

Social and Reproductive Behavior of the Smooth-billed Ani

SOCIAL AND REPRODUCTIVE BEHAVIOR OF THE
SMOOTH-BILLED ANI

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*A Thesis Submitted to the School of Graduate Studies in the Partial
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Lay Abstract

Sentinels are vigilant animals that watch over foraging group members. While sentinels has been reported in many animal species, minimal work has been done to test for coordination of sentinel behaviour. I tested for coordination of sentinel behaviour in Smooth-billed Anis and show that they do not coordinate sentinel bouts. I also examined sex ratios of Smooth-billed Anis. Adaptive sex ratio allocation in relation to hatching order has been suggested to reduce asymmetrical sibling competition and increase offspring survival. Females may also bias sex ratios of their offspring depending on their condition to maximize their fitness benefits. Here I report a male bias in last hatched chicks and no significant influence of rainfall (proxy for maternal condition) on brood sex ratios.

Abstract

Sentinel behaviour has been widely described in a variety of species. The term sentinel behaviour is typically used to describe a vigilant animal that watches over foraging conspecifics. Several studies have examined the advantages of sentinels in providing early detection of predators while increasing uninterrupted foraging opportunities. However, very little effort has been made to perform formal tests of coordination of sentinel bouts (i.e. the defining feature of sentinel behaviour). For the first portion of this thesis (Chapter II), I tested for sentinel coordination in Smooth-billed Anis. By examining differences between observed and expected proportion of time without a sentinel, and overlap between multiple sentinels, I show that Smooth-billed Anis do not possess a coordinated sentinel system and may have multiple sentinels overlapping at once.

Competitive dynamics of broods have been suggested to influence how adaptive sex ratios allocation can occur across hatching order. Furthermore, the influences of maternal condition and variance in reproductive success (i.e. Trivers-Willard hypothesis) have also been argued to inform sex ratio allocation of offspring sex ratios in a variety of animals. In the latter part of my thesis (Chapter III), I report a sex ratio bias of last-hatched Smooth-billed Anis chicks and show that rainfall (a proxy for maternal condition) does not have a statistically significant influence on the sex ratio of broods. While a male bias in last hatched chicks may represent a tactic to mitigate asymmetrical sibling competition, the mechanisms and adaptive advantage of this strategy remains to be explored.

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Declaration of Authorship

I, Jing Sheng Hing JING SHENG HING, declare that this thesis titled, Social and Reproductive Behaviour of the Smooth-billed ani “Social and Reproductive Behavior of the Smooth-billed Ani” and the work presented in it are my own. Chapter 1 details a general introduction of a brief description of Smooth-billed Anis, sentinel behaviour, and sex ratios studies of cooperative breeders. Each subsequent chapter is then written and formatted according to specific journal guidelines for submission for publication in peer-reviewed journals. Chapter 1 has been submitted to the Journal of Ornithology and Chapter 2 will be submitted to the Journal of Avian Biology. I hereby confirm the following:

- Chapter 1: General Introduction

Author: Jing Sheng Hing

- Chapter 2: Testing for Sentinel coordination in Smooth-billed Anis

Authors: Jing Sheng Hing, Gregory Schmaltz, James S. Quinn

Author Contributions: JSH wrote the first draft of the manuscript and conducted all statistical analyses. Statistical guidance provided by Ben Bolker. GS and JSQ provided comments and revised the manuscript. Field data collected by JSH, GS, Josh Robertson, Morgan Parks, Lilla Barabas, and Adrienne Boon.

- Chapter 3: Hatching order but not maternal condition shows sex ratio bias in joint-nesting Smooth-billed Anis

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Chapter 1

General Introduction

1.1 Sentinel Systems

Social living in gregarious species can confer many benefits such as better access and acquisition of resources (i.e. territory, food, mates: Reyer 1984, Bednarz 1988, Stander 1992, Connor 2000, reviewed in Johnson et al. 2002), alloparental care (Riedman 1982, Reyer 1984, Gilchrist 2007, Brouwer et al. 2012), territory defense (Brown 1964, Macdonald 1983, Farabaugh et al 1992), predator detection (Pulliam 1973, Ridley et al. 2013) and so forth. Anti-predator benefits of group living can manifest as alarm calling (Sommer 2011, Grieves et al. 2014), reduced vigilance per individual (Roberts 1996, Wright et al. 2001, Ridley and Rahani 2007), and dilution and confusion effects (Foster and Treherne 1981, Inman and Krebs 1987). One unique form of shared vigilance that has evolved in a select number of species is a coordinated sentinel system. While sentinel systems can be characterized by the use of vocal signals (Gaston 1977, Wickler 1985, Bednekoff 1997, 2001) and vigilant sentries situated in elevated, exposed positions (Bednekoff

1997, 2001), this is markedly differently from a coordinated sentinel system. A coordinated sentinel system/coordinated sentinel behaviour involves individuals of social groups performing sentinel duty in a coordinated manner whereby sentinel bouts are spread across time more evenly than expected by chance (McGowan and Woolfenden 1989, Bednekoff 1997, 2015).

A coordinated system can provide many benefits by maintaining a fixed level of vigilance, decreasing per capita vigilance and subsequently increasing foraging time (Bednekoff and Lima 1998, Bahr and Bekoff 1999, Rodriguez-Girone and Vasquez 2002). Theoretical models have also demonstrated that adopting a coordinated system in lieu of an uncoordinated system of antipredator vigilance can also increase the probability of survival of group members (Rodriguez-Girone and Vasquez 2002). A coordinated system however does also come with its costs. Studies have argued that the time costs involved in monitoring the behaviour of conspecifics to achieve coordination will outweigh the benefits unless predation risk is high and group size is small (Ward 1985). Coordinating sentinel behaviour can also be costly due to the risk of “cheating” as individuals must choose between foraging or engaging in sentinel behaviour (Wickler 1985). Individuals that defect and do not coordinate their sentinel bouts may still enjoy the benefits of a coordinated system (Wickler 1985, Rodriguez-Girone and Vasquez 2002). These costs and benefits are likely to have a strong influence on the evolution of coordination of sentinel bouts, particularly in species where survival of conspecifics may be important for territory defense, alloparental care, and future reproductive success (e.g. cooperative breeding species).

To date coordinated sentinel systems have only been confirmed in two species

(Florida Scrub Jay (*Aphelocoma coerulescens*) McGowan and Woolfenden 1989, and Meerkats (*Suricata suricatta*) Clutton-Brock et al. 1999) but have been suggested in a multitude of other mammalian, avian and aquatic species (reviewed in Bednekoff 2015). This dearth of evidence for coordinated vigilance requires more attention given the wealth of species that have been observed to utilize sentinels but have not tested these systems for coordination (reviewed in Bednekoff 2015). With more quantitative and experimental studies of sentinel behaviour, studies will be able to move beyond descriptions of sentinel behaviour and test for sentinel coordination so that we can begin to better understand the prominent evolutionary routes of coordinating sentinel behaviour.

1.2 Sex Ratios in Cooperative Breeders

Sex ratios studies have proliferated ever since the advent of molecular tools (i.e. Sexing via polymerase chain reaction (PCR); see Ellegren and Sheldon 1997, Komdeur and Pen 2002) that have facilitated rapid and accurate sex determination by researchers. Since then behavioural ecologists have used molecular tools to test prominent sex ratio theories such as the Trivers-Willard hypothesis (Trivers and Willard 1973) and Fisher's 1:1 sex ratio principle (Fisher 1930). Sex ratio studies of cooperative breeders have also garnered much attention with a focus on helper-at-the-nest-systems.

Helper-at-the-nest systems are typically composed of a dominant breeding pair that raise young with the aid of "helpers" (Skutch 1935, Brown 1978, Cockburn 1998). These helpers, otherwise known as auxiliaries, are predominantly kin (but

see pied kingfisher (*Ceryle rudis*), for a non-kin example; Reyer 1984) of either sex, can gain indirect fitness (e.g. through alloparental care of related offspring) or direct fitness (i.e. reproduction of offspring) benefits by choosing to participate in this reproductive strategy (Dickinson and Hatchwell 2004). While several studies have highlighted the benefits of helpers (Hatchwell et al. 2004, Hodge 2005, Ridley 2007, Russell et al. 2007, Brouwer 2012), the presence of helpers can also prove costly for parental reproductive success when food and other resources are scarce (Legge et al. 2000, Koenig et al. 2001, Komdeur et al. 2002, Clarke et al. 2002). As a result, sex allocation strategies of these cooperative breeders can vary depending on current social-ecological conditions and available resources (Clark 1978, Komdeur 1992, Komdeur and Pen 2002, Komdeur 2004, Rubenstein 2007). Two competing hypotheses emerge from such studies and are known as the helper repayment hypothesis (HRP) and helper competition hypothesis (HCP). The HRP predicts a sex ratio bias towards the helping sex and/or the philopatric sex to increase parental fitness (Clark 1978, Emlen et al. 1986; Pen and Weissing 2000) when resources are plentiful. Conversely, the HCP predicts that the dispersing sex and/or non-helping sex will be preferred when resources are scarce and/or the presence of helpers is detrimental towards parental fitness (Clark 1978, Dickinson 2004). Both hypotheses have received critical review with mixed results (reviewed in Koenig and Walters 1999, reviewed in Komdeur 2004) but does not necessarily apply to other forms of cooperative breeding (e.g. plural cooperative breeding systems with no helpers).

