillay et al. 2019). We included only resident species in our analyses, and removed any passage migrants (see Results). Given that we were interested in understanding how birds use forest sites of different ages, we chose to remove all parrots and parakeets from our analyses because they were consistently detected while flying over our autonomous recording units rather than landing within the recording sites.

Comparison of bird communities across decades

In addition to assessing bird communities along a chronosequence of tropical dry forest sites, we also sampled 30 sites in Sector Santa Rosa that were surveyed originally in 1996 (Gillespie 2000). These forests were 40 – 60 years old at the time of the 1996 survey (Gillespie 2000, Gillespie and Walter 2001), and 60 – 80 years old at the time of the 2019 survey. In early to mid-June of 1996, a single observer (TWG) conducted 10-minute point-count surveys at 30 sites in a grid that was approximately 500 meters (east-west) by 600 meters (north-south) with 100-150 meters between each point-count station (Gillespie 2000). This observer surveyed between 0530 and 0800 h for 10 minutes during fair weather days and included detections of all bird species within a 25 meter radius (Ralph et al. 1995, Gillespie 2000, Gillespie and Walter 2001). We replicated these point-count surveys in same forest in June 2019. Two observers (KCO and a field assistant) visited 30 sites within three days in mid-June approximately one month after the beginning of the wet season. Both observers completed practice point-count surveys together

prior to the survey days to ensure they had similar abilities in bird identification and distance estimates. Seasonal timing was similar between the two sampling years with the onset of heavy rains arriving in mid-May in both 1996 and 2019. We documented all birds detected during the surveys, however, for consistency with our automated recordings, we removed passage migrants and flyover species from final analyses. For consistency with the 1996 surveys, we only included birds detected within 25 meters.

Statistical analyses

We conducted all analyses in R (v3.6.1, R Development Core Team 2019). To test relationships among forest maturity, season, and bird biodiversity, we first created a Pearson's correlation matrix using R package "PerformanceAnalytics" (Peterson et al. 2019). Four habitat variables were positively correlated with forest age ($r > 0.35$, $p < 0.001$) including tree species richness, total basal area, canopy height, and canopy cover, while tree abundance was negatively correlated with forest age ($r = -0.34$, $p < 0.0001$). Additionally, during model selection, forest age was the only habitat variable that consistently showed a relationship with our bird response variables across all models. Therefore, for ease of interpretation we chose to use forest age to represent forest maturity in all of our final analyses.

We chose three variables related to bird biodiversity to use in our analyses: estimated total species richness (the Chao1 estimator; Chao 1984, Latta et al. 2018), effective number of species (Jost 2006, Latta et al. 2018), and observed bird abundance. Estimated total species richness was calculated using the function *estimateR* in R package "vegan" (Oksanen et al. 2019). The Chao1 estimator provides a lower bound estimate but has been shown to be a good estimator of true species richness (Walther and Moore 2005). We log transformed the total species richness values to correct for non-normality. We obtained Shannon diversity values for

each survey using the function *diversity* in R package 'vegan' (Oksanen et al. 2019). We used these values to calculate a diversity estimate accounting for species evenness called "effective number of species," by exponentiating Shannon entropy (Jost 2006, Latta et al. 2018). Observed bird abundance was simply the number of individuals identified on each recording.

To understand how bird communities respond to increasing forest maturity and season, we created linear mixed models using the R package "lme4" (Bates et al. 2015). In our models, we used season and forest age as fixed effects and we included the interaction between the two. Our models included both site identity and year, with a nested effect of ordinal day, as random effects. For the purpose of our linear mixed models, we square root transformed observed bird abundance, which lessens the influence of the most abundant species (Latja et al. 2016). We visually assessed our data for normality and homoscedasticity in R. We calculated marginal and conditional R^2 values for each of our models using *rsquared* in R package "piecewiseSEM" (Table 2.2; Lefcheck 2016), and p-values using R package "lmerTest" (Kuznetova et al. 2017). Additionally, to examine differences in our response variables by season without the influence of forest age, we used paired t-tests to compare bird diversity and abundance in the dry versus wet seasons (with data from both years pooled for the sites that were sampled in both 2018 and 2019).

To compare community composition of regenerating forests (i.e. sites <100 years old) to our reference forests (i.e. sites >100 years old), we pooled species lists to create a total count of the number of species across all surveys in reference forests during both seasons, and then accounted for sampling effort by dividing by the number of surveys (n = 18). We used the pooled species list to represent our reference community to compare to bird communities in the regenerating sites. We calculated Bray-Curtis dissimilarity values using the function *vegdist* in

package "vegan" (Oksanen et al. 2019) for all 132 surveys in regenerating sites as a percent difference from the reference community. We subtracted these from one, and converted to a percentage, to obtain percent similarity values (i.e. Sørenson Similarity Index). We also calculated percent similarity scores for all 18 surveys in reference sites to examine variability within the reference forests. To answer our question of whether bird communities in regenerating forests are becoming more similar to those in our reference forests, we used linear mixed models to examine the relationship between forest age, season, and percent similarity to reference forests.

To evaluate differences between point counts conducted in 1996 and 2019, we compared species lists between the two years. Data from 1996 exists as total counts for species and individuals for all 30 sites combined, therefore, we compared counts of species from 1996 and 2019 as an observational analysis. As in other studies using point-count surveys, we excluded passage migrants and highly mobile species (i.e. parrots that were detected flying over the forest canopy) from our total species lists (Edwards et al 2017, Darras et al. 2018b).

Results

Biodiversity assessments from automated recordings

We recorded a total of 4,884 individuals and 84 identifiable bird species across 62 sites in the tropical dry forests of Guanacaste, Costa Rica. We detected 2,533 individuals of 77 species during the dry season, and 2,351 individuals of 75 species during the wet season. We excluded two detections of passage migrants (one Swainson's Thrush, *Catharus ustulatus*, and one Yellow Warbler, *Setophaga petechia*), and we excluded 575 detections of parrots and parakeets detected flying over the recording sites (all detections are provided in Appendix 2.2). Any sounds that

were not identified to species were also removed from our analyses (n = 124). This resulted in a final count of 4,183 individuals of 77 species included in our final analyses.

Bird community recovery by forest age

We found that total species richness, effective number of species, and observed bird abundance were positively related to forest maturity (Table 2.1; Figure 2.2), such that older forests had higher species richness, diversity, and abundance. We found that communities in regenerating sites showed 18.5 – 58.9% similarity to the reference community, and that communities within individual reference sites used to create the reference community showed 49.1 - 62.4% similarity to the overall reference community. We found a positive relationship between percent similarity to the reference community and forest age (Table 2.1; Figure 2.3), such that bird communities became more similar to the reference community with increasing forest age. We did not find a relationship between percent similarity to reference community and season, nor an interaction between season and age (Table 2.1).

Biodiversity assessments in different seasons

We found that total species richness was higher in the dry season than the wet season (Figure 2.4a; paired t-test: $t = 3.9$, $df = 74$, $p = 0.0002$). Similarly, effective number of species was also higher in the dry season than the wet season (Figure 2.4b; $t = 3.1$, $df = 74$, $p = 0.003$;). Finally, we found that observed bird abundance was also higher in the dry season than the wet season (Figure 2.4c; $t = -2.1$, $df = 74$, $p = 0.041$). We found no other relationships with season, and the interaction terms between forest age and season did not show a relationship with any of our measures of biodiversity (Table 2.1).

Comparison of bird communities across decades

In June 1996, 267 individuals of 37 species were detected during fixed-radius point-count surveys (Gillespie 2000). In June 2019, 238 individuals of 35 species were detected in the same area, suggesting similar species richness and abundance across a 23 year interval. Although species and individual counts between the 1996 and 2019 surveys were similar, we did notice changes in community composition. Several species associated with open and young forest habitats were present in 1996 surveys but absent from 2019 surveys, including White-throated Magpie-Jay (*Calocitta formosa*), Hoffmann's Woodpecker (*Melanerpes hoffmannii*), and White-lored Gnatcatcher (*Polioptila albiloris*). Conversely, several species associated with mature forest habitats were absent or rare in 1996 surveys but present or abundant in the 2019 surveys, including Great Curassow (*Crax rubra*), Lesser Greenlet (*Pachysylvia decurtate*), and Yellow-olive Flycatcher (*Tolmomyias sulphurescens*; see Appendix 2.3 for counts from 1996 and 2019).

Discussion

Using automated recordings of bird vocalizations to assess avian biodiversity in neotropical dry forests, we found that bird species richness, diversity, and abundance increased as regenerating forests became more mature. However, even the oldest regenerating sites in our study (approximately 70 to 80 years old), still had lower total species richness, effective number of species, and observed abundance compared to the mature reference sites. This suggests that while bird biodiversity is recovering as neotropical forests regenerate, additional time is required before forest patches fully recover to pre-disturbance levels. The results from our point-count survey comparison showed minimal change in richness and abundance between surveys conducted 23 years apart. Overall, our results are consistent with the prediction that bird

biodiversity increases with increasing forest age, although the relationship was not perfectly linear, suggesting that richness and abundance have the highest increase in the first few decades after regeneration begins, as has been observed in previous studies (Dunn 2004, Pejchar et al. 2018). While some of our sampling locations exhibited richness and abundance levels similar to those observed in our mature reference sites, many younger regenerating sites will still require many decades to reach richness and abundance levels similar to our mature reference sites.

Our results show that bird communities in older regenerating sites became more similar to those in our reference forests, suggesting that community composition is recovering in regenerating forests. We also observed differences in community composition between the two point-count survey years, further supporting our hypothesis that different species use regenerating forests of different ages. We observed some overlap in community composition similarity values between regenerating forests and reference forests when comparing them to the reference community, suggesting that older regenerating sites may be recovering species composition to resemble primary forest communities. The high variability in similarity values within reference communities makes it challenging to evaluate the community composition recovery progress. These results follow similar conclusions from prior work on regenerating forest communities, where bird community composition was expected to take more than a century to fully recover (Shankar Raman et al. 1998, Dunn 2004). Specific to our study area, the complete recovery of these forests (i.e. including biotic and abiotic components) is a process that has been argued to require centuries (Allen 1988, Janzen 1988). It could take equally long for bird communities to follow suit. Composition of wildlife communities is an important element in the recovery of an ecosystem. The health and integrity of an ecosystem depend not only on the number of species and individuals living in the ecosystem, but also on which species it contains (Sekercioglu 2012, Rempel et al. 2016). For a restored ecosystem to have ecological integrity, it

must be able to support a community of species similar to that of undisturbed ecosystems (Karr and Dudley 1981, Carignan and Villard 2002).

Our results provide some interesting examples of how community composition changes with increasing forest age. Several species were detected in our youngest sites but did not occur in surveys from forests greater than 40 years old. These included Crested Bobwhite (*Colinus cristatus*), Double-striped Thick-knee (*Burhinus bistriatus*), Grey-crowned Yellowthroat (*Geothlypis poliocephala*), Lesser Ground-cuckoo (*Morococcyx erythropygus*), and Plain Chachalaca (*Ortalis vetula*). Similarly, many species were only detected in more mature regenerating sites (greater than 40 years old), including Long-billed Gnatwren (*Ramphocaenus rufiventris*), Rufous-and-white Wren (*Thryophilus rufalbus*), Royal Flycatcher (*Onychorhynchus mexicanus*), and Stub-tailed Spadebill (*Platyrinchus cancrominus*). One species, the Great Curassow (*Crax rubra*), was only detected in our oldest regenerating sites (~70-80 years old) and our reference sites (>100 years old). This species is considered a forest specialist and is listed by the International Union for the Conservation of Nature as vulnerable (BirdLife International 2019). Interestingly, no Great Curassows were detected in the 1996 point-count surveys, whereas eight individuals were detected in the 2019 surveys in the same area. This is an encouraging example of a forest specialist species using an older regenerating forest as it begins to resemble a mature forest. These examples further illustrate that restoration efforts in the Área de Conservación Guanacaste are promoting the return of forests and bird communities to their pre-disturbed states. Our point-count surveys were completed by different researchers in 1996 and 2019, and it is possible that variation in the detection and identification abilities of the researchers contributed to the differences we observed. However, given that all of the researchers involved had extensive experience with the vocalizations of birds in this region, we make the assumption that observer effects did not have a significant impact on our results. Using

bioacoustic surveys instead of traditional in-person surveys in future research would reduce any observer effects (Campbell and Francis 2011).