With molecular tools in hand, studies have found evidence for a myriad of other

influences on sex ratios. Factors such as paternal attractiveness (Burley 1986, Ellegren et al. 1996, Kolliker et al. 1998, Sheldon et al. 1999), hatching order (Badyaev et al. 2002, Arnold and Griffiths 2003, Fargallo et al. 2006, Darolova et al. 2008), size dimorphism (Bednarz and Hayden 1991, Blanco et al. 2002, Blanco et al. 2003, Legge et al. 2001, Magrath et al. 2003), food availability/maternal diet (Bradbury and Blakely 1998, Nager et al. 1999, Komdeur, Magrath, Krackow 2002), weather (Cooch et al. 1997, Weatherhead 2005, Rubenstein 2007), age (Blank et al. 1983, Cooch et al. 1997, Weimerskirch, Lallemand, Martin 2005) and so forth have been suggested to play a role in sex allocation. While these variables are not exclusive to cooperative breeders, they are important to investigate alongside the unique and complex life history traits of cooperative breeders that may also influence sex ratios. For example, social hierarchies are typical in cooperative breeders and can dictate access to breeding in social groups (Wiley and Rabenold 1984, Goldizen et al. 2002, Griesser et al. 2007). If producing one sex lends a greater adaptive value, this could potentially influence sex allocation "decisions" that impact individual fitness based on social status (Doutrelant et al. 2004). Thus with so many possible influences, sex ratio studies of cooperative breeders outside the helper at the nest framework are worth investigating to obtain a more holistic picture of prominent factors contributing to biased broods and the adaptive value of sex ratio adjustments.

1.3 Study Species: Smooth-billed Ani (*Crotophaga ani*)

Smooth-billed Ani (*Crotophaga ani*) are cooperatively breeding, neotropical cuckoos that reside in a range that spans central and Northern parts of South America, areas of Central America, the Antillean islands, and Southern Florida (Erritzoe et al. 2012). While Smooth-billed Ani can breed independently in pairs, they are also known to participate in a social mating system known as joint nesting (Vehrencamp and Quinn 2004). In a joint-nesting framework, multiple individuals share a single nest and females contribute to a communal clutch (Vehrencamp 2000). In joint-nesting Smooth-billed Ani, all members of the social group contribute to provisioning of young, nest building and territory defense (Quinn and Startek-Foote 2000). Diurnal incubation appears to be performed by both sexes while nocturnal incubation is predominantly performed by a single male (Quinn and Startek-Foote 2000), akin to the closely related Groove-billed Ani (*Crotophaga sulcirostris*) (Vehrencamp 1977) and Greater Ani (*Crotophaga Major*) (Riehl 2012). This male has been proposed to be the dominant male in these social groups and sires a greater proportion of the brood in joint nests (Robertson et al. 2016.).

As observed in other members of genus *Crotophaga*, joint-nesting Smooth billed Ani can experience severe competition at the nest that includes egg burial or tossing (Schmaltz et al. 2008). Previous research has identified that as group size increased, the number of eggs lost per capita also increased, with multi-female groups losing more eggs than single-female groups (Schmaltz et al. 2008). More

recent endocrine research has indicated these larger groups may be experiencing higher levels of stress (corticosterone used as proxy) as a result of heightened reproductive conflict (Schmaltz et al. 2016, Robertson et al. 2016).

1.4 Thesis Goals

Smooth-billed Anis at the Cabo Rojo National Wildlife Refuge have been studied intensively over the last 18 years. Research has focused on multiple aspects of behavioural and molecular ecology. Even with such research rigour, there still remains a plethora of questions to investigate, particularly related to the benefits of group living in these species amidst intense reproductive conflict (see Grieves et al. 2014). For this thesis, I explore sentinel behaviour and sex ratios of Smooth-billed Anis in an effort to help fill this gap in our knowledge.

In chapter 2 I report on my study of sentinel behaviour of Smooth-billed Anis observing and testing for coordination of sentinel bouts. I explored this by following social groups throughout the breeding season and analysing times spent by individuals as sentinels while comparing observed and expected proportion of times when a) no sentinel was present and b) when two or more individuals were acting as sentinels.

In chapter 3 I explored sex ratios of Smooth-billed Anis broods over a period of 17 years in relation to rainfall (as a proxy for maternal condition) and hatching order. I specifically tested Trivers-Willard hypothesis by examining the relationship between the amount of pre-breeding rainfall one month prior to when a nest was

found (proxy for maternal condition), to sex ratios of the nest. I also investigated whether females bias sex ratios in relation to hatch order to mitigate the negative effects of hatching asynchrony.

On a broad scale these studies provide insight into the biology and behaviour of a unique neotropical bird, contributing to the sparse literature that currently exists on neotropical species. More specifically these studies 1) explore the existence of a coordinated sentinel system as a potential benefit to group living to better our understanding the evolution and maintenance of group living in these species, 2) highlight the complex nature of reproductive decisions and influences in a neotropical, joint-nesting, cooperative breeding species.

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Chapter 2

Testing for Sentinel coordination in Smooth-billed Anis (*Crotophaga ani*)

“...Their wariness and their habit of going in groups does not permit any of the hawks to surprise them. Although the small boy with a sling shot is a constant source of danger, the birds seem to know the exact range and remain beyond it.”

(Davis 1940)

2.1 Abstract

Sentinel behaviour has been widely described, but whether or not it is coordinated among group members requires careful examination. The Smooth-billed Ani *Crotophaga ani*, a social, joint-nesting, cooperative breeding bird, appears to have a sentinel system; however, it is unknown whether that vigilance is coordinated, a defining feature of sentinel behaviour. We followed social groups during two breeding seasons to observe and time their individual sentinel bouts. We then tested for coordination by quantifying the observed and expected proportions of time during which a) no sentinel was present during observations and b) two or more sentinels were present. The majority of social groups spent less time than expected under the coordination hypothesis with at least one sentinel, indicating evidence against coordinated vigilance. This was significant for social groups observed in 2015, but not in 2002. Furthermore, across both years majority of individuals from these social groups also overlapped on sentinel duty in groups of 4 birds, more than expected under the coordination hypothesis. On the other hand, overlap between 2 individuals occurred less than expected under the coordination hypothesis and was also significant across both years. This study contributes to a gap in our knowledge of sentinel behaviour and suggests that Smooth-billed Anis lack organized vigilance, possibly due to low within group relatedness and group instability.

2.2 Introduction

Descriptive studies of several species report sentinel behaviour (reviewed in Bednekoff 2015). Currently, most of these descriptive studies have focused on the presence or absence of sentinel behaviour. However, there is a dearth of studies that have quantitatively tested for coordinated sentinel behaviour in which group members coordinate activities. Coordinated sentinel behaviour is defined as coordinated vigilance of sentinel bouts that are spread across time more evenly than expected by chance to reduce the amount of time when no sentinel is present (McGowan and Woolfenden, 1989; Bednekoff 1997, Bednekoff 2015). Bednekoff (2015) revealed that empirical evidence of a coordinated sentinel system in birds has only been reported in Florida Scrub Jays *Aphelocoma coerulescens* (McGowan and Woolfenden 1989), highlighting a large gap in our knowledge of this intriguing behaviour.

The use of sentinels in many social species is known to function as an anti-predator adaptation that confers a variety of benefits. Sentinels can decrease per capita time spent scanning for predators, allowing more dedicated time spent foraging, and earlier detection and avoidance of predators (McGowan and Woolfenden 1989; Manser 1999; Hollen et al. 2008; Ridley et al. 2010; Sorato et al. 2012). These advantages may be amplified by a coordinated system. The cost of being a sentinel and the trade-off between foraging and the risk of predation are also likely to influence the evolution and maintenance of a coordinated sentinel system. For example, sentinel activity is known to correlate with satiation and energetic state. Although sentinels have the advantage of early detection of predatory threats,

there is a cost in foraging opportunities such that the benefits of foraging increase the longer an individual is a sentinel. Studies have shown that individuals are more likely to be sentinels and remain so for longer periods of time when satiated (Bednekoff 2003) and when in possession of greater energy reserves (Wright et al. 2001a). It is also worth noting that sentinels are not necessarily safer than their foraging conspecifics, as one study showed that sentinels are further from cover than foragers and were more likely to be targeted by predators (Ridley et al. 2013). Therefore, a coordinated sentinel system whereby individuals coordinate sentinel bouts might allow individuals to share costs and benefits among individuals (Wickler 1985).

Apparent sentinel behaviour and coordinated sentinel behaviour have been observed in cooperative breeders across mammalian (Clutton-Brock et al. 1999; Rasa 1986) and avian (McGowan and Woolfenden 1989; Yasukawa and Cockburn 2009; Sorato et al. 2012; Ridley et al. 2013) taxa. Cooperative breeding brings individuals together in a situation in which kin selection and/or reciprocity may set the stage for organized vigilance (McGowan and Woolfenden 1989). One reason why cooperative breeding may favour selection for coordinated sentinel behaviour relates to the potential fitness benefits sentinels may accrue (Bednekoff 1997; 2015). For example, in providing protection of adult genetic offspring in kin groups, individuals may obtain delayed benefits such as alloparental care and increased reproductive success (Komdeur et al. 1995; Ekman et al. 2004). Kin selection may also lead to the formation of small and stable groups that favours coordinating vigilance as the time cost involved in monitoring group members is reduced (Bednekoff 1997, Ward 1985). However kin selection may not be necessary for the

evolution of coordinated sentinel behaviour (Bednekoff 1997; Clutton-Brock et al. 1999; Wright et al. 2001b; Bednekoff 2003). Using a theoretical model, Bednekoff (1997) showed that coordination of sentinel bouts can be maintained when individuals take the role of sentinel when sufficiently satiated and when no one else is on duty. The model did not require kin selection as coordination here is based on by-product mutualism. Since then this model has received strong support from empirical studies (Clutton-Brock et al. 1999; Bednekoff 2003).