We predicted that season would influence how bird communities use tropical dry forests, and in support of this prediction, we detected more birds in the dry season than the wet season. However, we did not find support for the idea that birds use mature forests as refugia in the dry season because there was no interaction between forest age and season in our models. These results may reflect a change in vocal behaviour of birds between the two seasons. Many birds time their breeding activities so that they are feeding young when resource availability is high, which means breeding commences at the onset of the wet season in this habitat (Janzen 1983a). It is possible then that birds become more vocal towards the end of the dry season as they begin to defend territories, find mates, and build nests (Janzen 1983a), thus becoming easier to detect by automated recorders. Season may also affect detection rates if song transmission is influenced by changes in vegetation, possibly due to higher leaf density in the wet season. Due to our observed differences in detection rates between dry and wet seasons, we recommend that future research collecting bioacoustic data in tropical dry forests consider how season might influence vocal and breeding behaviour in their species or communities of interest. Collecting recordings at other times of year, including the early dry season and late wet season, might help to further reduce seasonal biases. In addition to differences in detection rates, it is also possible that changes in community composition could arise from local movements of animals within our study area or movements to other ecosystems such as nearby rainforests or cloud forests. Although we removed long-distance migrants from our analyses, some dry forest animals are known to move between different areas in our study area to take advantage of different resources such as moist and cool refuges or fruiting trees (Janzen 1988, Moline 1999). Although our results suggest a difference in bird vocalizations between dry and wet seasons, we have not determined

whether these results reflect changes in detection rates or changes in community composition. Future research might consider using occupancy models with bioacoustic data to determine the differences between detection rates and community composition to address this ambiguity.

Our bioacoustic surveys reveal that species richness, abundance, and community composition change as forests mature. Similarly, our point-count surveys reveal that more forest specialist species and fewer open habitat species are present when forests mature. Many studies have highlighted the benefits of bioacoustic surveys over traditional methods (Celis-Murillo et al. 2018, Darras et al. 2018a), including the ability to review recordings as many times as necessary (Shonfield and Bayne 2017). With continued improvements in bird recognition software, these types of surveys will likely become increasingly efficient and popular in wildlife monitoring. Future research might consider pairing acoustic surveys with other emerging tools such as remote sensing. Comparing biodiversity data from regenerating forests over time presents a singular opportunity to understand how wildlife respond to habitat change. As ecological restoration and monitoring continue to be applied around the world, these types of comparative studies can be used to assess the success of habitat restoration efforts. With the addition of acoustic recording in wildlife monitoring regimes, a permanent record of biodiversity surveys can be created and used by researchers who wish to directly compare these recordings to data collected in the future. We recommend future surveys of vocalizing wildlife should include an acoustic recording component for this reason.

Our research adds to the growing body of literature highlighting the benefits that large-scale restoration and conservation projects have for biodiversity (Crouzeilles et al. 2016). It is important to recognize that tropical dry forests are particularly susceptible to impacts from climate change because species in these forests have unique life histories and breeding

behaviours that strongly couple their survival and reproduction with intense seasonality (Janzen 1988, Woodworth et al. 2018). Climate change is expected to cause more unpredictable and extreme weather, further increasing the severity of drought and fire in tropical dry forests (Miles et al. 2006). Given this, it is critically important that conservation initiatives continue to be implemented and monitored in the Neotropics. Our results showed that bird diversity and abundance were greatest in the most mature forest patches, which are thought to serve as refugia for animals seeking to escape the extreme conditions of adjacent young forests, particularly during the dry season (Janzen, 1986). These refugia may become more important to wildlife as climate change continues to affect tropical dry forests.

Our results reveal the ways in which the restoration efforts in the Área de Conservación Guanacaste are successfully promoting bird community recovery. However, despite these encouraging findings, full recovery of tropical dry forests and their associated bird communities may not be reached for several centuries. Based on our results, we recommend that conservation initiatives focus on further protection of undisturbed forests and reconnecting these forest patches through habitat restoration. We also recommend continued monitoring of bird communities in the Área de Conservación Guanacaste to fill remaining gaps in our understanding of how bird communities recover in late successional forests.

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Tables and Figures

Table 2.1. Results from linear mixed effects models with fixed effects of forest age and season, as well as the interaction between these two variables, on features of the bird community the Área de Conservación Guanacste, Costa Rica. Random effects of site and day nested within year are included in the model. Response variables include total species richness, effective number of species, observed bird abundance (square root transformed), and percent similarity to reference community. Marginal and conditional R^2 values are provided for each model. Results with $p < 0.05$ are indicated with an asterisk.

Response variable	Fixed effects	Estimate	SE	t	P
Total species richness $R^2_m = 0.27$ $R^2_c = 0.45$	Intercept	14.4	1.6	9.1	<0.001*
	Forest age	0.2	0.03	5.7	<0.001*
	Season	1.1	2.6	0.3	0.78
	Forest age×season	0.07	0.04	1.5	0.14
Effective number of species $R^2_m = 0.22$ $R^2_c = 0.50$	Intercept	10.1	0.7	14.7	<0.001*
	Forest age	0.06	0.01	5.3	<0.001*
	Season	1.9	1.1	1.7	0.09
	Forest age×season	-0.01	0.02	-0.59	0.56
Observed bird abundance (square root transformed) $R^2_m = 0.20$ $R^2_c = 0.55$	Intercept	4.6	0.1	33.9	<0.001*
	Forest age	0.01	0.002	5.1	<0.001*
	Season	0.2	0.2	1.2	0.25
	Forest age×season	-0.001	0.004	-0.4	0.73
Percent similarity to reference community $R^2_m = 0.40$ $R^2_c = 0.79$	Intercept	24.8	2.6	9.6	<0.001*
	Forest age	0.4	0.5	6.7	<0.001*
	Season	2.1	2.5	0.8	0.38
	Forest age×season	0.02	0.05	0.3	0.74
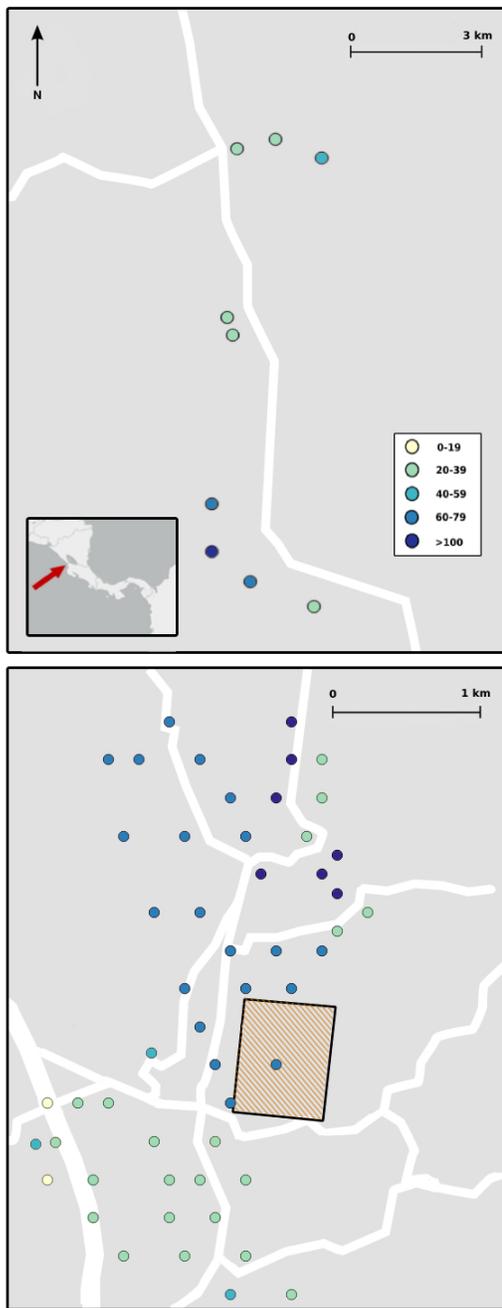
Figure 2.1. Maps of the Área de Conservación Guanacaste, Costa Rica, showing the location of 62 sites monitored with autonomous recording units in 2018 and 2019, as well as the area where point counts were conducted in 1996 and 2019 (orange shaded rectangle). Upper map shows the more northerly sites, and bottom map shows the more southerly sites (these regions are separated by approximately 1 km). White lines represent roads. Red arrow in the inset map shows the location of the study site in Central America.
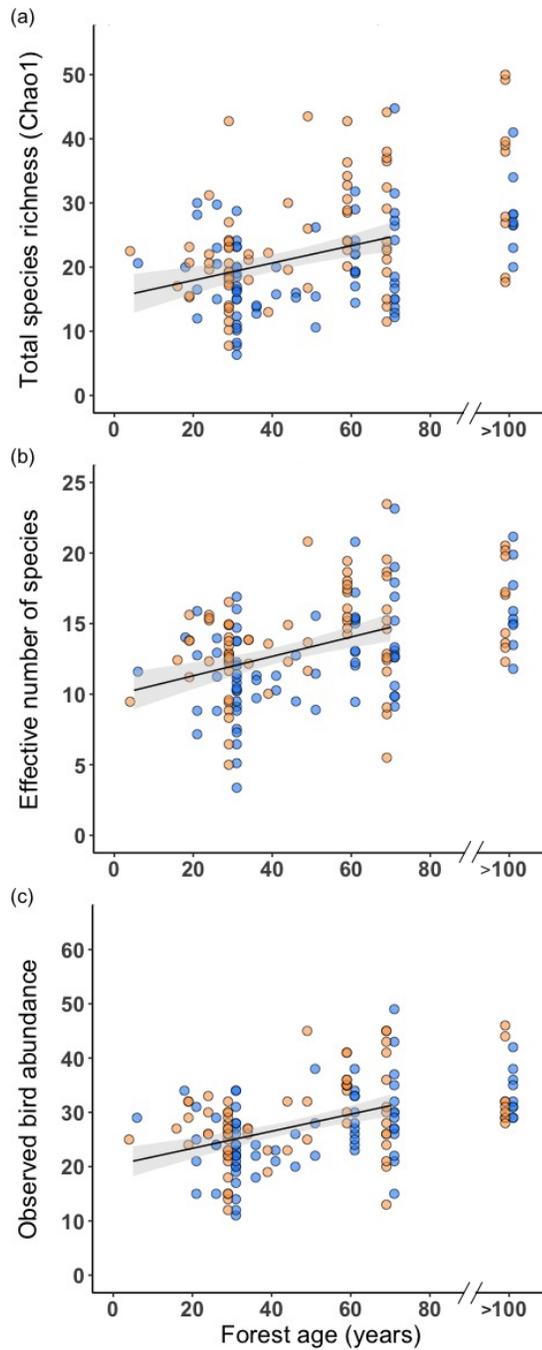
Figure 2.2. (a) Estimated total species richness (Chao1), (b) effective number of species, and (c) observed bird abundance versus forest age at 62 sites sampled in the Área de Conservación Guanacaste, Costa Rica. References sites are denoted here as >100 years. Lines represent best fit based on linear mixed effect models of each bird response variable by forest age (excluding >100 year old forests) and season with 95% confidence intervals. Sites sampled in the dry season are shaded orange, and sites sampled in the wet season are shaded blue. All dry-season points are shifted one value to the left, and all wet-season points are shifted one value to the right, to reduce the number of overlapping points.
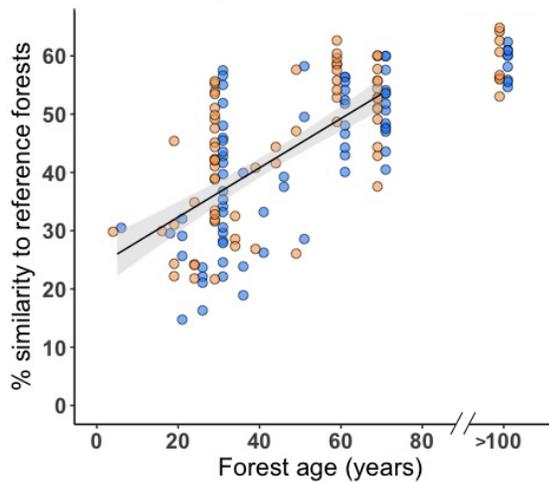
Figure 2.3. Percent similarity between bird communities in each site compared to the reference community (pooled data from sites >100 years old) in Área de Conservación Guanacaste. Line represents best fit based on linear mixed effects model of percent similarity by age of regenerating forest and season with 95% confidence intervals. Sites sampled in the dry season are shaded orange, and sites sampled in the wet season are shaded blue. All dry-season points are shifted one value to the left, and all wet-season points are shifted one value to the right, to reduce the number of overlapping points.
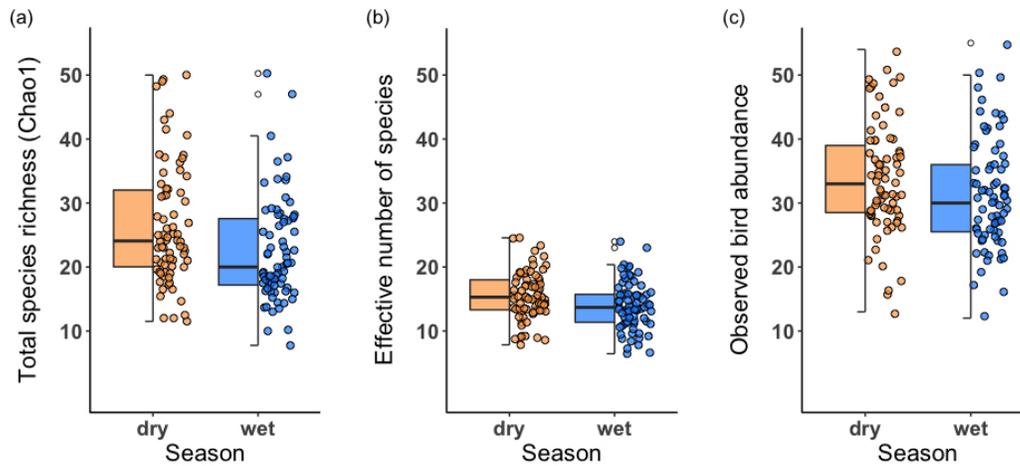
Figure 2.4. (a) Estimated total species richness (Chao1 estimator), (b) effective number of species, and (c) observed bird abundance in both dry season (orange) and wet season (blue) for birds in the Área de Conservación Guanacaste, Costa Rica. Data are shown both as box plots (central line shows the median, box boundaries represent the interquartile range; whiskers show the interquartile range multiplied by 1.5 with any values beyond these limits are represented as dots) with the raw data shown beside the box plots.