Environmental and ecological conditions may also facilitate the evolution of a coordinated sentinel system (Bednekoff 1997, 2015). Coordinated sentinel systems and apparent sentinels systems have been primarily observed to occur in dry, open, tropical regions, where perches readily offer available prime vantage points (Bednekoff 1997, 2015). Suitable habitats include deserts, (Wright et al. 2001a) and scrub-oak woodland (McGowan and Woolfenden 1989). These arid environments can be unpredictable and harsh, whereby food becomes limited and all foraging opportunities must be fully exploited. Coordinated sentinel systems would allow extended periods of uninterrupted foraging much needed in such environments. Species demonstrating coordinated and apparent sentinel systems often forage near the ground, where they are susceptible to predation and have limited predator detection abilities (Gaston 1977, McGowan and Woolfenden 1989, Wright et al. 2001a). While these foraging behaviours and environmental characteristics may be important preconditions favouring sentinel behaviour, there also seems to be a phylogenetic element. For example, 5 Babbler species (*Turdoides*; reviewed in Bednekoff 2015) with apparent sentinel systems, coexist with many other non-sentinel species in the same habitat. Evolutionary factors responsible

for the pervasiveness of sentinel behaviour in this genus remain unknown (Bednekoff 2015).

Smooth-billed Anis (*Crotophaga ani*) have been observed to use a functional referential alarm call system (Grieves et al. 2014), a trait that would support a coordinated sentinel system (Bednekoff 1997, 2015). An apparent sentinel system in Smooth-billed Anis was first described by Loflin (1983) however, whether or not the system is coordinated has not yet been examined. Smooth-billed Anis are joint-nesting cooperatively breeding neotropical birds that form social groups ranging from 2-17 individuals, composed mostly of socially monogamous pairs (Quinn and Startek-Foote 2000). In these social groups, members share a joint nest, defend an all-purpose territory and forage together during the breeding season. Smooth-billed Anis usually forage in open areas where trees and snags are available as perches for sentinels (Quinn and Startek-Foote 2000).

We tested the hypothesis that Smooth-billed Anis have a coordinated sentinel system. This hypothesis predicts that sentinel bouts across time are spread out more evenly than expected by chance. Specifically, we predicted the observed proportion of time without a sentinel would not exceed the calculated expected (optimal) proportion of time without a sentinel. In addition, we predicted the observed proportion of time with overlap between individuals would not exceed the expected proportion (optimal) of time where more than one individuals would act as sentinels simultaneously (prediction 2).

2.3 Methods

Field Site

Smooth-billed Anis were studied at the Cabo Rojo National Wildlife Refuge (CRNWR) in southwestern Puerto Rico (17°59"N, 67°10"W) from September – December of 2002 and 2015. The CRNWR has a dry tropical climate with a pronounced rainy season typically from September to December and a dry season from January to April. A short increase in rain typically occurs in May, followed by dry conditions until September. During the rainy season of 2015, average rainfall was significantly lower (mean = 40.7 mm, SE = \pm 15.5 mm) in comparison to average rainy season rainfall calculated from 1998 – 2015 (mean = 114.5 mm, SE = \pm 13.1 mm) (Hing unpubl. data.; $t = 3.63$, $P = 0.0037$). Average rainy season rainfall was greater in 2002 (mean = 73.7 mm, SE = \pm 24.2 mm) than in 2015, however this was not statistically significant (Hing unpubl. data. $t = 1.145$, $P = 0.29$). CRNWR can be described as a subtropical, dry, scrubland in secondary succession with large open areas (Schmaltz et al. 2008). Here Smooth-billed Anis typically breed during the rainy season and are residents year round, although they do not consistently hold territories during the dry season, roaming larger areas in loose, large foraging flocks (Quinn and Startek-Foote 2000).

Sentinel Observations

We selected groups for observation based on how effectively group members could be distinguished. Banding efforts at CRNWR have been ongoing since 1998 and banded individuals are distinguishable by a unique combination of one metal

band and 3 colour bands. To test for coordination in sentinel behaviour we chose groups where there were no more than 2 unbanded individuals. In each sentinel observation of groups that contained 2 unbanded individuals ($n = 5$), we were able to distinguish between the 2 individuals for the duration of the observation by tracking spatial location and movements of each unbanded individual relative to one another.

Each social group was followed over the following periods: December 6th – 29th of 2002 and October 7th – December 14th of 2015. Sentinel observation bouts were conducted for 1-2 hour periods using a Swarovski scope (ATX 25-60x65) and binoculars (Vortex Crossfire, 8 x 42) for 2015 and binoculars (Pentax, 8x42 and 10x42) for 2002. The use of a telescope and binoculars allowed us to maintain a fair distance (100-200m) so that we did not affect the behaviour of the subjects. Observations in which the social group was lost from view before 1 hour had elapsed were excluded from the analysis. In 2002, a total of 5 groups were followed and a total of 15 hours of foraging observations were collected. In 2015, a total of 5 groups were followed during the breeding season of 2015 and a total of 20 hours of group foraging observations were successfully collected. All sentinel observations were conducted from 0800-1100 hours in the morning and 1530-1800 hours in the afternoon/evening.

Individuals were classified as sentinels if they met the following requirements: a) perched at least 1m off the ground for at least 30 seconds in a conspicuous location near foragers and b) scanning the surrounding area. A stopwatch was used to keep track of observation times. The observation period would begin when the stopwatch started and lasted for 1-2 hours. The observer would record the

identity of the individuals acting as sentinels for the duration of the observation, taking note of the start and stop times of sentinel bouts to the nearest second.

2.4 Statistical Analyses

All analyses were conducted in R version 3.1.2. (R Core Team 2016). First, we calculated the total amount of time spent as sentinels by each individual and the total amount of time without sentinels during each observation. To calculate the expected proportion of time without a sentinel for each observation, the following formula was used:

$$\prod_{i=1}^n (1 - p_i)$$

where p_i is the proportion of time spent by an individual, i , on sentinel duty (Bednekoff 2015). This value was then compared to the observed proportion of time without a sentinel during that observation. We calculated the average observed and expected time without sentinels across sessions for each social group. Coordination was then measured as the difference between the average observed and expected time without a sentinel per group.

We also looked at the observed and expected proportions of overlap between all combinations of $n = 2-5$ birds (i.e. overlap between pairs, triplets etc) for each observation session. To calculate the expected proportion of overlap for each combination of birds, we first multiplied the proportion of time each individual was a sentinel. We then multiplied that value by the proportion of time the remaining group members were not sentinels. So for example in a group of 3 birds, (a, b, c),

to calculate the expected proportion of overlap time between a and b:

$$(p_a \times p_b) \times (1 - p_c)$$

where p is the proportion of time spent by an individual on sentinel duty. We calculated the average observed and expected proportions of overlap time for every combination of $n = 2-5$ birds across observation sessions for each group to test for differences between observed and expected values.

To assess the difference between observed and expected proportion of time with no sentinel across both years we used a linear mixed effects model (LMM) using the “nlme” package (Pinheiro et al. 2016). In this model, average proportion of time without sentinel was the response variable. Year, “type” (coded as a binary variable, i.e. observed or expected), and the interaction between these two variables were included as fixed effects. Social group was included as a random intercept in this model to account for statistical non-independence of our observations for each group. This LMM corresponds to a pooled t-test on “type”.

To assess the overall difference between observed and expected proportions of overlap time, we conducted separate LMM for overlap between $n = 2-5$ birds. For each model, the response variable was average proportion of time with overlap. Year, “type”, and the interaction between these two variables were included as fixed effects. Social group was included as a random intercept for each model. These LMM are also similar to conducting a pooled t-test on "type".

2.5 Results

Overall across both years, there was a significantly greater proportion of the average observed time with no sentinel than expected (estimate = 0.0642, 95% CI = [0.00739, 0.121], $P = 0.0313$) (Fig 2.1). Looking at the difference between the average observed and expected proportions of time with no sentinel in each year, there was a greater proportion of time observed than expected with no sentinel in 2002, however, this was non-significant (estimate = 0.0182, 95% CI = [-0.0621, 0.0985], $P = 0.615$) (Fig 2.1). In 2015, there was a significantly greater proportion of the average time observed with no sentinel than expected (estimate = 0.110, 95% CI = [0.0299, 0.191], $P = 0.0133$) (Fig 2.1). There was no significant difference between the average observed and expected proportions of time with no sentinel in 2015 compared to 2002 (estimate = -0.0920, 95% CI = [-0.206, -0.0920], $P = 0.0988$) (Fig 2.1).

Across both years, the average proportion of time observed with multiple sentinels overlapping was significantly greater than expected for combinations of 4 birds (estimate = 0.0676, 95% CI = [-0.000904, 0.134], $P = 0.0478$) (Fig 2.2). Across both years, the average observed proportion of time with multiple sentinels overlapping was significantly less than expected for combinations of 2 birds (estimate = -0.125, 95% CI = [-0.169, -0.0805], $P < 0.01$) (Fig 2.2). Across both years, there was also no significant difference between the average observed and expected proportion of time for combinations of: 3 birds (estimate = -0.0170, 95% CI = [-0.103, 0.0685], $P = 0.659$), and 5 birds (estimate = 0.0423, 95% CI = [-0.0195, 0.104], $P = 0.130$) (Fig 2.2).

In 2002, there was no significant difference between the average observed and expected proportion of overlap time for combinations of overlap between 2-5 birds (Table 2.1, Fig 2.2).

In 2015, the average observed proportion of time with multiple sentinels overlapping was significantly greater than expected for combinations of 4 birds (Table 2.2, Fig 2.2). The average observed proportion of time with multiple sentinels overlapping was significantly less than expected for combinations of 2 birds (Table 2.2, Fig 2.2) There was no significant difference between the average observed and expected proportion of overlap time for combinations of 3 and 5 birds (Table 2.2, Fig 2.2)

There was no significant difference between the between average observed and expected proportions of time with multiple sentinels overlapping in 2015 compared to 2002 for combinations of: 3-5 birds (Table 2.2, Fig 2.2). There was a significantly smaller difference between the average observed and expected proportion of time with pairs of sentinels overlapping in 2015 compared to 2002 (Table 2.2, Fig 2.2).