Appendix 2.1. Geographic coordinates of 62 sites sampled with autonomous recording units in the dry and wet seasons of 2018 and 2019 in the Área de Conservación Guanacaste, Costa Rica (latitude and longitude given in the WGS84 coordinate system).

Site	Approximate forest age (years)	Dry season sampling date(s)	Wet season sampling date(s)	Latitude	Longitude
1	>100	2018-05-12	2018-06-25	10.85939	-85.60964
2	30	2019-04-19	2019-06-03	10.84795	-85.60508
3	30	2018-04-30	2018-06-16	10.84680	-85.60691
4	70	2019-04-19	2019-06-30	10.84566	-85.60782
5	70	2018-05-02	2018-06-16	10.84566	-85.61055
6	70	2018-06-03	2018-06-16	10.84566	-85.61328
7	17	2019-04-13	2019-05-30	10.83652	-85.62420
8	30	2018-04-24	2018-06-03	10.83652	-85.62238
9	30	2018-04-25	2018-06-03	10.83652	-85.62056
10	70	2018-04-29	2018-06-10	10.83652	-85.61328
11	30	2018-04-28	2018-06-11	10.82507	-85.60964
12	60	2018-05-07	2018-06-21	10.85939	-85.61692
13	50	2019-04-18	2019-06-01	10.82507	-85.61328
14	70	2018-04-29	2018-06-12	10.84110	-85.61510
15	60	2018-05-10	2018-06-22	10.84338	-85.61601
16	70	2019-04-18	2019-06-02	10.84338	-85.61237
17	60	2018-05-07	2018-06-21	10.85710	-85.61510
18	30	2018-04-28	2018-06-10	10.83423	-85.61783
19	5	2019-04-13	2019-05-30	10.83194	-85.62420
20	30	2018-04-23	2018-06-02	10.83194	-85.62147
21	30	2019-04-24	2019-06-08	10.85710	-85.60782
22	30	2019-04-24	2019-06-08	10.85481	-85.60782
23	>100	2018-05-13	2018-06-25	10.85710	-85.60964
24	>100	2019-04-25	2019-06-07	10.85481	-85.61055
25	60	2019-04-25	2019-06-07	10.85481	-85.61328
26	35	2019-04-17	2019-05-31	10.83423	-85.61419
27	70	2018-04-30	2018-06-03	10.83881	-85.61055
28	30	2018-04-26	2018-06-08	10.83194	-85.61237
29	30	2018-04-24	2018-06-08	10.83194	-85.61692
30	30	2018-04-25	2018-06-08	10.83194	-85.61510
31	30	2019-04-17	2019-05-31	10.82965	-85.61419
32	30	2018-04-23	2018-06-04	10.82965	-85.61692
33	30	2018-04-22	2018-06-02	10.82965	-85.62147
34	60	2018-05-06	2018-06-21	10.85710	-85.61874
35	60	2018-05-05	2018-06-19	10.85710	-85.62056
36	70	2018-05-02	2018-06-14	10.84338	-85.60964
37	30	2018-04-22	2018-06-04	10.82736	-85.61601
38	30	2019-04-18	2019-06-01	10.82736	-85.61965
39	30	2018-04-28	2018-06-11	10.82736	-85.61237
40	60	2018-05-08	2018-06-23	10.84795	-85.61783
41	60	2018-05-08	2018-06-23	10.84795	-85.61510

42	>100	2019-04-22	2019-06-06	10.85024	-85.61146
43	>100	2018-05-04	2018-06-15	10.85024	-85.60782
44	30	2019-04-21	2019-06-06	10.85253	-85.60873
45	>100	2019-04-21	2019-06-04	10.85138	-85.60691
46	>100	2019-04-20	2019-06-04	10.84910	-85.60691
47	60	2018-05-05	2018-06-18	10.85253	-85.61965
48	60	2019-04-22	2019-06-06	10.85252	-85.61238
49	60	2018-05-06	2018-06-18	10.85253	-85.61601
50	35	2018-04-12 2019-04-21	2018-05-31 2019-05-28	10.83418	-85.62372
51	25	2018-04-06 2019-04-14	2018-05-26 2019-05-24	10.92188	-85.61287
52	70	2018-04-17 2019-04-09	2018-05-30 2019-05-26	10.88872	-85.61542
53	25	2018-04-20 2019-04-06	2018-05-28 2019-05-24	10.91862	-85.61195
54	20	2018-04-25 2019-04-07	2018-05-29 2019-05-23	10.95352	-85.60473
55	>100	2018-04-20 2018-05-31	2018-05-31 2019-05-25	10.83945	-85.61420
56	50	2018-04-20 2019-04-11	2018-05-31 2019-05-25	10.95017	-85.59703
57	>100	2018-04-18 2019-04-10	2018-05-30 2019-05-26	10.88017	-85.61535
58	30	2018-04-26 2019-04-10	2018-05-31 2019-06-02	10.87042	-85.59830
59	20	2018-04-05 2019-04-07	2018-05-29 2019-05-23	10.95185	-85.61115
60	40	2018-04-21 2019-04-12	2018-06-01 2019-05-28	10.83407	-85.62492
61	70	2018-04-17 2019-04-10	2018-05-31 2019-05-26	10.87488	-85.60902
62	45	2018-04-19 2019-04-11	2018-05-31 2019-05-25	10.83952	-85.61802

Appendix 2.2. Total counts of birds detected in bioacoustic surveys in the Área de Conservación Guanacaste, Costa Rica, during bioacoustic monitoring in the dry and wet season of 2018 and 2019 (some sites sampled in both years); counts are summed across sites of similar age.

| Scientific Name[1] | Common name | Approximate forest age in years (number of surveys per group) | | | | | | | Season totals | | Grand Total |
		20 (20)	30 (48)	40 (8)	50 (6)	60 (22)	70 (24)	>100 (22)	Dry	Wet	
Crypturellus cinnamomeus	Thicket Tinamou	37	83	19	15	40	27	22	125	118	243
Ortalis vetula	Plain Chachalaca	1	1	1	0	0	0	0	3	0	3
Penelope purpurascens	Crested Guan	6	17	0	1	11	8	7	30	20	50
Crax rubra	Great Curassow	0	0	0	0	0	1	6	4	3	7
Colinus cristatus	Crested Bobwhite	7	3	2	0	0	0	0	6	6	12
Patagioenas flavirostris	Red-billed Pigeon	7	21	1	4	19	19	13	43	41	84
Columbina inca	Inca Dove	4	7	6	4	0	1	1	17	6	23
Columbina passerine	Common Ground Dove	4	11	4	0	2	1	2	16	8	24
Claravis pretiosa	Blue Ground Dove	0	0	0	0	5	0	0	0	5	5
Leptotila verreauxi	White-tipped Dove	28	89	18	17	26	45	25	150	98	248
Zenaida asiatica	White-winged Dove	6	10	4	2	4	1	3	14	16	30
Morococcyx erythropygus	Lesser Ground-Cuckoo	3	2	0	0	0	0	0	2	3	5
Piaya cayana	Squirrel Cuckoo	0	1	0	2	4	7	3	12	5	17
Trochilidae sp.	Hummingbird sp.	0	11	0	3	5	3	4	13	13	26
Burhinus bistriatus	Double-striped Thick-knee	0	1	2	0	0	0	0	1	2	3
Vanellus chilensis	Southern Lapwing	0	5	1	0	0	0	0	5	1	6
Leptodon cayanensis	Gray-headed Kite	0	1	0	0	0	0	0	0	1	1
Rupornis magnirostris	Roadside Hawk	3	7	0	2	1	0	0	8	5	13
Trogon melanocephalus	Black-headed Trogon	18	28	8	10	8	25	5	39	63	102
Trogon caligatus	Gartered Trogon	0	2	0	1	1	7	1	7	5	12
Trogon elegans	Elegant Trogon	8	40	14	12	32	53	22	73	108	181
Momotus lessonii	Lesson's Motmot	0	6	0	0	10	14	13	10	33	43
Eumomota superciliosa	Turquoise-browed Motmot	6	11	4	2	6	12	7	30	18	48
Notharchus hyperrhynchus	White-necked Puffbird	0	2	0	0	1	1	2	4	2	6
Ramphastos sulfuratus	Keel-billed Toucan	0	1	0	1	0	2	1	4	1	5

Melanerpes hoffmannii	Hoffmann's Woodpecker	14	49	11	18	18	24	8	73	69	142
Dryocopus lineatus	Lineated Woodpecker	0	0	0	0	0	0	2	0	2	2
Campephilus guatemalensis	Pale-billed Woodpecker	0	6	5	1	12	7	6	23	14	37
Herpetotheres cachinnans	Laughing Falcon	2	0	0	2	0	0	0	0	4	4
Micrastur semitorquatus	Collared Forest-Falcon	0	1	0	0	2	1	1	3	2	5
Eupsittula canicularis	Orange-fronted Parakeet *	32	88	21	8	17	22	12	133	67	200
Brotogeris jugularis	Orange-chinned Parakeet *	13	44	5	14	77	37	21	106	105	211
Amazona albifrons	White-fronted Parrot *	6	33	5	8	15	6	4	32	45	77
Amazona farinose	Mealy Parrot *	0	0	1	0	0	0	0	1	0	1
Amazona auropalliata	Yellow-naped Parrot *	11	35	7	12	7	9	5	39	47	86
Thamnophilus doliatus	Barred Antshrike	0	5	0	1	18	24	24	40	32	72
Sittasomus griseicapillus	Olivaceous Woodcreeper	1	6	0	2	9	6	11	22	13	35
Dendrocincla homochroa	Ruddy Woodcreeper	0	1	0	0	2	0	3	3	3	6
Dendrocolaptes sanctithomae	Northern Barred Woodcreeper	0	4	0	0	1	7	2	9	5	14
Xiphorhynchus flavigaster	Ivory-billed Woodcreeper	2	14	0	2	13	8	12	19	32	51
Lepidocolaptes souleyetii	Streak-headed Woodcreeper	0	7	1	9	16	18	20	45	26	71
Chiroxiphia linearis	Long-tailed Manakin	9	41	4	11	37	45	66	103	110	213
Tityra semifasciata	Masked Tityra	0	0	0	0	0	1	2	3	0	3
Pachyramphus aglaiae	Rose-throated Becard	0	1	0	0	0	0	0	0	1	1
Onychorhynchus coronatus	Royal Flycatcher	0	0	0	0	1	0	3	3	1	4
Platyrinchus cancrominus	Stub-tailed Spadebill	0	0	0	0	0	3	2	1	4	5
Oncostoma cinereigulare	Northern Bentbill	0	1	0	0	4	2	7	6	8	14
Poecilotriccus sylvia	Slate-headed Tody-flycatcher	0	2	0	0	3	8	11	9	15	24
Todirostrum cinereum	Common Tody-Flycatcher	0	0	0	0	1	0	1	2	0	2
Tolmomyias sulphurescens	Yellow-olive Flycatcher	4	66	4	26	46	61	37	117	127	244
Camptostoma imberbe	Northern Beardless-Tyrannulet	1	4	1	2	3	1	1	10	3	13
Myiopagis viridicata	Greenish Elaenia	1	1	0	1	2	12	8	15	10	25
Attila spadiceus	Bright-rumped Attila	2	4	0	1	6	9	12	16	18	34
Myiarchus tuberculifer	Dusky-capped Flycatcher	0	3	1	1	3	5	11	15	9	24
Myiarchus nuttingi	Nutting's Flycatcher	0	3	0	0	1	0	0	0	4	4

Myiarchus tyrannulus	Brown-crested Flycatcher	17	42	9	12	11	10	1	67	35	102
Pitangus sulphuratus	Great Kiskadee	2	5	3	1	1	0	0	8	4	12
Megarynchus pitangua	Boat-billed Flycatcher	0	1	0	0	1	2	1	3	2	5
Myiodynastes luteiventris	Sulphur-bellied Flycatcher	3	11	3	5	7	9	7	29	16	45
Legatus leucophaius	Piratic Flycatcher	0	5	0	0	0	0	0	4	1	5
Pachysylvia decurtata	Lesser Greenlet	0	24	2	8	18	26	25	43	60	103
Vireo flavoviridis	Yellow-green Vireo	6	99	8	22	80	109	56	194	186	380
Calocitta formosa	White-throated Magpie-Jay	6	9	9	3	1	0	0	22	6	28
Troglodytes aedon	House Wren	1	0	0	0	0	0	0	1	0	1
Campylorhynchus rufinucha	Rufous-naped Wren	35	95	22	27	43	54	16	149	143	292
Thryophilus rufalbus	Rufous-and-white Wren	0	0	0	2	2	1	22	12	15	27
Thryophilus pleurostictus	Banded Wren	39	158	37	30	58	65	20	198	209	407
Cantorchilus modestus	Cabanis's Wren	0	1	0	0	4	8	11	14	10	24
Ramphocaenus melanurus	Long-billed Gnatwren	0	4	0	0	9	5	10	13	15	28
Polioptila albiloris	White-lored Gnatcher	3	35	9	3	0	5	1	33	23	56
Polioptila plumbea	Tropical Gnatcatcher	2	9	1	7	28	35	19	61	40	101
Catharus ustulatus	Swainson's Thrush *	0	1	0	0	0	0	0	1	0	1
Turdus grayi	Clay-coloured Thrush	1	4	1	1	1	4	2	7	7	14
Euphonia affinis	Scrub Euphonia	6	27	1	8	20	10	4	50	26	76
Peucaea ruficauda	Stripe-headed Sparrow	1	6	2	0	3	0	0	3	9	12
Arremonops rufivirgatus	Olive Sparrow	1	6	1	0	5	7	2	7	15	22
Icterus pustulatus	Streak-backed Oriole	0	2	0	1	0	0	0	3	0	3
Dives dives	Melodious Blackbird	0	4	0	0	0	0	0	0	4	4
Quiscalus mexicanus	Great-tailed Grackle	2	2	1	0	0	0	0	2	3	5
Setophaga petechia	Yellow Warbler *	0	0	0	1	0	0	0	1	0	1
Basileuterus rufifrons	Rufous-capped Warbler	6	38	4	6	41	33	19	67	80	147
Passerina caerulea	Blue Grosbeak	0	2	0	0	0	0	0	2	0	2
Eucometis penicillata	Gray-headed Tanager	0	1	0	1	1	0	1	2	2	4

[1] Naming follows the American Ornithological Society's Birds of North and Middle America Checklist (Chesser et al. 2019).

* Species marked with asterisks are migratory birds (2 species of songbird) or highly mobile species (5 species of parrot) that were not included in the analyses presented in the manuscript, for reasons stated in the Methods.

Appendix 2.3. Species counts from point-count surveys completed in Sector Santa Rosa of the Área de Conservación Guanacaste, Costa Rica, in 1996 and 2019, as well as differences in counts per species.

Scientific name[1]	Common name	1996	2019	Difference
Crypturellus cinnamomeus	Thicket Tinamou	10	3	-7
Crax rubra	Great Curassow	0	8	+8
Patagioenas flavirostris	Red-billed Pigeon	1	2	+1
Columbina passerina	Common Ground-dove	0	1	+1
Leptotila verreauxi	White-tipped Dove	5	10	+5
Zenaida asiatica	White-winged Dove	3	0	-3
Piaya cayana	Squirrel Cuckoo	7	4	-3
Amazilia spp.	Hummingbird sp.	5	3	-2
Rupornis magnirostris	Roadside Hawk	3	1	-2
Trogon melanocephalus	Black-headed Trogon	5	5	0
Trogon caligatus	Gartered Trogon	1	0	-1
Trogon elegans	Elegant Trogon	7	9	+2
Motmotus lessonii	Lesson's Motmot	4	1	-3
Eumomota superciliosa	Turquoise-browed Motmot	0	1	+1
Melanerpes hoffmannii	Hoffmann's Woodpecker	22	7	-15
Campephilus guatemalensis	Pale-billed Woodpecker	2	1	-1
Herpetotheres cachinnans	Laughing Falcon	0	1	+1
Thamnophilus doliatus	Barred Antshrike	3	7	+4
Sittasomus griseicapillus	Olivaceous Woodcreeper	5	3	-2
Dendrocolaptes picumnus	Northern Barred-Woodcreeper	2	2	0
Xiphorhynchus flavigaster	Ivory-billed Woodcreeper	2	5	+3
Lepidocolaptes souleyetii	Streak-headed Woodcreeper	1	2	+1
Chiroxiphia linearis	Long-tailed Manakin	7	16	+9
Tityra semifasciata	Masked Tityra	3	0	-3
Onychorhynchus coronatus	Royal Flycatcher	0	1	+1
Oncostoma cinereigulare	Northern Bentbill	0	1	+1
Poecilotriccus sylvia	Slate-headed Tody-Flycatcher	2	0	-2
Tolmomyias sulphurescens	Yellow-olive Flycatcher	7	18	+11
Camptostoma imberbe	Northern Beardless Tyrannulet	2	0	-2
Elaenia flavogaster	Yellow-bellied Elaenia	2	0	-2
Attila spadiceus	Bright-rumped Attila	0	1	+1
Myiarchus tuberculifer	Dusky-capped Flycatcher	27	4	-23
Myiarchus nuttingi	Nutting's Flycatcher	3	0	-3
Myiarchus tyrannulus	Brown-crested Flycatcher	9	4	-5
Megarynchus pitangua	Boat-billed Flycatcher	10	0	-10

Myiozetetes similis	Social Flycatcher	1	0	-1
Myiodynastes maculatus	Streaked Flycatcher	2	0	-2
Legatus leucophaius	Piratic Flycatcher	0	1	+1
Pachysylvia decurtata	Lesser Greenlet	0	14	+14
Calocitta formosa	White-throated Magpie-Jay	11	0	-11
Campylorhynchus rufinucha	Rufous-naped Wren	20	31	+11
Thryophilus rufalbus	Rufous-and-white Wren	0	1	+1
Thryophilus pleurostictus	Banded Wren	17	37	+20
Ramphocaenus melanurus	Long-billed Gnatwren	0	4	+4
Polioptila albiloris	White-lored Gnatcatcher	29	0	-29
Polioptila plumbea	Tropical Gnatcatcher	0	5	+5
Turdus grayi	Clay-coloured Thrush	0	1	+1
Euphonia affinis	Scrub Euphonia	0	2	+2
Arremonops rufivirgatus	Olive Sparrow	11	2	-9
Basileuterus rufifrons	Rufous-capped Warbler	16	21	+5
Eucometis penicillata	Grey-headed Tanager	1	0	-1
Cyanerpes cyaneus	Red-legged Honeycreeper	2	0	-2
	Total species	37	35	-2
	Total individuals	267	238	-29

[1] Naming follows the American Ornithological Society's Birds of North and Middle America Checklist (Chesser et al. 2019).

Chapter 3: The effects of neighbours, time of day, and seasonal variation on vocal

behaviours of female and male Rufous-and-white Wrens

Chapter Summary

Songbird vocalizations serve many functions including territory defence against neighbouring conspecific animals. In tropical and south-temperate regions, songbirds commonly use female song and coordinated duetting in addition to male song to defend their territories. The Área de Conservación Guanacaste in Costa Rica is the site of an ambitious conservation effort to protect and regrow tropical dry forests, and represents a mosaic of regenerating and mature forest patches, presenting a special opportunity to study the effects of fragmentation and population density on male song, female song, and male-female duets. We analyzed recordings of Rufous-and-white Wrens (*Thryophilus rufalbus*) over a 17-year period, focusing on variation in the number of territorial neighbours in the fragmented landscape in the conservation area. We hypothesized that the number of conspecific neighbours would influence vocal behaviours, including female and male independent song rate, duet responsiveness rate, repertoire use, and song-switching rate. For females, we found that wrens change song-types more often in areas with more neighbours, whereas the other aspects of female vocal behaviour did not vary with the number of neighbours. For males, we found no aspect of vocal behaviour varied with the number of neighbours. For both sexes, we found variation in some vocal behaviours with time of day and time of year, in keeping with previous research in this and other species. Our results underscore the important idea that we must explore female and male birds independently, because the sexes may respond differently to external factors. While we did not find strong links between wren vocal behaviour and variation in the number of neighbours, behavioural studies on populations in fragmented landscapes are necessary to understand the impact of habitat change on animals, and to inform conservation initiatives.

Introduction

Habitat change has a profound influence on animals, with the most immediate response often being behavioural (Bro-Jørgensen et al. 2019). Habitat fragmentation, habitat loss, or, conversely, habitat restoration, can lead to changes in the environment that result in different population densities and altered behavioural dynamics (Tuomainen and Candolin 2011). In species where vocalizations are used to communicate with nearby animals, habitat change may influence territorial defence behaviours due to variation in population densities (Olinkiewicz and Osiejuk 2003), which can alter the number of available territories. Territorial behaviour may be influenced by forest fragmentation if the amount of suitable habitat differs among fragments, and results in different numbers of individuals that competitively interact.

In many taxa of birds, vocal activities are influenced by the number of nearby conspecific neighbours. For example, in a removal experiment with Chipping Sparrows (*Spizella passerina*), males greatly reduced their dawn singing rate when all of their conspecific neighbours were removed, and increased their song output when those neighbours were subsequently released (Liu 2004). Conversely, male Corn Buntings (*Emberiza calandra*) showed higher song output when they had one neighbour compared to two or more neighbours, suggesting that males with many neighbours spend more time engaged in physical interactions with other males or listening to singing of their neighbours (Olinkiewicz and Osiejuk 2003). In Chaffinches (*Fringilla coelebs*), repertoire size was negatively correlated with number of territorial neighbours, an unexpected result that the author could not provide an explanation for (Slater 1981). Although varying results were found across different species, these studies share a common theme: they focus solely on male birds in temperate locations. Many questions in bird behaviour that have been addressed only in north-temperate bird species need to be revisited in tropical and south-

temperate taxa, and in birds where females sing and exhibit different life histories and behaviours.

Birdsong research has traditionally focused on male song, but, more recently, a growing body of research has revealed that female song is widespread across taxa, especially in the tropics and south-temperate regions, and is an ancestral trait in songbirds (Odom et al. 2014). The lack of understanding on the topic of female song has largely been the consequence of historical biases towards research in north-temperate ecosystems where female song is less common (Stutchbury and Morton 2001, Odom et al. 2014, Tobias et al. 2016). Several large-scale analyses have revealed that female song is associated with life history traits that include year-round territory defence, social monogamy, and sexual monochromatism (Najar and Benedict 2015, Odom et al. 2015, Tobias et al. 2016), and that female song serves similar functions to male song including territory defence, mate attraction, and mate guarding (Langmore 1998, Hall 2004, Cain and Langmore 2015, Najar and Benedict 2015). Following a call for increased focus on female song more than two decades ago (Langmore 1998), there has been growing documentation of female singing behaviour, and yet there is still much research to be done and many unanswered questions about female song (Odom and Benedict 2018). In particular, little is known about how variation in neighbouring territories due to habitat fragmentation influences female singing behaviour.