Smooth-billed Anis were not observed to use unique vocalizations to signal sentinel presence or to signal the end of a sentinel bout.

2.6 Discussion

Smooth-billed Anis did not demonstrate a coordinated sentinel system despite the benefits associated with social behaviour such as cooperative nest defence or referential alarm calling systems. Most Smooth-billed Ani social groups were

observed to have a greater proportion of time without a sentinel than expected indicating a lack of coordination.

Most social groups were also observed to have groups of 4 sentinels for a significantly greater proportion of time than expected, but not for other numbers of simultaneous sentinels. On the other hand, social groups were observed to have pairs of sentinels for a significantly less proportion of time than expected. One possible explanation of this pattern of overlap in foursomes versus pairs may be related to an imminent/immediate predatory threat whereby probability of capture is high. Such may cause the majority of individuals in social group members to become vigilant sentinels. Overall, these results also indicate a lack of coordination (as defined by Bednekoff 2015 and Woolfenden and McGowan 1989). Our analysis also detected a smaller difference between observed and expected proportion of time when 2 sentinels were overlapping in 2015 than in 2002. We do not have an explanation for this difference between years.

Smooth-billed Anis did not appear to use any unique vocalizations that might aid coordination of sentinel bouts as observed in other species (Gaston 1977, Manser et al. 1999, Hollen et al. 2008). The absence of such vocalizations however has been observed in coordinated sentinel systems (e.g. Florida-Scrub Jay, Bednekoff et al. 2008) suggesting that the evolution of coordinated sentinel behaviour may not be dependent on such vocal signals.

The lack of coordinated sentinel behaviour in Smooth-billed Ani social groups may be attributed to a variety of factors. Low relatedness among adults in social groups (Quinn and Startek-Foote 2000, Robertson et al. in review) may explain

the absence of a coordinated system. Kin selection has been suggested to be one of the drivers behind coordinated vigilance (Clutton-Brock et al. 1999). Delayed dispersal of kin may allow for small and stable long-lived groups to form (Bednekoff 1997) whereby the costs of monitoring conspecifics is low and the benefits of coordination high (Ward 1985). Kin selection may also be a strong evolutionary force in promoting coordination where kin groups rely on the survival of young of the year for reproductive success or territory acquisition, as seen in some helper-at-the-nest cooperative breeders (e.g. Florida scrubjays, Woolfenden and Fitzpatrick 1984). However, since Smooth-billed Anis typically form groups of unrelated adults (Quinn and Startek-Foote 2000, Robertson et al. in review), kin selection benefits promoting sentinel behaviour are likely non-existent except during periods when fledglings are with the group.

Another character influencing the evolution and maintenance of a coordinated system is the stability of group composition across years. Coordination of sentinel behaviour has been suggested to be an example of reciprocal altruism (Bednekoff 1997, Clutton-Brock et al. 1999). This is likely the case for highly social species, whereby individuals are able to recognize one another to keep track of sentinel bouts and detect defectors of sentinel duty (Blumstein 1999). Continuous cooperation among the same individuals can promote reciprocity that leads to coordinated vigilance (Brandl and Bellwood 2015). Indeed, coordinated vigilance is known to be favoured when group composition is stable as this makes cheating unlikely (Wickler 1985). Furthermore, by maintaining consistent group membership, this may also confer greater reliability of alarm calls of sentinels as a result of familiarity versus unfamiliar or unreliable individuals/signals (Hare and Atkins

2001), facilitating more effective sentinel coordination over time. Unstable group composition in Smooth-billed Anis persists as social groups that form during the breeding season, typically dissolve into large loose flocks at the end of the season. These foraging flocks roam freely as territory boundaries become blurred (Quinn and Startek-Foote 2000). Come the following breeding season, group composition and territory boundaries are often different. While fledglings and adults may occasionally remain on natal/breeding territory between breeding seasons (Quinn and Startek-Foote 2000), group membership often changes as juvenile retention is low (Robertson 2016). Furthermore, flexibility in group composition/size may also arise as Smooth-billed Anis are not obligatory cooperative breeders and can nest as lone pairs (Quinn and Startek-Foote 2000). Indeed, group membership was not consistent across years for the majority of social groups (see Appendix A, Table A.1). Such dynamic group composition across years in Smooth-billed Anis may serve as a barrier for the evolution of coordinated sentinel behaviour predicated on reciprocal altruism.

Arguably the length of a breeding season (~4 months [as per length of rainy season]) could provide sufficient time for a reciprocal altruistic system of coordinating sentinel bouts to evolve as group membership is stable during this period (Quinn and Startek-Foote 2000). The time required for individuals to remain together that would facilitate a coordinated sentinel system however remains unknown. Species in which a coordinated sentinel system has been tested and positively identified (i.e. Florida Scrub-Jays and Meerkats, *Suricata suricatta*), form long-lived, stable, kin groups whereby offspring may delay dispersal for up to 2, and 5 years, respectively (Woolfenden and Fitzpatrick 1984, Clutton-Brock et al. 2008). In both

species, some individuals may remain on the natal territory and eventually inherit dominant breeding positions (Woolfenden and Fitzpatrick 1978, Woolfenden and Fitzpatrick 1984, Clutton-Brock et al. 2008). Based on these studies, the stability of group composition/membership across multiple years could facilitate a coordinated system via reciprocal altruism. Such stability in group membership and composition across that time scale is lacking in our study species.

The absence of coordinated sentinel behaviour could also be attributed to the ability of foraging Smooth-billed Anis to detect predators sometimes without lifting their heads (Fernandez-Juricic et al. 2004). While theoretical studies have claimed that foraging and scanning for predators are mutually exclusive activities (Pulliam et al. 1982, Ward 1985), empirical studies have shown that individuals with heads down (i.e. foraging) retain the ability to scan and detect predators (Lima and Bednekoff 1999, Guilleman et al. 2001). This ability is widespread among other ground foragers owing to the visual configuration of avian eyes that provide peripheral vision and the simultaneous view of the ground and distant horizon at the same time (Martin and Katzir 1999, Fernandez-Juricic et al. 2004). Using a simulated model, Fernandez-Juricic et al. (2004) showed that when individuals can occasionally detect predators in a head down position (e.g. foraging), and there is a low probability of being warned when another group member detects a predator, coordinating vigilance would not be favoured. With a functionally referential alarm call system, Smooth-billed Anis in social groups undoubtedly have a high probability of being warned of the type of threat (terrestrial vs. aerial predator) without relying on a visual cue (i.e. can be foraging/head down) and perform the appropriate, response relative to the alarm call given (see Grieves et al. 2014).

However, the ability of foraging Smooth-billed Anis to detect predators without raising their heads remains equivocal as they often forage in a variety of microhabitats, from short to medium length grass (Quinn and Startek-Foote 2000), as well as deep grass. In medium to deep grass, there is a greater probability of being unable to detect predators while foraging that may not allow for independent scanning while reducing the reliance of a sentinel. Therefore it is unlikely that the ability to detect predators explains why Smooth-billed Anis have not evolved a coordinated sentinel system.

In conclusion, we show that Smooth-billed Anis do not perform coordinated sentinel behaviour. While Smooth-billed Anis are vigilant and use referential alarm calls, they must be added to the negative column of a scant list of studies that have quantitatively tested for coordination of sentinel behaviour. Some environmental and behavioural factors associated with Smooth-billed Anis might favour the evolution of a coordinated sentinel system, while other ecological and life history traits may hinder its evolution. This research demonstrates the importance of quantitative testing of coordination to help distinguish between coordinated sentinel systems/behaviour from apparent sentinel systems/behaviour.

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2.8 Figures and Tables

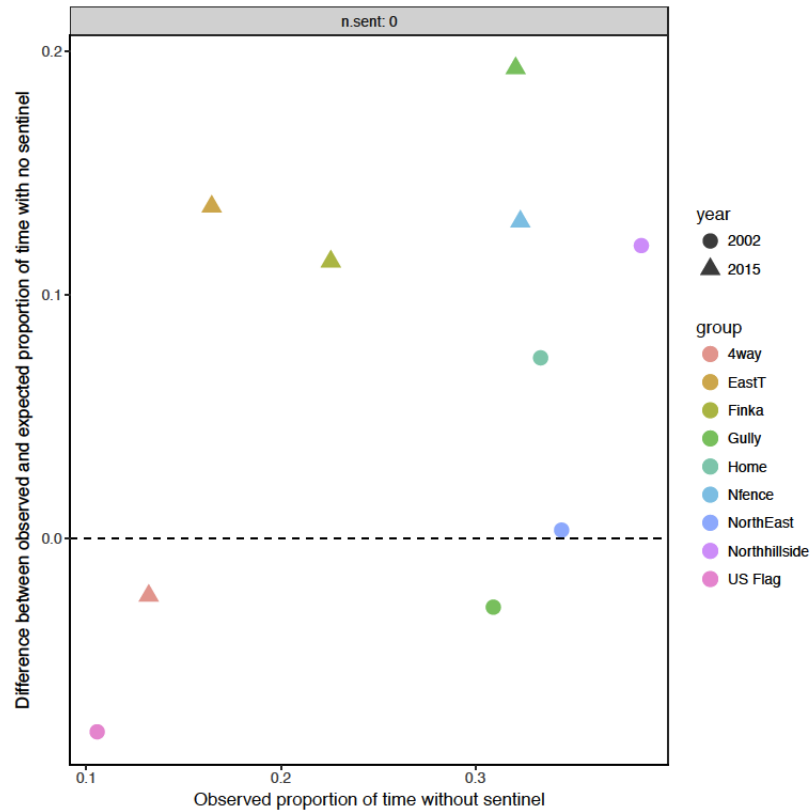


FIGURE 2.1: Difference between the average observed and expected proportion of time (y-axis) with no sentinel. X-axis represents the average proportion of time with no sentinel. Triangles represent social groups observed in 2015 while circles represent groups observed in 2002. The dotted line represents a difference of 0, (i.e. Obs = Exp).