In species where both sexes sing, breeding partners may combine their vocalizations into duets, which occur when one member of a pair responds to the song of its mate, thus coordinating their behaviour in a jointly-produced vocalization (Hall 2004). Duets serve multiple functions that vary with context and species (Hall 2004, Mennill and Vehrencamp 2008). For example, in Rufous Horneros (*Furnarius rufus*), males and females use duets to cooperatively

defend year-round territories, and to mutually guard mates (Diniz et al. 2018). In Venezuelan Troupials (*Icterus icterus*), duets are used to defend territory and maintain contact (Odom et al. 2017). In Barred Antshrikes (*Thamnophilus doliatus*), pairs produce duets to defend territories of rival pairs (Koloff and Mennill 2013). These examples indicate that duetting behaviour serves multiple functions, with joint territory defence being a common function across the diverse avian taxa in which duetting occurs (reviewed in Hall 2004). Whether duets, as a joint territory defence behaviour, varies with different numbers of neighbours, is a question that has not been addressed previously.

We studied how variation in the number of territorial neighbours impacts vocal behaviours of Rufous-and-white Wrens (*Thryophilus rufalbus*), a species that defends year-round territories in the Neotropics and exhibits both female song and vocal duets. Males and females are monochromatic but can be distinguished by morphometric features, behaviour, and voice (Mennill and Vehrencamp 2008). Both female and male Rufous-and-white Wrens sing with eventual variety, repeating a given song type many times before switching to a new song type (Mennill and Vehrencamp 2005). Males sing more often than females and display a larger repertoire of song types (Mennill and Vehrencamp 2005). Both sexes create duets by responding to their partner's songs, although females create duets more often than males (Mennill and Vehrencamp 2005). Independent song rate and duet responsiveness are highest in males when their breeding partners are fertile, and in females, rates are highest during the pre-breeding season (Topp and Mennill 2008). Playback experiments reveal that duet use is especially high during territorial encounters (Mennill 2006, Mennill and Vehrencamp 2008). Rufous-and-white Wrens provide a special opportunity to compare intersexual differences in vocal behaviours, and the effects of number of neighbours on the vocal behaviour of both sexes.

Based on 17 years of field recordings of Rufous-and-white Wrens living in fragmented dry forests in northwestern Costa Rica, we tested the hypothesis that female and male vocal behaviours are influenced by the number of territorial neighbours. We predicted that with an increase in the number of neighbours in larger forest patches, we would find differences in (1) independent song rate, (2) duet responsiveness, (3) repertoire use, and (4) song-type switching rate. We also tested the hypothesis that male and female vocal behaviours would vary with time of day and time of year, in keeping with widely-recognized patterns in this and other species. We predicted that we would find temporal variation in the aforementioned four features of singing behaviour for both males and females.

Methods

Study system

Between 2003 and 2019, we studied a population of Rufous-and-white Wrens living in Sector Santa Rosa of the Área de Conservación Guanacaste (10°52'N, 85°36'W) in northwestern Costa Rica. We collected data between April and July of each year, which coincides with the onset of heavy rains and the breeding activities for this population of Rufous-and-white Wrens (Topp and Mennill 2008, Woodworth et al. 2018). Each year, our team captured and banded birds, monitored breeding activities, and collected recordings (details below). On a daily basis, we traveled through the habitat, detecting all birds based on their vocalizations and by observing their unique colour band combinations.

The Área de Conservación Guanacaste is the result of a decades-long conservation effort to protect some of the last remaining fragments of mature dry forest, and regrow much of the surrounding forests (Allen 2001). Within Sector Santa Rosa of the Área de Conservación

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Guanacaste, patches of mature primary forest exist in a matrix of regenerating forest and are particularly evident during the driest times of the year when deciduous trees lose their leaves. The mature semi-evergreen forest fragments are home to a resident population of Rufous-and-white Wrens (Figure 3.1). The size of this population, and therefore the number of wrens within each forest patch, varies each year with annual survival, with a heavy influence of changes in temperature (Woodworth et al. 2018). Rufous-and-white Wrens provide a compelling species for this study because they defend year-round territories from conspecifics in mature forest fragments of Sector Santa Rosa. Most of these mature forest patches are home to one or more Rufous-and-white Wrens, resulting in wrens with different numbers of neighbours. Consistent with definitions from previous research on this study population, we define "neighbours" as wrens with territory boundaries less than 50 m apart, and we define "territory boundaries" as areas where two or more neighbours were observed having vocal or physical interactions (Battiston et al. 2015). Across the 17 years of this study, we found wrens with zero to four neighbours. Most wrens in our study population have one or two neighbours, and four neighbours was rare. We focused our analysis of our historical dataset on a subset of pairs with similar representation for birds with 0, 1, 2, or 3+ neighbours, limited by the number of pairs with zero and three and four neighbours. We chose to analyze birds with three and four neighbours together (i.e. three or more neighbours).

Recording techniques

To study the vocalizations of Rufous-and-white Wrens, our research team recorded wrens on their territories, visiting each territory at least once every two weeks, and more often whenever possible. We recorded and observed each pair for approximately one hour during the early morning hours (0500 to 0700 h; sunrise occurs at approximately 0515 h). Wrens were recorded

using two approaches. First, we recorded wrens on their territories by following animals and using digital recorders (Sennheiser MKH70 or ME67) and shotgun microphones (Marantz PMD660 or PMD670; 22,050 Hz sampling rate, 16-bit encoding accuracy, WAVE format). In some recordings, playback was used, or the recordist whistled to imitate Rufous-and-white Wren song, to draw birds near to observe colour bands; given that playback influences song rate (Mennill 2006; Mennill and Vehrencamp 2008), we excluded any sections of recordings where playback or imitation was evident. If a period of playback or whistling was followed by an hour or longer of silence from the recordist, we included the recordings in our analysis (previous research has confirmed that response to playback by Rufous-and-white Wrens decline to baseline levels at intervals less than 1 h; Mennill and Vehrencamp 2008). Approximately 73% of recordings were collected through in-person, focal recordings.

In addition to focal recordings, we also used passive acoustic monitoring to collect recordings of wrens. Passive acoustic monitoring equipment was placed within a pair's territory, usually in the approximate center of the pair's territory or near a nest. Passive acoustic monitoring equipment varied over the 17 years: in 2003 and 2004, recordings were collected used eight-channel microphone arrays (details in Mennill et al. 2006, Mennill and Vehrencamp 2008); from 2007 to 2010, recordings were collected using automated recorders with elevated omni-directional microphones (Sennheiser ME62) with solid-state digital recorders (Marantz PMD670; details in Mennill and Vehrencamp 2005); and from 2011 to 2019 recordings were collected using autonomous recorders (Song Meter models SM1 and SM2+; Wildlife Acoustics Inc. Concord, Massachusetts, USA; details in Mennill et al. 2012). No automated recordings were collected in 2005 and 2006. Our team collected autonomous recordings at different times of day, however, the majority of data used in our analyses are from the morning hours (Harris et al. 2016). Approximately 27% of recordings were collected using passive acoustic monitoring.

Song analysis

We analysed songs and duets of 45 pairs of Rufous-and-white Wrens. We chose 90 unique

individuals for vocal behaviour comparisons, and included at least one pair from each of the 17

years of the study. All pairs had at least 2 hours of audio recordings (average ± SE recording

length: 13.1 ± 2.5: $n = 45$).

We analyzed recordings using SYRINX-PC sound-analysis software (J. Burt, Seattle,

Washington). Each Rufous-and-white Wren has a unique repertoire of song types, and for each

bird, we built a library of sound files representing all of the song types for that individual. Song

types can be differentiated on the basis of fine-structural features on the sound spectrograms. For

each recording, individual birds were identified by visualizing their songs on a sound

spectrogram, and each song was annotated by the individual's identity (i.e. the unique colour

band combination as dictated by the recordist), and the individual's song type. For each wren, we

counted the number of independent songs they produced (i.e. solo songs or songs where a bird

sang and then its partner responded, turning the song into a duet). If a wren sang within one

second of its mate, we deemed this to be a duet and we counted the number of male-created

duets (i.e. duets where the male sang in response to a female song, turning her song into a duet)

and female-created duets (i.e. duets where the female sang in response to a male song, turning

his song into a duet), as in previous studies of duetting in this population (Mennill and

Vehrencamp 2005, Topp and Mennill 2008). When duets involved more than one song from the

male or the female, we considered only the first contribution of each bird to the duet in our

analysis of number of duets created.

Our analysis of vocal behaviour focused on four features of songs for each sex:

independent song rate, duet responsiveness, repertoire use, and song-switching rate. We

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calculated independent song rate by dividing the number of independent songs per recording by the total length of the recording (measured from first song to last song). We calculated duet responsiveness by dividing the number of duet-creation songs by one bird by the total number songs sung by its partner (i.e. total number of opportunities to create a duet). We calculated repertoire use by counting the total number of unique song types used in a single recording and dividing by the combination of all songs (solo and duet songs) produced by that individual during that same recording. Finally, we calculated song-type switching rates by dividing the total number of song type changes in a recording by the total number of independent songs (Molles and Vehrencamp 1999).

Analyses

We conducted all analyses in R v3.6.1 (R Development Team 2019). We created eight linear mixed models (four for each sex, for the four variables listed above) using R package 'lme4' (Bates et al. 2015). Dependent variables were independent song rate, duet responsiveness, repertoire use, and song type switching rate. All models included fixed effects of number of neighbours, time of day, and ordinal day. We included pair identity as a random effect. We chose to include time of day and ordinal day as fixed effects because previous research on this population showed that male and female vocal behaviours were influenced by time of day and time of year (Topp and Mennill 2008). We used R package 'lmerTest' to obtain P-values for our models (Kuznetsova et al. 2017).

Previous research on this population of wrens showed that male and female vocalization rates differ substantially (Mennill and Vehrencamp 2005). To confirm this, we created four additional linear mixed models comparing males and females for each of the four response variables. These four models tested each independent variable by sex without any fixed effects,

but with the random effect of pair identity. Because the results from these models confirmed that males and females have very different vocalization rates (Table 3.1), we chose to analyze males and females separately in our final analyses.

Female wrens vocalize less often than male wrens, and males create fewer duets than females (Mennill and Vehrencamp 2005), resulting in an inflation of zeros in our dataset. We used Tukey's Ladder of Powers (Tukey 1977) in R package 'rcompanion' (Mangiafico 2016), a transformation technique for addressing violations of assumptions including normality of residuals and equality of variances. We used Levene's Tests and variance inflation factors from R package 'car' (Fox and Weisberg 2019) to assess variance equality and collinearity. We visually assessed Q-Q plots of residuals for normality, and we plotted residuals by fitted values to evaluate homoscedasticity.

To test our hypothesis that vocal behaviour is influenced by number of neighbours, we first examined our data using ANOVA on our eight models with package 'car' (Fox and Weisberg 2019). To test our hypothesis that vocal behaviour would change with time of day and time of year, we first examined our models using ANOVA, and then looked at results from our linear mixed models to determine the direction of the effect. We ran post-hoc tests on any models showing differences in neighbour groups from the ANOVA using R package 'emmeans' (Lenth et al. 2020).

Results

Based on repeated recordings of 45 pairs of Rufous-and-white Wrens collected over a 17 year period, we found that several aspects of singing behaviour varied with time of year and time of day, and one singing behaviour varied with number of conspecific neighbours (Table 3.2; Figure

3.2). We found limited support for the hypothesis that vocal behaviour changes with the number of neighbours; for female wrens, song-type switching rates change with different numbers of neighbours (Table 3.2; Figure 3.2). Tukey-Kramer post-hoc tests showed differences at 0.95 confidence levels in one comparison: female wrens with one neighbour had lower song-switching rates than those with two neighbours ($p = 0.02$). No other comparisons yielded results supporting the hypothesis that vocal behaviour varies with number of neighbours.

We found that males and females exhibited different time-of-day and time-of-year effects. Male independent song rate and repertoire use were positively related to time of year (Table 3.2; Figure 3.3), such that males sang more songs and used a larger proportion of their vocal repertoire as the breeding season progressed. Male independent song rate showed negative relationships with time of day (Table 3.2; Figure 3.4), such that males sang less often as the day progressed. Male duet-response and song-type switching rates were not influenced by time of day or time of year.

Females showed different patterns of temporal variation in independent song rate and duet responsiveness compared to males. Female independent song rate and duet responsiveness were negatively related to time of year, such that females reduce their song output and duet-responsiveness as the breeding season progressed (Table 3.2; Figure 3.4). Interestingly, females showed a similar pattern to males in their repertoire use; repertoire use by female wrens increased with ordinal day, such that females, like males, use a greater proportion of their song repertoire as the breeding season progressed (Table 3.2; Figure 3.4). Female song-type switching rate was not influenced by time of day or time of year.

Discussion

In this study, we tested the influence of variation in number of neighbours on vocal behaviour of Rufous-and-white Wrens living in fragmented forests. Although we did not find that male song or duetting behaviour, for both females and males, was influenced by number of neighbours, we found an effect on female singing behaviour: females with more neighbours switch song types at higher rates. While our results do not strongly support our hypotheses, we did find that number of neighbours in fragmented habitat influences female singing behaviour in these wrens. We conclude that the number of territorial neighbours in a fragmented landscape has no effect on the male vocal behaviours, but an effect on song-type switching rates in female Rufous-and-white Wrens.

Most studies on temperate species have suggested that vocalization rates change with different numbers of neighbours, although the pattern varies across taxa. In Chipping Sparrows (*Spizella passerine*; Liu 2004), Willow Warblers (*Phylloscopus trochilus*; Gorestskaia 2004), and Orange-crowned Warblers (*Oreothlypis celata*; Yoon et al. 2012), vocalization rates increased with higher population density or neighbours, similar to our result in female wren song-type switching rates. Conversely, Corn Buntings (*Emberiza calandra*) showed a decrease in song output with more neighbours (Olinkiewicz and Osiejuk 2003). Similar to our observations in male Rufous-and-white Wrens, House Wrens (*Troglodytes aedon*; Wilson and Bart 1985) and Black-throated Blue Warblers (*Setophaga caerulescens*; Sillett et al. 2004) showed no effect of population density or neighbours on vocalization rates. Our research highlights the importance of continued research on tropical species, including revisiting questions concerning bird behaviour that have only been addressed with temperate or migratory species.

Female Rufous-and-white Wrens showed higher song-type switching rates with higher number of neighbours, particularly in a post-hoc comparison of females with one neighbour versus two neighbours. Previous research has found that song-type switching rates play a role in aggressive signalling in male songbirds (Searcy and Beecher 2009, Deoniziak and Osiejuk 2020), and may be associated with song-type matching interactions (Vehrencamp 2001, Akçay et al. 2013), although there has been very little study of song-type switching behaviour in female birds. If higher rates of song-type switching are associated with aggressive interactions with neighbours, and given that we found some evidence that female wrens have higher song-type switching rates with more neighbours, then we suggest that female wrens may be using song-type switching in territorial defence against conspecifics. We did not observe the same pattern in male wrens, and therefore this explanation does not apply to both sexes, raising the idea that the sexes may show different responses to living with different population densities in fragmented landscapes. Our results emphasize the increasingly popular idea that female behaviour needs to be addressed in studies of bird behaviour, which has traditionally focused solely on male birds (Topp and Mennill 2008; Odom et al. 2014, Riebel et al. 2019). Our results also suggest there is a need to test hypotheses in females and males separately, and that it is important not to assume that behaviours from both sexes will be influenced in the same way by external factors. Our results corroborate previous findings that male and female Rufous-and-white Wrens exhibit dramatic differences in singing behaviour, not just in song output, but also in duet responsiveness, repertoire use, and song-type switching rate (Mennill and Vehrencamp 2005; Table 3.1). Previous research on female song has shown stronger physical responses by females to playback of conspecific females rather than males (Mennill and Vehrencamp 2008, Krieg and Getty 2013), but similar levels of vocal responses to both sexes. We recommend future research

into the role of neighbours should focus on disentangling the effect of male and female neighbours on female vocal behaviours.

There are a number of possible explanations for the results we observed. Given that male and female vocalizations serve multiple functions, it is likely that the diverse functions of both male and female song (including mate attraction or communication between mates) complicate or obscure the effects of territorial neighbours. Over the 17 years of study on this population of wrens, our research team has mainly collected data in the weeks before and following the onset of the breeding season when male and female wrens are focused on breeding activities. Because songs and duets serve multiple functions (Hall 2004, Catchpole and Slater 2008), it is possible that the effects of neighbours are masked by the influence of other breeding activities (e.g. attracting an extra-pair mate, or communication between mates). Studying vocal behaviour in these wrens at other times of year could shed light on whether neighbours influence vocal behaviours in the non-breeding season. Another possibility is that Rufous-and-white Wrens do not respond strongly to familiar neighbours, a phenomenon known as the "dear enemy effect" (Temeles 1994), although one previous experimental study on this population suggests that Rufous-and-white Wrens do not exhibit this effect (Battiston et al. 2015). In the same study, the authors examined aggressive non-vocal behaviours of Rufous-and-white Wrens to neighbours, such as distance to closest approach (Battiston et al. 2015). Rufous-and-white Wrens may use non-vocal behaviours in territorial disputes differently than vocalizations. Therefore, future research on the effect of neighbours might consider including these non-vocal behaviours.

We observed relationships between singing behaviours and both the time of day and the time of year for both males and females. For males, independent song and song-type switching rates decreased later in the day, while independent song rate and repertoire use increased as the season progressed. In females, independent song rate and duet responsiveness were highest

earlier in the season, while repertoire use was highest later in the season. Similar to previous research on Rufous-and-white Wrens in this population, we found that independent song rate had a positive relationship with time of year in male wrens, and a negative relationship in female wrens (Topp and Mennill 2008). Previous research on female song, including in Rufous-and-white Wrens, has shown that female song peaks before the start of the breeding season (Topp and Mennill 2008). For males, this peak in independent song rate occurs later in the year at the onset of the breeding season when the female becomes fertile (Topp and Mennill 2008). We did not find relationships with time of day or year and song-type switching, and in males, we also did not find that duet responsiveness was related to time of day or year. However, we did observe that females were less likely to respond to male songs to form duets later in the season, a phenomenon that probably reflects the increasing attention that females devote to nesting and parental care later in the year (Topp and Mennill 2008). Despite an overall reduction in vocal output by females, we found that female wrens increased their repertoire use with time of year, exhibiting more song types later in the year. Later in the year, females may produce more song types during the period when young birds are learning to sing. More research into repertoire use by females is needed to better understand this relationship.

Habitat fragmentation has been shown to have different effects on population density or territory size in different bird species and functional groups. Generalist and edge species tend to increase in population size following fragmentation, while forest specialist species generally decline (Bender et al. 1998). In fragmented tropical forests of southeastern Brazil, understorey species showed different responses to habitat fragmentation: some species increased their home range sizes to increase access to resources, thereby reducing population density, while more sensitive species were restricted to larger forests patches or contiguous forests, thereby increasing density (Hansbauer et al. 2008). The impacts of habitat fragmentation on population

sizes in birds is complex, and can be influenced by changes in resource availability, predator populations, and vegetation structure (Robinson and Sherry 2012). Therefore, exploring how population density or number of neighbours influences social behaviour can improve understanding of how these behaviours may be affected by habitat change. Due to the fragmented nature of mature tropical dry forests in Costa Rica, Rufous-and-white Wrens share suitable habitat patches with zero to several neighbours. Although most vocal behaviours were not influenced by the number of neighbours in our analysis, we did find that female song-type switching was higher in females with more neighbours. Therefore, habitat fragmentation may be indirectly influencing singing behaviour in female wrens through population dynamics. It will be important to continue monitoring this population of wrens over time as this habitat continues to change.

Bird conservation research often focuses on measures of species diversity and abundance to monitor impacts or changes in a particular community or population. Less frequently, behaviour is used as a measure to understand how birds are influenced by changes in their environment (Lewis et al. 2020). More recently, researchers have shown how animal behaviour studies are important in conservation research (Bro-Jørgensen et al. 2019), and have suggested that changes in vocal behaviour can act as early warning signs of impacts on animals from changes in their environment (Tuomainen and Candolin 2011, Teixeira et al. 2019). For example, research on the impact of habitat fragmentation on song in the threatened Dupont's Lark (*Chersophilus duponti*) found that habitat patch size, male population, and dispersal distance reduced cultural variety in song repertoires (Laiolo and Tella 2007). Fragmentation may even lead to cultural erosion, and serve as a prelude to population extirpation (Laiolo and Tella 2007). Although Rufous-and-white Wrens are not a species of conservation concern (BirdLife International 2018), our study population lives in some of the most imperilled forests of the

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tropics (Janzen 1988). Wrens of this population have unique song characteristics and behaviours that differ from nearby populations of the same species (Graham et al. 2017). Our results suggest that female singing behaviour of Rufous-and-white Wrens living in a fragmented landscape is influenced by their number of neighbours, however, most vocal behaviours in male and female wrens are not affected. If habitat fragmentation, or future forest regeneration, influences Rufous-and-white Wren density in this population, we expect that some wren vocal behaviours, particularly in female wrens, could be affected. By studying behaviours of animals living in changing habitats, we can better inform conservation initiatives while increasing our understanding of how different species respond to habitat changes.

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Tables and Figures

Table 3.1. Results from linear mixed effects models examining relationships between four response variables, separately (independent song rate, duet responsiveness, repertoire use, song-type switching rate) by sex (male and female). Random effect of pair ID is included in each model. Marginal and conditional R^2 values are provided for each model. Results with $p < 0.05$ are indicated with an asterisk.

		Estimate	SE	t	P
Independent song rate	Intercept	5.3	4.1	1.3	0.2
$R^2_m = 0.4$; $R^2_c = 0.4$	Sex	107.3	5.2	20.6	<0.001*
Duet responsiveness	Intercept	-0.5	0.05	-9.1	<0.001*
$R^2_m = 0.3$; $R^2_c = 0.4$	Sex	1.2	0.07	15.8	<0.001*
Repertoire use	Intercept	0.6	0.06	9.8	<0.001*
$R^2_m = 0.3$; $R^2_c = 0.3$	Sex	-1.1	0.07	-14.4	<0.001*
Song-type switching rate	Intercept	0.2	0.2	1.5	<0.001*
$R^2_m = 0.09$; $R^2_c = 0.2$	Sex	1.2	0.2	7.3	<0.001*

Table 3.2. Results from eight linear mixed models examining relationships between four response variables separately (independent song rate, duet responsiveness, repertoire use, song-type switching rate) by number of neighbours for both male and female Rufous-and-white Wrens using ANOVA. Results from regression are provided for fixed effects of ordinal day and time of day only. Random effect of pair ID is included in each model. Marginal and conditional R^2 values are provided for each model. Results with $p < 0.05$ are indicated with an asterisk.