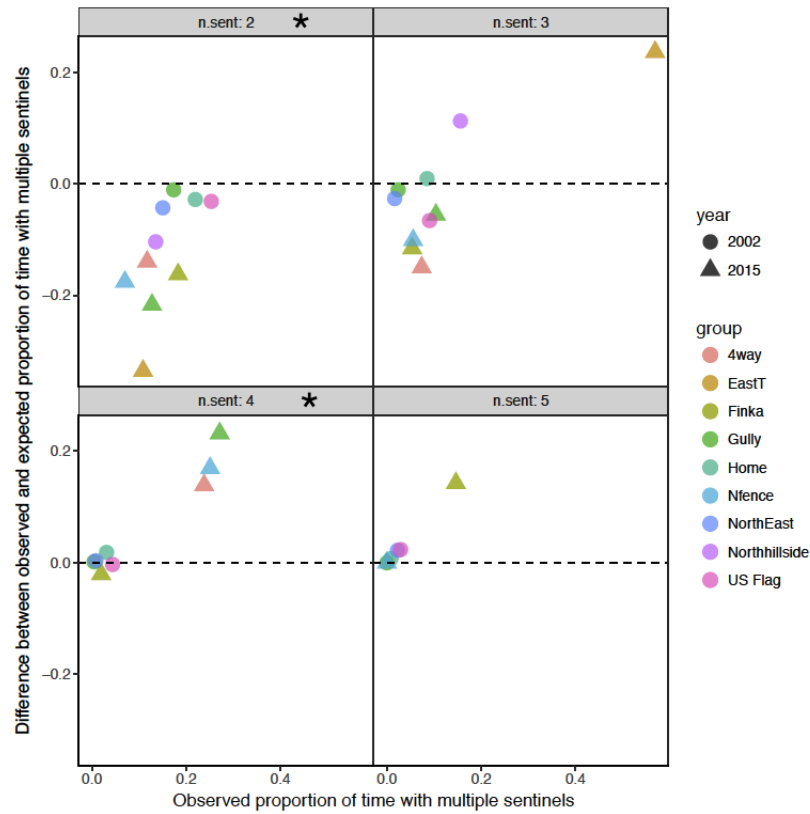


FIGURE 2.2: Difference between the average observed and expected proportion of time (y axis) for 2-5 sentinels overlapping. X axis represents the average observed proportion of time with overlapping sentinels. Triangles represent social groups observed in 2015 while circles represent groups in 2002. The dotted line represents a difference of 0, (i.e. Obs = Exp). Asterisk indicates a significant difference between average observed and expected values for overlap between multiple sentinels.

TABLE 2.1: Linear mixed model (LMM) results used to examine differences between observed and expected proportion of time with multiple sentinels (i.e. overlap between 2-5 sentinels) for the year 2002. For each LMM: Fixed effects are Year, Type (i.e. observed or expected), and the interaction between these two fixed effects. Average proportion of time with multiple sentinels overlapping was the response variable. Model sample size includes 5 socials groups.

Fixed Effect	Estimate	95% CI	<i>p</i> – value
<i>Overlap between 2 sentinels</i>			
Year	0.106	0.0309, 0.181	0.0116
Type	-0.0437	-0.106, 0.0190	0.1471
Year * Type	-0.162	-0.251, -0.073	<0.01
<i>Overlap between 3 sentinels</i>			
Year	0.132	-0.0233, 0.289	0.0854
Type	0.00352	-0.117, 0.125	0.948
Year * Type	-0.041	-0.212, 0.130	0.5956
<i>Overlap between 4 sentinels</i>			
Year	0.0538	-0.0498, 0.157	0.2512
Type	0.00518	-0.0891, 0.0995	0.8973
Year * Type	0.125	-0.00856, 0.258	0.062
<i>Overlap between 5 sentinels</i>			
Year	0.000159	-0.09000, 0.0904	0.996
Type	0.0132	-0.0581, 0.0846	0.633
Year * Type	0.0579	-0.0656, 0.182	0.262

TABLE 2.2: Linear mixed model (LMM) results used to examine differences between observed and expected proportion of time with multiple sentinels (i.e. overlap between 2-5 sentinels) for the year 2015. For each LMM: fixed effects are Year, Type (i.e. observed or expected), and the interaction between these two fixed effects. Average proportion of time with multiple sentinels overlapping was the response variable. Model sample size includes 5 socials groups.

Fixed Effect	Estimate	95% CI	<i>p</i> – value
<i>Overlap between 2 sentinels</i>			
Year	-0.106	-0.181, -0.0310	0.0116
Type	-0.206	-0.269, -0.143	<0.01
Year * Type	0.162	0.0735, 0.251	<0.01
<i>Overlap between 3 sentinels</i>			
Year	-0.132	-0.289, 0.0233	0.0854
Type	-0.0374	-0.158, 0.0835	0.495
Year * Type	0.041	-0.130, 0.212	0.596
<i>Overlap between 4 sentinels</i>			
Year	-0.0538	-0.157, 0.0498	0.2512
Type	0.129	0.0356, 0.224	0.015
Year * Type	-0.125	-0.258, 0.00856	0.062
<i>Overlap between 5 sentinels</i>			
Year	0.000159	-0.0904, 0.09004	0.122
Type	0.0713	-0.0296, 0.172	0.996
Year * Type	0.0579	-0.182, 0.0656	0.262

Chapter 3

Hatching order but not maternal condition shows sex ratio bias in joint-nesting Smooth-billed Anis

3.1 Abstract

Maternal condition, maternal investment, and hatching order reportedly influence offspring sex ratios. Sex ratio studies of cooperatively breeding species, however, have predominantly focused on helper-at-the-nest systems. Here we provide the first data on sex ratios in relation to maternal condition and hatching order in the Smooth-billed Ani, a neotropical, joint-nesting cuckoo. Using rainfall as proxy for maternal condition, we examined sex ratios across broods and in chicks from eggs that hatched first, in the middle, and last. We found a sex ratio bias favouring males only in last hatched eggs. There was no statistically significant influence of rainfall on brood sex ratios. This suggests maternal condition does not influence sex ratio of broods, which was predicted given the non-significant difference in reproductive variance between sexes. While it is difficult to pinpoint the significance and cause of male-bias in last hatch eggs, we discuss multiple factors worth further investigation that may help elucidate this sex ratio bias.

3.2 Introduction

Sex ratio analyses in avian species have been rampant since the development of molecular sexing tools (Ellegren and Sheldon 1997, Komdeur and Pen 2002), often driven by two monumental theories on sex ratio proposed by Trivers and Willard (1973), and Fisher (1930). Since then, a multitude of studies have been published (see reviews: Pike and Petrie 2003, Ewen et al. 2004, Komdeur 2004) investigating sex ratio manipulation in birds. Sex ratio studies of cooperatively breeding species have focused largely on helper-at-the-nest systems (Ligon and Ligon 1990, Komdeur et al. 1997, Komdeur 2004, Griffin et al. 2005, Rubenstein 2007, Canestrari et al. 2012) with the goal of addressing the adaptive significance of facultative adjustments of sex ratios (Komdeur and Pen 2002).

Helper-at-the-nest systems have been suggested to favour sex ratio adjustments in accordance to either the helper-repayment-hypothesis (HRP) or the helper-competition-hypothesis (HCP) (Clark 1978). The HRP predicts a sex ratio bias towards the philopatric/helping sex when the helping sex increases parental reproductive success and when resources are plentiful (Clark 1978, Emlen et al. 1986; Pen and Weissing 2000). Conversely, the HCP predicts that the dispersing sex and/or non-helping sex will be preferred when the presence of helpers is detrimental towards parental reproductive success and when resources are scarce (Clark 1978, Dickinson 2004). Other aspects of cooperative breeding have also inspired sex-ratio studies. For example, some studies have examined how sex ratios can be influenced by dominance status (Brown and Silk 2002, Sheldon and West 2004), habitat quality (Komdeur et al. 1996), sexual dimorphism (Bednarz and Hayden

1991, Blanco et al. 2002, Blanco et al. 2003, Legge et al. 2001, Magrath et al. 2003) and hatching order (Bednarz and Hayden 1991, Woxvold and Magrath 2008, Arnold et al. 2001, Legge et al. 2001, Blanco et al. 2002, Krebs et al. 2002, Blanco et al. 2003, Magrath et al. 2003, Mullner 2004).

Several studies that highlight the importance of sex ratio biases across hatching order have shown that this pattern tends to be pronounced in species where sexual dimorphism is observed in nestlings (Bednarz and Hayden 1991, Blanco et al. 2002, Blanco et al. 2003, Legge et al. 2001, Magrath et al. 2003) and in highly asynchronous clutches (Bortolotti 1986, Stoleson and Beissinger 1995; Cook and Monaghan 2004, Arnold et al. 2001, Fargallo et al. 2006, Saino et al. 2011). Sex ratio bias studies across hatching order in cooperative breeding species have suggested that biasing sex ratio patterns can be a proximate mechanism to temper brood reduction (Bednarz and Hayden 1991, Legge et al. 2001, but see Mullner 2004). By leveling competition for food, the disparity in growth and size may be reduced. Even in species that are not size dimorphic, differences in growth and size can arise from hatching asynchrony (Ostreiher 1997), if the onset of incubation occurs prior to clutch completion (Stoleson and Beissinger 1995). In large and/or asynchronous clutches, a brood hierarchy may also develop where younger nestlings (i.e. hatch last or smallest) are at a disadvantage in comparison to older nestlings (i.e. hatch first) when competing for food (Ostreiher 1997, Schmaltz 2008). As a result, biased mortality can occur at specific points of the hatching sequence and studies have shown a tendency for last hatched nestlings to suffer as a result of the brood hierarchy (Lack 1968, Stoleson and Beissinger 1995).