|		Males				Females			
		F	P	Estimate	SE	F	P	Estimate	SE
Independent song rate	Number of neighbours	0.9	0.4			1.6	0.2		
	Ordinal day	46.5	<0.001*	0.01	0.002	7.6	0.006*	-0.007	0.003
	Time of day	36.3	<0.01*	-0.3	0.05	0.6	0.5	-0.05	0.06
		$R^2_m = 0.24$; $R^2_c = 0.29$				$R^2_m = 0.05$; $R^2_c = 0.2$			
Duet responsiveness	Number of neighbours	0.1	0.9			2.2	0.1		
	Ordinal day	0.9	0.4	0.003	0.003	40.4	<0.001*	-0.01	0.002
	Time of day	0.8	0.4	-0.06	0.07	0.003	0.9	-0.003	0.06
		$R^2_m = 0.01$; $R^2_c = 0.04$				$R^2_m = 0.2$; $R^2_c = 0.3$			
Repertoire use	Number of neighbours	0.2	0.9			1.4	0.3		
	Ordinal day	19.0	<0.001*	-0.01	0.002	4.6	0.03*	0.005	0.002
	Time of day	1.4	0.2	0.07	0.06	0.4	0.5	-0.04	0.06
		$R^2_m = 0.07$; $R^2_c = 0.2$				$R^2_m = 0.05$; $R^2_c = 0.1$			
Song-type switching rate	Number of neighbours	0.8	0.5			3.5	0.02*		
	Ordinal day	1.7	0.2	-0.002	0.002	0.7	0.4	-0.002	0.002
	Time of day	2.1	0.2	-0.06	0.05	1.8	0.18	-0.08	0.06
		$R^2_m = 0.03$; $R^2_c = 0.2$				$R^2_m = 0.06$; $R^2_c = 0.06$			
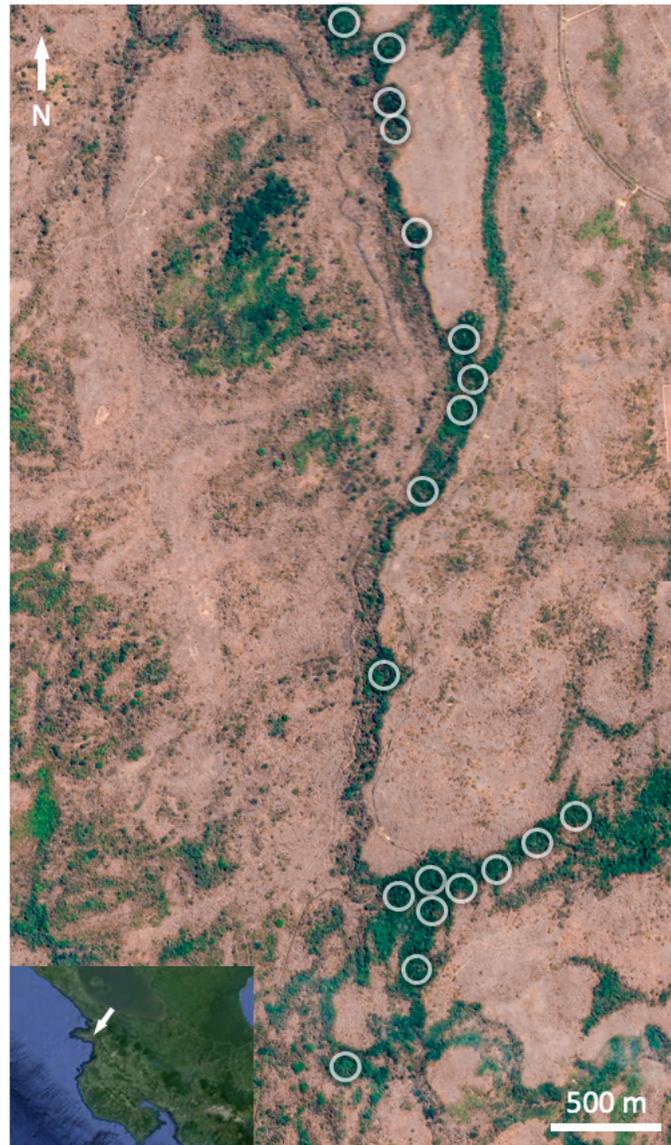
Figure 3.1. Map of Sector Santa Rosa of the Área de Conservación Guanacaste with approximate Rufous-and-white Wren territories for a single year represented in circles. Imagery is from the end of a dry season in May 2013, and shows mature forest patches in green. Inset map shows the location of the study area within Central America.
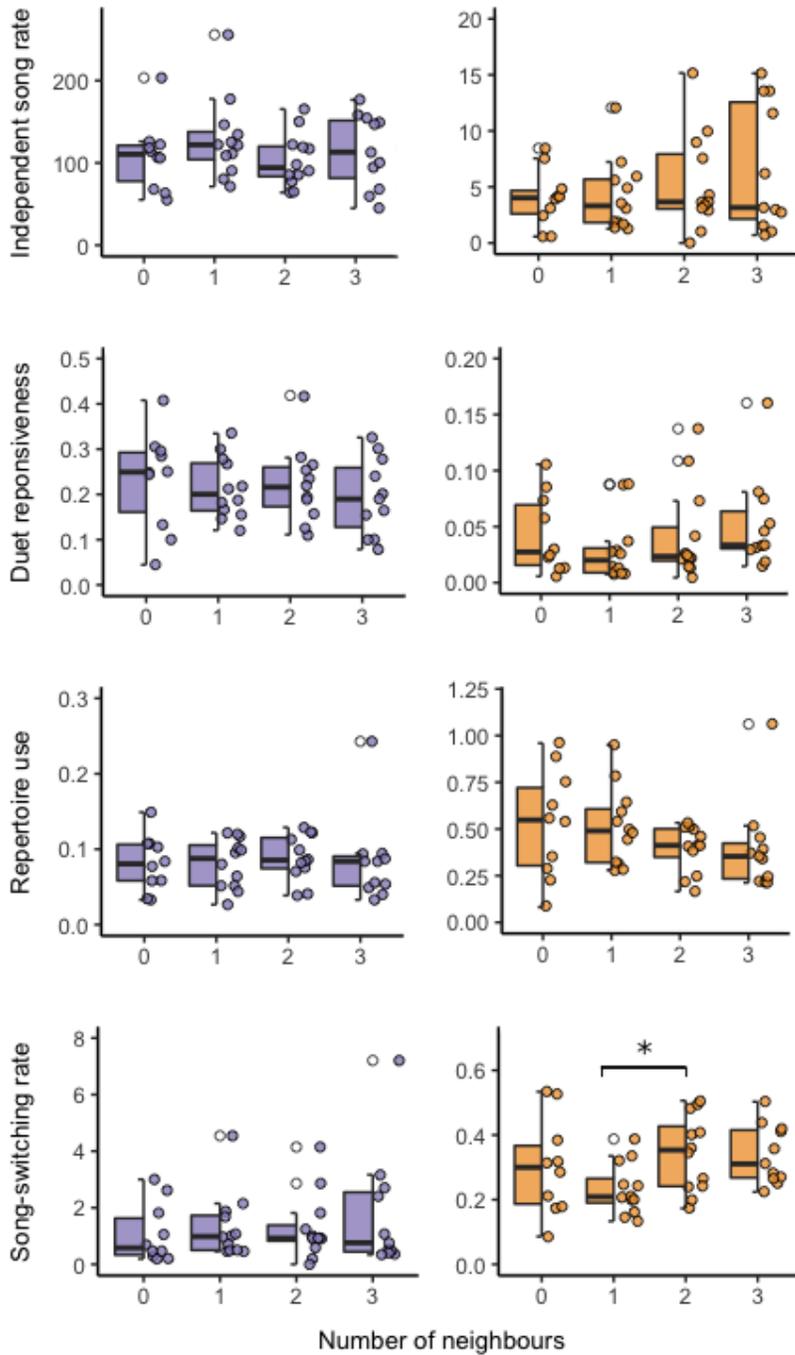
Figure 3.2. Male (left, purple) and female (right, orange) vocalization responses (top to bottom: independent song rate, duet responsiveness, repertoire use, and song-switching rate) to number of conspecific neighbours. Boxplots summarize the mean and interquartiles ranges for each vocalization rate in all males and females in the study sample. Individual points represent mean vocalization rates per bird. Asterisk denotes comparison between groups that are different at 0.95 confidence intervals, open circles represent boxplot outliers.
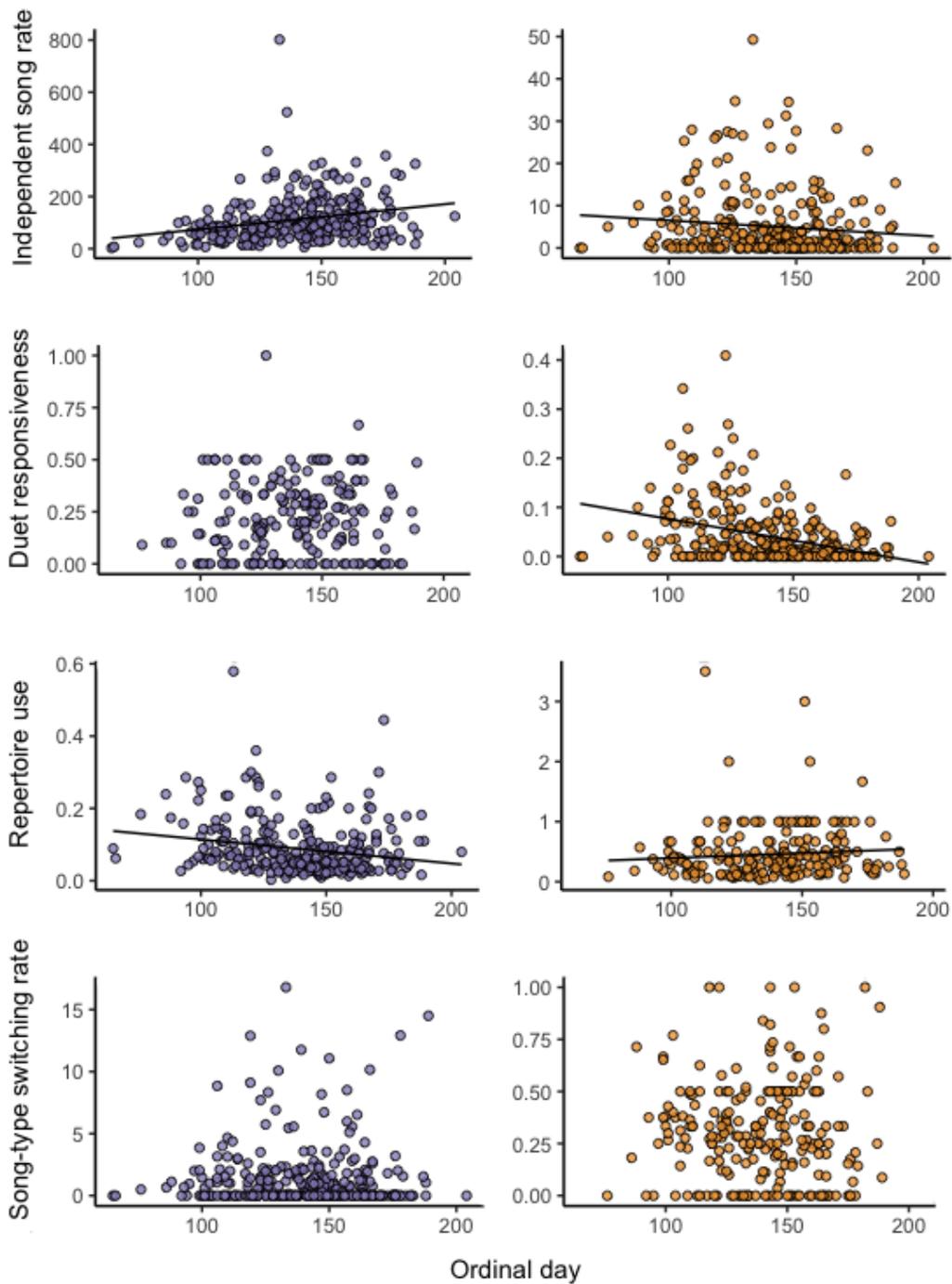
Figure 3.3. Male (left, purple) and female (right, orange) vocalization responses (top to bottom: independent song rate, duet responsiveness, repertoire use, and song-switching rate) by ordinal day. Day 100 is April 10 (April 11 in leap years). Line of fit is shown for any plots where there was an effect with $p < 0.05$.
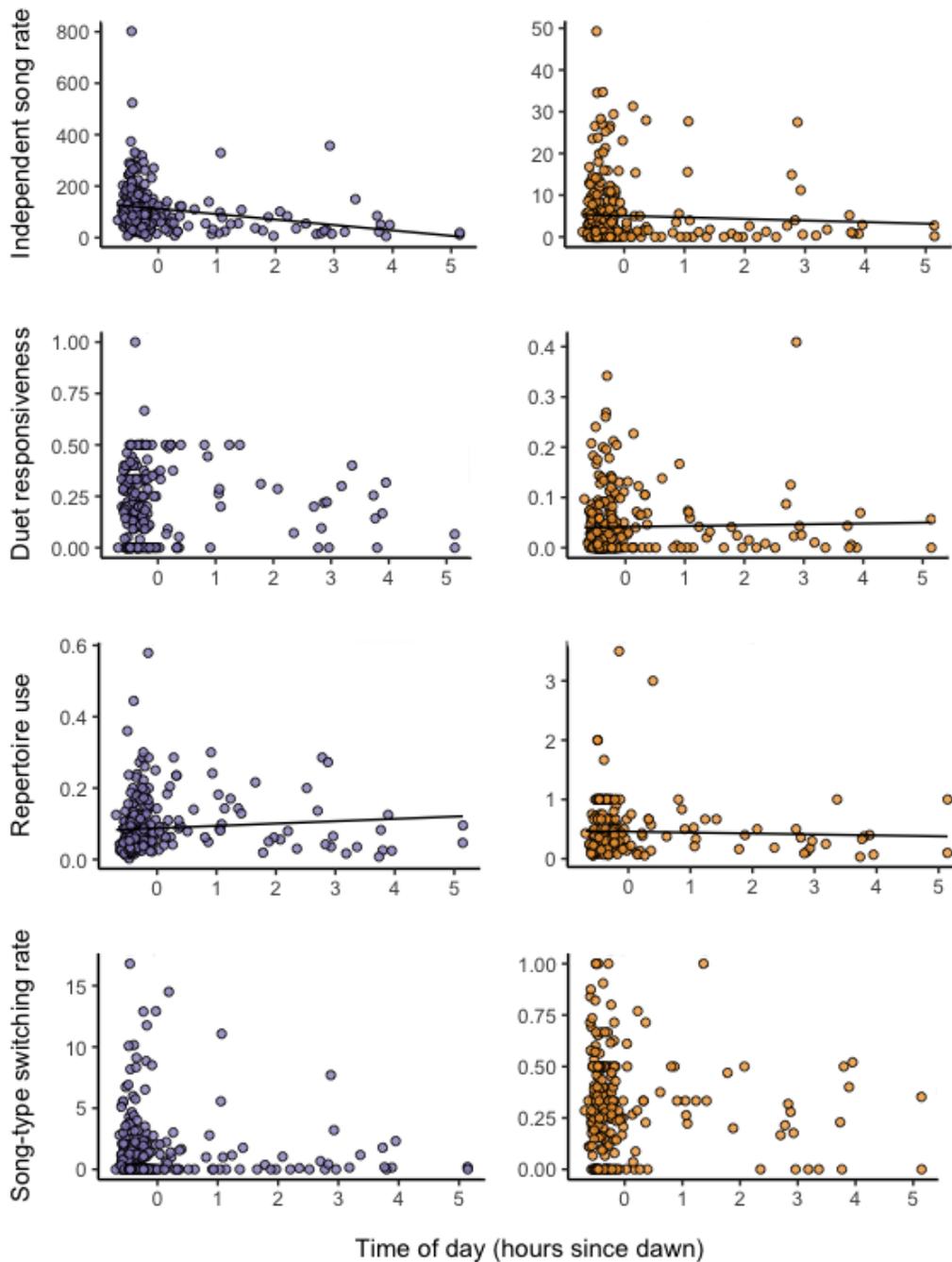
Figure 3.4. Male (left, purple) and female (right, orange) vocalization responses (top to bottom: independent song rate, duet responsiveness, repertoire use, and song-switching rate) by time of day in hours since dawn (approximately 0500h). Line of fit is shown for any plots where there was an effect with $p < 0.05$.

Chapter 4: General Discussion

In this thesis, I have shown how bioacoustic monitoring of wildlife can help us understand how birds respond to changes in their environment, including variation in habitat age and variation in the number of conspecific neighbours. In Chapter 2, I showed that bird communities become more diverse, abundant, and similar to those in mature primary forests when regenerating forests grow older. I highlighted species that appear to be benefitting from dry forest restoration efforts, including the Great Curassow, a threatened species (BirdLife International 2016), and the Rufous-and-white Wren, a mature-forest specialist. In Chapter 3 of this thesis, I showed that female Rufous-and-white Wrens display higher song-type switching rates with greater numbers of neighbours, but that vocal behaviours in males and duetting in both sexes are not affected. I also showed that vocal behaviour of Rufous-and-white Wrens living in fragmented forests varies with time of year and time of day. In this General Discussion, I briefly summarize the results of these two data chapters, I draw connections between the two chapters, and I offer ideas for future research.

In this thesis, I promoted the use of bioacoustic approaches by providing two examples of questions that can be addressed with bioacoustic methods, and I explained the benefits of these approaches. Using sound recordings, I studied bird diversity, abundance, and species composition across diverse dry-forest bird species (Chapter 2), as well as the vocal behaviour of a single species (Chapter 3). Both chapters involved passive acoustic monitoring, which allows for data collection that minimizes observer influences on animal behaviour, thereby reducing bias resulting from having an observer present or handling the birds. Passive acoustic monitoring and focal recordings allow for large datasets to be archived, with raw data that can be reviewed repeatedly if necessary. Notably, in Chapter 3, I analyzed recordings collected over 17 years of bioacoustic research; the creation of a permanent set of recorded audio data is another benefit of using bioacoustic methods. Through two very different topics covered in this thesis, my research

demonstrated that bioacoustic methods can appropriately address a wide range of questions related to bird conservation and behaviour.

In Chapter 2, I explored how bird communities respond to tropical dry forest regeneration using passive acoustic monitoring. I recorded bird communities in forests at different stages of succession, as well as in primary mature forests. I also resampled a 60 to 80 year-old forest using traditional point-count surveys to compare to survey data from 23 years earlier. By using passive acoustic monitoring and point-count surveys, I showed that bird species richness, diversity, abundance, and community composition are recovering in these regenerating forests (Figure 4.1). There are relatively few studies that examine bird communities across a chronosequence that includes forests from 5 to more than 100 years old. This chapter contributes to our growing understanding of the importance of monitoring in conservation and restoration research. This chapter was recently published in the June 2020 issue of the journal Avian Conservation and Ecology (Owen et al. 2020).

In Chapter 3, I used bioacoustic methods to study vocal behaviour of Rufous-and-white Wrens living in the same fragmented mature forests that I used as reference sites to study bird communities in Chapter 2. Across 17 years of acoustic recordings of Rufous-and-white Wrens, I examined how male and female wrens respond to conspecific neighbours of which they defend their territory against. Particularly, I found that number of neighbours influenced song-type switching rate in female wrens, such that females with two neighbours switched song types more than females with a single neighbour. I did not find any influence of neighbours on male wrens or on the duetting behaviour of both sexes. My results highlight the importance of expanding the focus of bird song research to include female singing behaviour, because female birds may respond differently to changes in their environment, as I have shown in Chapter 3. Using a much

larger dataset than previous research, I also found an influence of time of day and time of year on vocal behaviours, corroborating previous research (Topp and Mennill 2008), and furthering our understanding of how specific vocal behaviours in female and male wrens change over time and season.

Rufous-and-white Wrens live and breed in some of the most restricted habitat in Central America: mature patches of neotropical dry forests (Janzen 1988). Although Rufous-and-white Wrens are not a species of conservation concern (BirdLife International 2016), it is important to monitor the behaviour of bird populations in rapidly changing landscapes. Understanding behaviour of birds in fragmented habitat could shed light on possible impacts of further fragmentation, or the reversal of fragmentation through restoration, like the efforts highlighted in Chapter 2. In fragmented forests of Central Amazonia, lekking behaviour of White-throated Manakins (*Corapipo gutturalis*) was negatively affected by forest fragmentation, with manakins not using the smallest forest patches in the study area (Tolentino and Anciães 2020). Similar to Rufous-and-white Wrens, White-throated Manakins are not a species of conservation concern, although they live in habitat that has been affected by forest fragmentation. By studying how habitat fragmentation affects avian behaviour, we gain insight into how continued fragmentation may threaten the persistence of now-common species (Tolentino and Anciães 2020). Behavioural changes are often the initial response by animals to habitat change (Tuomainen and Candolin 2011), and can help guide conservation efforts.

Throughout this thesis, I have highlighted the ecological importance of tropical dry forests, discussed efforts to protect and regrow them, and argued for the importance of monitoring wildlife within them. With their extreme seasonality, tropical dry forests present a unique ecosystem in which to conduct research on animal adaptations and transitions. Both of

my data chapters include data collected during both dry and wet seasons. In Chapter 2, I showed that season influences detection rates of birds, and is therefore an important consideration when monitoring birds in tropical dry forests. The onset of the wet season coincides with the breeding season for many species, including the Rufous-and-white Wren. In Chapter 3, in keeping with results from previous research on this population (Topp and Mennill 2008), I showed that time of year (i.e. from late in the dry season to early in the wet season) influences vocal behaviour of male and female wrens. In Chapter 3, I found that female wrens sing more often earlier in the year (i.e. dry season). In Chapter 2, I detected more bird vocalizations in the dry season than in the wet season. One possible explanation is that females of many tropical species sing, and like the Rufous-and-white Wren, they may do so much more often in the dry season rather than the wet season when they are on nests. Therefore, both of my data chapters reveal differences in avian ecology in dry and wet seasons of tropical dry forests.

As habitats continue to change around the world, including through fragmentation and restoration, it is important that we understand how these changes influence wildlife species and communities. Two areas of research that need further attention are long-term monitoring of biodiversity response to habitat restoration (Lindenmayer 2020), and monitoring changes in animal behaviours in response to anthropogenic change (Tuomainen and Candolin 2011, Teixeira et al. 2019). One important area for future research is an examination of the influence of population density and territory size of mature forest species, like the Rufous-and-white Wren, on vocal behaviours and reproductive success. As bird communities continue to recover in regenerating tropical dry forests, it will be worthwhile to not only monitor communities, but also individual species' behaviours to understand how they respond to habitat change. It would also be worthwhile to look more closely at how species within these communities respond to forest restoration, for example, by determining the timing of colonization of species such as Rufous-

and-white Wrens. By understanding the long-term effects of habitat change on specific species and their behaviours, scientists will be better able to ensure the continued persistence of these unique populations and behaviours. Through effective monitoring, we can help mitigate negative impacts to wildlife, and ensure that conservation initiatives are positively impacting wildlife species and communities.

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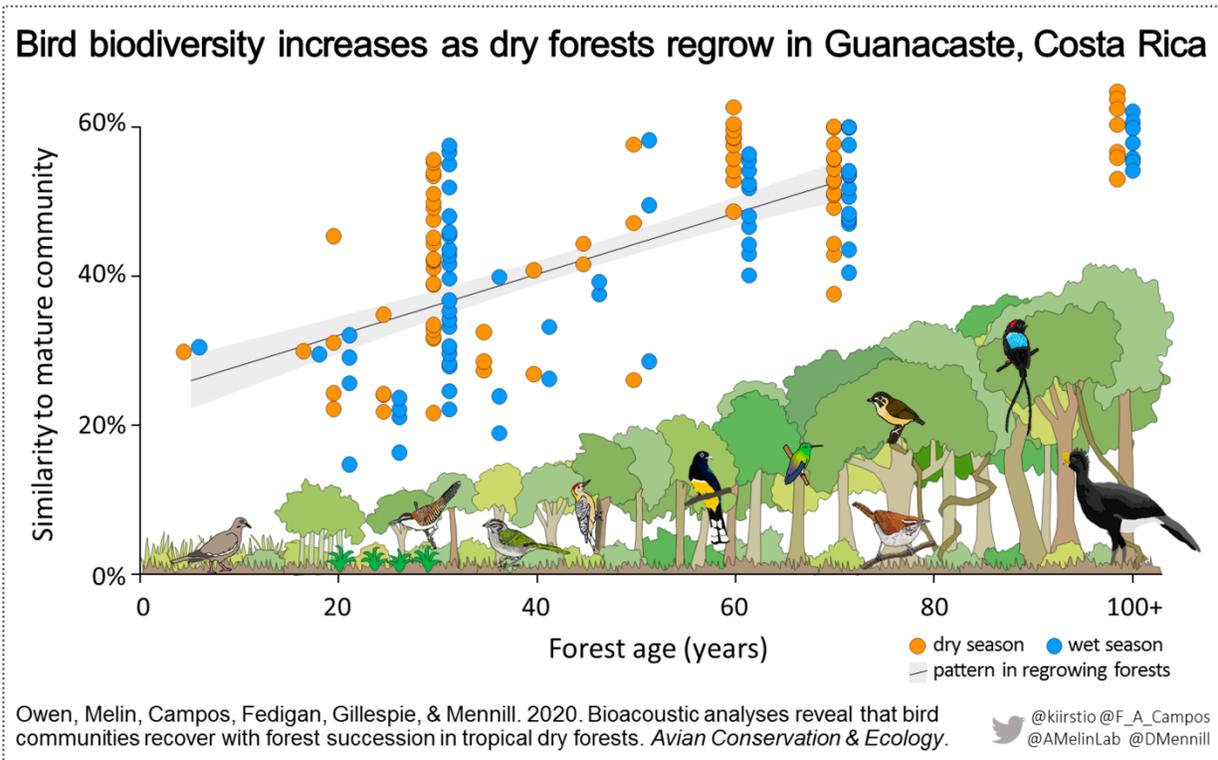
Figure 4.1. Infographic summarizing results from Chapter 2 of this thesis, as published in Avian Conservation and Ecology in the June 2020 issue. Infographic created by K. Owen and D. Mennill.

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