As a consequence of asymmetrical sibling competition and potential biased

mortality according to hatch order, females may adjust sex ratios at specific hatch orders to match brood conditions and/or increase the probability of survival across the brood (Bortolotti 1986, Nager et al. 1999, Legge et al. 2001, Darolova et al. 2008). Position in hatching order may not only influence survival but has also been shown to impact other traits such as growth rate (Darolova et al. 2008, Badyaev et al. 2002, Dey et al. 2014), reproductive success (Saino et al. 2012) and dominance status in adulthood (Dey et al. 2014). Strategic sex allocation across hatching order may therefore confer a variety of benefits to offspring and mothers, especially when there are sex-specific differences in offspring reproductive success. As the heterogametic sex, female birds have been suggested to be capable of modifying sex ratios of broods. To adjust sex ratios in relation to hatching order, one strategy females may employ is to vary maternal investment of hormones across the laying order. Differences in the deposition of maternal hormones according to sex across laying order has also been documented (Groothuis et al. 2005), and alludes to the involvement of hormones, more specifically the deposition of maternal androgens, in sex determination (Pike and Petrie 2003, Groothuis et al. 2005, Navara 2013). Androgens have been suggested to influence sex determination by direct mediation of meiotic segregation during pre-follicle development (Pike and Petrie 2003, Rutkowska and Badyaev 2008, Navara 2013) and some studies have shown greater concentrations of androgens to correlate with male eggs in comparison to female eggs (Petrie et al. 2001, Müller et al. 2002).

Sex ratios can also be influenced by maternal condition and environmental conditions (Komdeur and Pen 2002, Badyaev et al. 2002, Love et al. 2005, Rubenstein 2007, Bensch 1999). Trivers and Willard (1973) first reported this relationship and

proposed that maternal condition could influence offspring sex allocation if reproductive variance differed between sexes. According to Trivers and Willard (1973), mothers in relatively good condition are predicted to bias progeny in favour of the sex with highest reproductive variance, whereas females in relatively poor condition should produce an excess of the sex with lower reproductive variance. Condition in turn is arguably largely affected by other factors such as food availability and weather (Brown and Sherry 2006). For example, food abundance in habitats located near the equator is strongly influenced by the amount of rainfall (Grant and Boag 1980, Leigh et al. 1996, Ahumada 2001). With increases in rainfall resulting in increases in food availability, body condition is expected to also improve. Temporal environmental variation has previously been shown to affect maternal condition and sex ratios of broods (Rubenstein 2007, Berkeley 2010). As a result, one would expect females in long-lived species to bias their offspring as predicted by Trivers-Willard (1973), in accordance to the temporal variation of environmental and body conditions to maximize fitness (Rubenstein 2007).

Here we provide the first data on sex ratios of Smooth-billed Ani (*Crotophaga ani*) in relation to hatch order, and historic rainfall as a proxy for maternal condition. Sex ratio studies of joint nesting species are scarce in the literature (but see McRae 1996, Koenig et al. 2001, Mullner 2004, Magige 2012, Healey et al. in review). Currently such paucity is apparent for socially monogamous, joint nesting, species. The Smooth-billed Ani is a socially monogamous, joint-nesting, neotropical cuckoo that offers a unique opportunity to study how the social and mating system complexities inform sex allocation across hatching order and in relation to maternal condition. Adult Smooth-billed Anis often engage in ovicide

by egg tossing and burial, trying to increase the survival of their own offspring (Schmaltz et al. 2008a). In large multi-female groups, egg laying is prolonged and joint nests often contain large communal clutches that are asynchronous (Schmaltz et al. 2008a,b). One endocrine study of Smooth-billed Ani eggs revealed that last laid eggs tended to have higher levels of testosterone (T), potentially to mitigate detrimental effects of hatching asynchrony and the disadvantage of hatching last (Schmaltz et al. 2008b). However this study did not investigate sex ratios of broods in relation to hatching order.

We examined sex ratio differences across hatching order, and the influence of rainfall (i.e. maternal condition) on sex ratios in broods of Smooth-billed Anis. We hypothesized that females may bias sex ratios in relation to hatch order to mediate detrimental effects of hatching asynchrony. Specifically we predicted a bias in last hatched eggs as a strategy to level competitive interactions with older siblings and to increase their likelihood of survival. We also tested Trivers-Willard's hypothesis using rainfall as a proxy for maternal condition. With no evidence of a statistically significant difference in the variance of reproductive success in Smooth-billed Anis (Robertson 2016), we did not expect a sex ratio bias across broods in relation to rainfall (maternal condition).

3.3 Methods

Field Location

The study was conducted at the Cabo Rojo National Wildlife Refuge (hereon CRNWR) located in southwestern Puerto Rico (17°59'N, 67°10'W). The CRNWR is characterized as subtropical dry scrubland in secondary succession, composed of open and disturbed areas (Schmaltz et al. 2008a, b). The CRNWR experiences a rainy season during the months of August to December that coincides with the main breeding season of Smooth-billed Anis. Reproductive data used in this study were collected during the breeding season in the years of 1998-2014 with the exception of the year 2010 when the field site was not visited.

Nestling Sampling, Hatch Order Determination and Categorization

Nests were located by watching for nest building behavior and by searching potential nesting trees. Nests that were found during nest-building or nests with eggs were visited every one to three days until the onset of hatching. Nests were then visited daily during the hatching period. Smooth-billed Ani eggs are covered in white vaterite that wears off as eggs are moved around in the nest, revealing a blue-pigmented shell. Laying sequence of eggs that were already laid upon nest discovery was determined by the degree of wearing of the vaterite and overall colouration of shells (Riehl 2010b). Eggs were marked with a non-toxic marker to indicate order in the laying sequence. Any additional eggs added to clutches were marked as found during daily nest checks. Hatching order was determined by daily visits to nests and checking for chicks from the onset of hatching (2 days prior to the estimated first hatching) until the last chick hatched. When multiple chicks were found on the same day, hatching order was determined by relative differences in size and/or feather development. Following hatching, chicks were sampled for blood (approximately 20-100 μ L) by femoral veinpuncture and

collection in capillary tubes. Blood samples were stored in approximately 1.0 mL of Queen's Lysis Buffer (Seutin et al. 1991) until DNA was extracted. We used a system of clipped toenails at hatching to distinguish chicks in each nest until they were large enough to provide a unique band combination. We categorized hatching orders as "first", "middle" and "last" with all eggs hatching between the first and last chicks classified as "middle".

Molecular Sexing

DNA was extracted from blood samples via a standard phenol–chloroform–isoamyl alcohol (25:24:1) protocol or by salt extraction (Sambrook et al. 1989). Stock DNA samples were then diluted to create working solutions in preparation for molecular sexing via polymerase chain reaction (PCR) of intron 16 on the CHD (Chromosomal Helicase DNA) gene following conditions outlined in Griffiths et al. (1998). PCR amplicons were then viewed on 1-3% agarose gels.

Precipitation Data Collection

All historic rainfall data used in this study were collected from three different sources. During the years 1998-2001, breeding season rainfall data was compiled from manual readings of a rain gauge at the CRNWR collected by U.S. Fish and Wildlife Service (USFWS). From 2002-2004, breeding season rainfall data (August – December) was compiled from: 1) a weather station at the CRNWR (17°59' N, 67°10' W), operated by the United States Geological Survey and Natural Resources Conservation Service (USGS-NRCS) as part of the Soil Climate Analysis Network (SCAN), 2) manual readings of a rain gauge at the CRNWR. Since only total monthly rainfall data were available for these years (i.e. 1998-2004), we estimated

daily rainfall accumulations by taking monthly summations of rainfall data during the breeding season and dividing it by the number of days in that particular month (e.g. Total Rainfall for August: 127 mm, = 127 mm / 31 days). For the years 2005-2014, we used rainfall data collected from another weather station on site the CRNWR (17°58' 23"N, 67°09' 46"W) also operated by the USFWS. Rainfall and other weather data metrics (e.g. humidity, temperature etc.) have been uploaded on a government website daily since its inception in December 2004. Daily rainfall data from this particular weather station during the breeding season of these years was not available for 37 days, largely due to the nationwide government shutdown in the year 2013. To account for daily rainfall in these circumstances of missing data, we used rainfall data from the USGS-NRCS/SCAN weather station. We do not anticipate the use of rainfall data from these different sources to affect our results given the proximity of both weather stations on the CRNWR (1.215 km). Overall in our entire rainfall dataset, no data was available for 5 days from any of the three sources. For these 5 days, we estimated daily rainfall by taking the sum of rainfall for that month and dividing it by the total number of days in that month (see example above).

Using both the estimated daily rainfall and daily rainfall data, we decided a priori to use the sum of 30 days of rainfall prior to the beginning of egg laying as our proxy for maternal condition. The beginning of egg laying for each nest was estimated by first determining the date of clutch completion for each nest. We then subtracted 14 days (the average amount of time for clutch completion; Erritzoe et al. 2012) from this date of clutch completion to obtain an estimated date on which egg laying commenced. The sum of 30 days of rainfall prior to

this date was then used as our proxy for maternal condition. Using pre-breeding rainfall as a proxy for female condition has also been documented elsewhere in the literature (e.g. Rubenstein 2007). For our study, this particular cutoff of 30 days of pre-breeding rainfall was based on a former study on Smooth-billed Anis that observed egg-laying behaviour across breeding seasons. In this study, first signs of egg laying were observed 2 weeks after breeding commenced, with several more nests initiating egg laying at 4 weeks (Davis 1940). These observations suggest that our chosen time frame of 30 days of rainfall prior to the beginning of egg laying should capture a measure of condition prior to laying in nests during which females acquire sufficient amounts food to make reproductive decisions related to sex ratio allocation. In addition, we also validated this assumption by showing that 30 days of lagged rainfall was significantly predictive of maternal condition (see statistical Appendix B, Table B.1, Figure B.3). The timing of reproduction of Smooth-billed Anis is known to be highly contingent on rainfall (Loffin 1983; Blanchard 2000; Quinn and Startek-Foote 2000; JSQ, JSH, JKR pers. obs.) which is likely due to the increase in insect abundance commonly observed in dry tropical scrubland habitats (Quinn and Startek-Foote 2000, Strong and Sherry 2000, Dugger et al. 2004, JSH, JKR pers. obs.). Given that Smooth-billed Anis are primarily insectivorous (Quinn and Startek-Foote 2000), it is expected that rainfall is a valid proxy for maternal condition.

Statistical Analyses

Analyses were conducted in R version 3.1.3 (R Core Team 2016), using the lme4 (Bates et al. 2014), prcomp (R Core Team 2016), and car (Fox and Weisberg 2011) packages.

We used a generalized linear mixed model (GLMM) to investigate sex ratio deviations from parity for each specific hatching order category. Here, the response variable was sex, coded as a binary variable (F = Female, M = Male) and hatching order was the sole fixed effect. Year and nest were included as random intercepts. Nest was included as a random intercept to account for statistical non-independence of chicks that hatched from the same nest and year was included as a random intercept to account for random variation between years.

To verify the influence of precipitation (sum of rainfall 30 days prior to date of capture) on body condition, we first assigned all adults a value of the sum of rainfall accumulated over 30 days preceding their date of capture. We then generated a condition index using a principal component analysis (PCA) of morphometric variables representative of body size for all adults with measurement data ($n = 305$). For the PCA we loaded measurements of wing chord (mm), head to tip (mm), exposed culmen (mm), and maximum bill depth (mm). We then selected the component that explained the greatest amount of variance and assigned each individual a unique body score (Body size PC1). All components were centered and scaled (0-1) to aid in visual interpretation.

Taking these body scores, we then performed an ordinary least squares regression against body mass. Body mass residuals were extracted from the model output to be used as a measure of individual condition (Jakob et al. 1996, Hayes and Shonkwiler 2001, Ardia 2005, Schulte-Hostedde et al. 2005). All residuals were scaled and centred as described above. We then used a linear mixed-effects model to test a correlation between condition (body mass residuals) and precipitation (sum of rainfall 30 days prior to date of capture). In this model the residuals

were used as the response variable and precipitation as the sole fixed effect. Year and territory ID were used as random intercepts to account for repeated sampling of territories across years and variation in rainfall patterns over the years.

To investigate the influence of precipitation (defined as the sum of 30 days of precipitation prior to the beginning of egg laying) on sex ratios in nests we used a GLMM. The response variable was the observed proportion of males (male offspring per nest divided by the total number of offspring per nest) and precipitation (defined as the sum of precipitation across 30 days prior to the beginning of egg laying) as the sole fixed effect. Nest was included as a random intercept to account for repeated sampling of territories across the years even though territories shifted in size and location somewhat between years. Year was also included as a random intercept. Since the number of chicks sexed was <5 for some nests, we fitted models with the Laplace approximation, as recommended by Bolker et al. (2008). We checked the assumptions of the model by looking at residuals versus fitted, quantile-quantile and scale location plots. We also looked at the ratio of residual deviance to residual degrees of freedom to check for over dispersion.

3.4 Results

Last hatched chicks were significantly more likely to be male ($Z = 2.17$, $P = 0.03$, Table 3.1; Figure 3.1). First hatched chicks trended towards a male-bias ($Z = 1.34$, $P = 0.18$) while middle-hatched eggs trended towards a female-bias ($Z = -0.39$, $P = 0.70$), but both results were statistically non-significant (Table 3.1, Figure 3.1).

The first principal component for the body size PCA explained 63.4% of the variance for measures of wing chord, head to tip, exposed culmen, and maximum bill depth (Appendix B, Figure B1-B2). Precipitation significantly predicted ordinary least squares residuals ($t = 2.99$, $P = 0.01$, Appendix B, Table B.1; Figure B.3) indicating 30 days of rainfall prior to date of capture to be a predictor of maternal condition.

There was no significant influence of precipitation (defined as the sum of 30 days of rainfall prior to the beginning of egg laying) on brood sex ratios ($Z = -0.220$, $P = 0.826$, Table 3.2; Figure 3.2).

3.5 Discussion

Our results demonstrate a significant relationship between sex and hatching order in Smooth-billed Anis, specifically in the last hatched eggs as we predicted. The possibility that males grow faster than females could drive a male bias in the last hatched ani chicks as this might offset the disadvantage of hatching late. This sex ratio bias would be an effective strategy if faster growth rates allow last hatched chicks to cope with initial size asymmetry and intense competition amongst nest mates. However, preliminary analyses showed no significant difference in growth rates between sexes (see Supplementary Table A.1, Figure A.1), suggesting that such may be unlikely for Smooth-billed Anis. While differences in growth rates between sexes have been observed (Velando, Graves, Freire 2000, Weimerskirch, Barbraud, Lys 2000, Martins 2003), last hatched chicks often suffer reduced growth rates in comparison to other nest mates (reviewed in Krebs 1999, but see Tilgar

and Mand 2006, Wecgrzyn 2012). In addition, other studies have also discovered no significant difference in growth rates between sexes (Schadt and Bird 1993, Goymann 2016). Further study is recommended given that measurements of chicks used were not consistently collected daily throughout the nestling period with the intention of conducting a formal growth analysis. Sample size was also limited as not all chick morphometrics were measured daily, nor for the same duration of time. This is likely to limit our analysis of differences in growth rate. Future research with more stringent, daily, data collection will be required to determine differences in growth rates between sexes.

Although we did not observe a significant difference in growth rates, this does not exclude the possibility of differences in embryonic developmental rate between sexes (Blanco et al. 2003, Cook and Monaghan 2004). Temporal patterns of hatching are crucial in determining the competitive nature of the nest owing to initial size differences that can persist throughout the nestling period (Cook and Monaghan 2004), arguably more so in species where sibling competition and hatching asynchrony is pronounced. Differences in embryonic development may therefore help to alleviate competitive disadvantage and chances of survival by reducing hatching asynchrony. Chicks that develop faster in ova may therefore have an opportunity to catch up with older nestmates. Currently there is some evidence in other species that last laid eggs undergo accelerated development to hatching independent of incubation regime, resulting in reduced hatching asynchrony (Davies and Cooke 1983, Chickens [*Gallus domesticus*]; Vince 1964; Bobwhite quail [*Colinus virginianus*] Japanese quail, [*Coturnix japonica*]). If embryonic development of male Smooth-billed Anis is faster than females, selection may favour last laid

offspring to be males to reduce hatching asynchrony and corresponding detrimental effects of hatching last. Exploration of growth during the embryonic stages will provide more resolution for differences in developmental rates.

Sexual size dimorphism reported in adult Smooth-billed Anis may also yield insight into our reported patterns of sex-biased hatching order. Robertson (2016) reported both a larger body and bill size in adult males, when compared to adult females. However, there is currently no evidence to suggest that similar patterns of sexual size dimorphism are present in Smooth-billed Anis nestlings at hatching (Supplementary Table A.2, Figure A.2). Other sex ratio studies of sexually dimorphic species have reported that the larger sex tends to hatch last/later in the sequence (Bednarz and Hayden 1991, Legge et al. 2001). Such a strategy has been suggested to be beneficial in preventing maladaptive brood reduction as the larger sex is more capable of competing with older siblings for food (Bednarz and Hayden 1991, Legge et al. 2001). Given the absence of sexual size dimorphism at hatching, such a tactic appears to be unlikely in Smooth-billed Anis and suggests that sexual size dimorphism may develop post-fledging.

While the proximate mechanism for this sex ratio bias in last hatched chicks remains unknown, one potential mechanism may be linked to patterns of maternal deposition of yolk androgens across the laying order. Yolk androgens have been reported to bias the fated offspring sex by influencing meiotic segregation of sex chromosomes during germ cell genesis (Meiosis I) (Pike and Petrie 2003, Rutkowska and Badyaeva 2008, Navara 2013). Specifically, greater concentrations of androgen deposition have been suggested to influence developing oocytes to retain the Z chromosome that then proceeds to become ova (Navara 2013). In

contrast, deposition of lower concentrations of yolk androgens result in the alternative W-Chromosome being retained in oocytes (Navara 2013). Such patterns of steroid deposition across laying order may therefore reflect sex ratio bias in respect to hatching order (Pike and Petrie 2003, Gil 2003, Love et al. 2005, Alonso-Alvarez 2006, Navara 2013).

A previous study on Smooth-billed Anis provided evidence for significantly higher concentrations of testosterone deposited in later laid eggs in comparison to early laid eggs (Schmaltz et al. 2008a). Schmaltz et al. (2008a) provided this pattern of maternal T deposition as support for the hatching asynchrony hypothesis as this strategy could increase the survival of later-hatched chicks by increasing competitive ability for resource acquisition (i.e. chick begging behaviour) (Schmaltz 2008, Schmaltz et al. 2008a). Alternatively, this may not function solely to alter chick behaviour, but could potentially bias sex ratios. With later laid eggs harbouring greater amounts of testosterone than early laid eggs (Schmaltz et al. 2008a), the observed sex ratio bias of last hatched chicks here may be a result of higher concentrations of androgen deposition favouring Z chromosome destined oocytes (Navara 2013). Other studies have also shown testosterone deposition in yolk to correlate with male biased sex ratios (Burley 1986, Gil et al. 1999, Müller et al. 2002) suggesting further investigation of the influence of yolk androgens is promising.

Our analyses did not detect a significant influence of the sum of precipitation 30 days prior to egg laying (maternal condition) on sex ratios. This result appears to be consistent with current estimates of reproductive variance that report no

significant difference between sexes (Robertson 2016). Thus it appears that maternal condition does not influence sex ratios in this species. It is, however, worth noting, that an accurate assessment of reproductive variance remains difficult to achieve in this species for a variety of reasons. First, current measures of reproductive variance measures for Smooth-billed Anis may be imprecise as ovicide can affect reproductive success independently of adult sex, increasing variance in both male and female reproductive success. In addition, a meta-analysis on cooperative breeders showed that numbers of non-breeding alloparents can increase variance in reproductive success such that females acquire greater reproductive variance than males (Hauber and Lacey 2005). Measures of reproductive variance were also not representative of lifetime reproductive success (Robertson 2016), which may hinder accurate inference of differences in reproductive variance between male and female adult Smooth-billed Anis. All these limitations may obfuscate how sex ratio bias might relate to maternal condition.

Given the dearth of studies that have found sex ratio biases in last hatched offspring (Woxvold and McGrath 2008), our sex ratio bias in last hatched chicks result could be representative of a type I statistical error. The majority of studies that have found significant sex ratio biases in relation to hatch order observe this deviation from parity in first hatched chicks (Bortolotti 1986, Bednarz and Hayden 1991, Leroux and Bretagnolle 1996, Emlen 1997, Arnold, Griffith and Goldizen 2001, Blanco et al. 2002, Blanco et al. 2003) and biases in last hatched chicks remain rare and difficult to interpret (Woxvold and McGrath 2008). However, if this hatching order effect is robust, this study demonstrates that sex ratio biases in last hatched offspring may warrant further investigation.

In conclusion, we show statistical support for hatching order effects on sex ratios of Smooth-billed Anis whereby last hatched chicks are statistically significantly more likely to be male. However, we did not observe a statistically significant relationship between rainfall (sum of rainfall 30 days prior to the beginning of egg laying), our proxy for maternal condition, and brood sex ratios. Future studies of offspring behaviour (e.g. chick begging) as well as pre- and post-natal growth may help examine if a male bias in last hatched chicks provides an adaptive benefit in mitigating the negative effects of hatching asynchrony.

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3.7 Figures and Tables

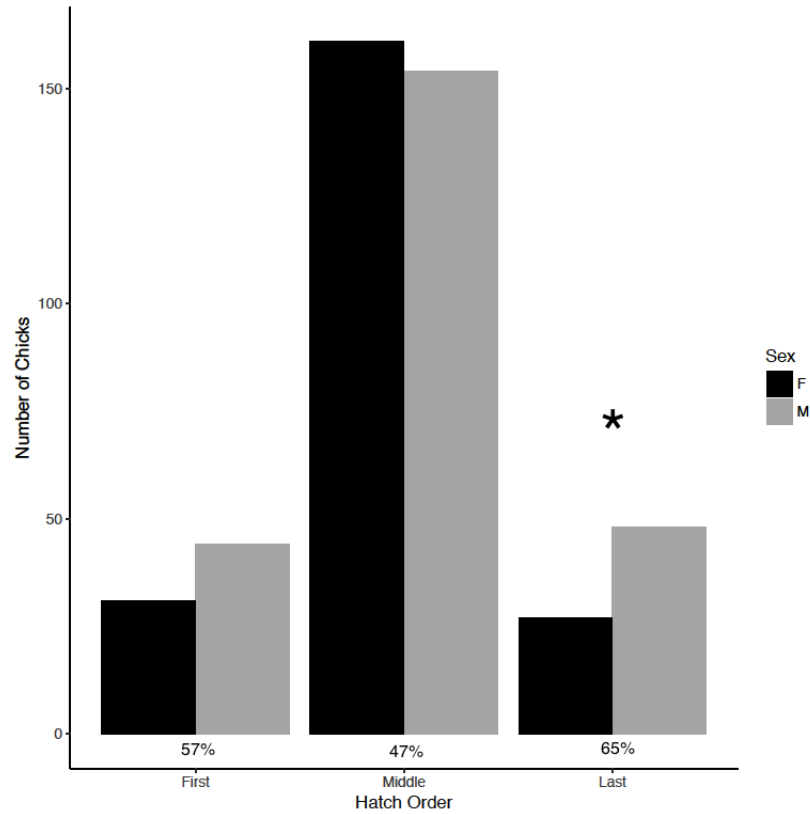


FIGURE 3.1: Frequency histogram showing the number of male and female chicks in each hatch category includes 465 chicks from 77 nests. Hatching order categories that differ significantly from parity are depicted with an asterisk above each set of bars. Percentage values represent proportion of males per hatch order.

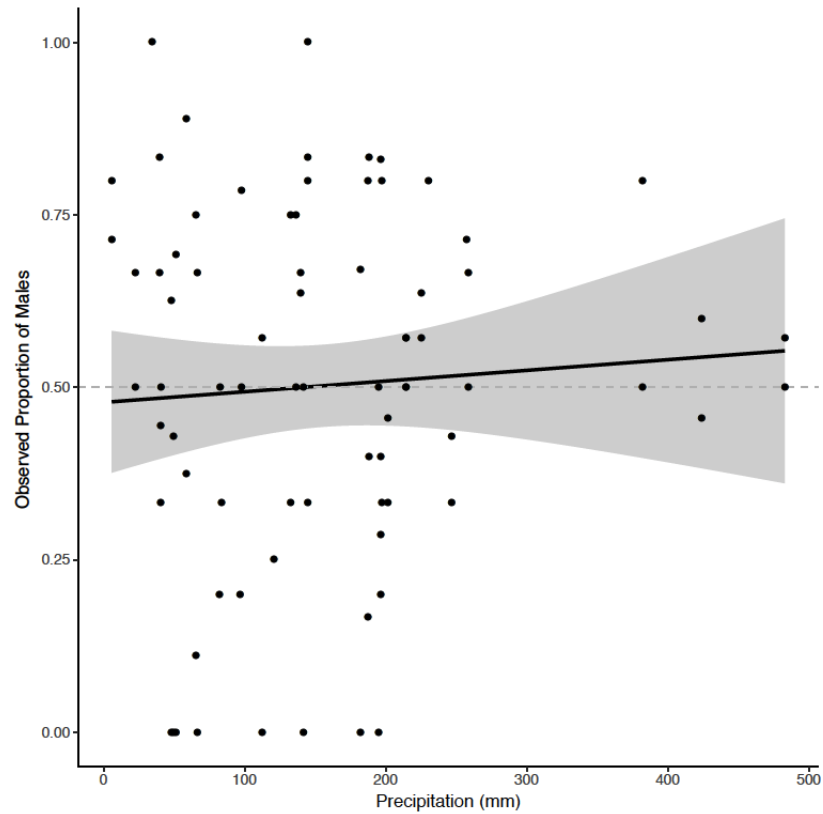


FIGURE 3.2: Relationship between the observed proportion of males per nest and precipitation (defined as the sum of 30 days of precipitation prior to the beginning of egg laying, i.e. maternal condition). Each data point represents a nest in a given year. The dotted line represents an even 50:50 sex ratio. $n = 77$ nests across $n = 17$ years. Shaded area represents 95% confidence intervals of regression line.

TABLE 3.1: Generalized linear mixed model with logit link functions examining if hatching order influences sex. Sex was coded as a binary response variable. Nest ID and year were included as random intercepts. 465 chicks from 77 nests.

Fixed effect	Estimate	95% CI	<i>p</i> -value
First Hatched	0.389	-0.180, 0.958	0.180
Second Hatched	-0.0805	-0.487, 0.327	0.698
Last Hatched	0.641	0.0617, 1.22	0.0301

TABLE 3.2: Generalized linear mixed-effects model examining if precipitation (defined as total precipitation 30 days prior to the beginning of egg laying) influences the observed proportion of male chicks in nests. The observed proportion of males was a continuous response variable and precipitation was the sole fixed effect. Year and territory were included as random intercepts. $n = 77$ nests across $n = 17$ years.

Fixed effect	Estimate	95% CI	<i>p</i> -value
Intercept	0.139	-0.141, 0.419	0.330
Precipitation	-0.0293	-0.237, 0.189	0.826

Appendix A

Chapter 2 Supplement

TABLE A.1: Number of individuals retained in the social groups over ± 3 consecutive years. Numbers in each Y-i column represent the total number of banded individuals from each social group observed in this study that retained in the same social group/territory in preceding years. Y-1, represents the preceding year from when sentinel observations were conducted, while Y+1 represents the subsequent year from when sentinel observations were conducted. Y-2 represents two years prior to the focal year(s) etc.

Social Group	Year Sentinel Observations Conducted (Y)	Y-1	Y-2	Y-3	Y+1	Y+2	Y+3
Gully	2015	0	0	0	NA	NA	NA
Finka	2015	1	0	0	NA	NA	NA
EastT	2015	0	0	0	NA	NA	NA
4way	2015	1	0	0	NA	NA	NA
Nfence	2015	0	0	0	NA	NA	NA
NEast	2002	0	0	0	0	0	0
USFlag	2002	1	0	0	1	0	0
Nhillside	2002	0	0	0	0	0	0
Gully02	2002	3	1	0	1	0	0
Home	2002	4	2	1	2	2	0

Appendix B

Chapter 3 Supplement

B.1 Supplementary Figures and Tables

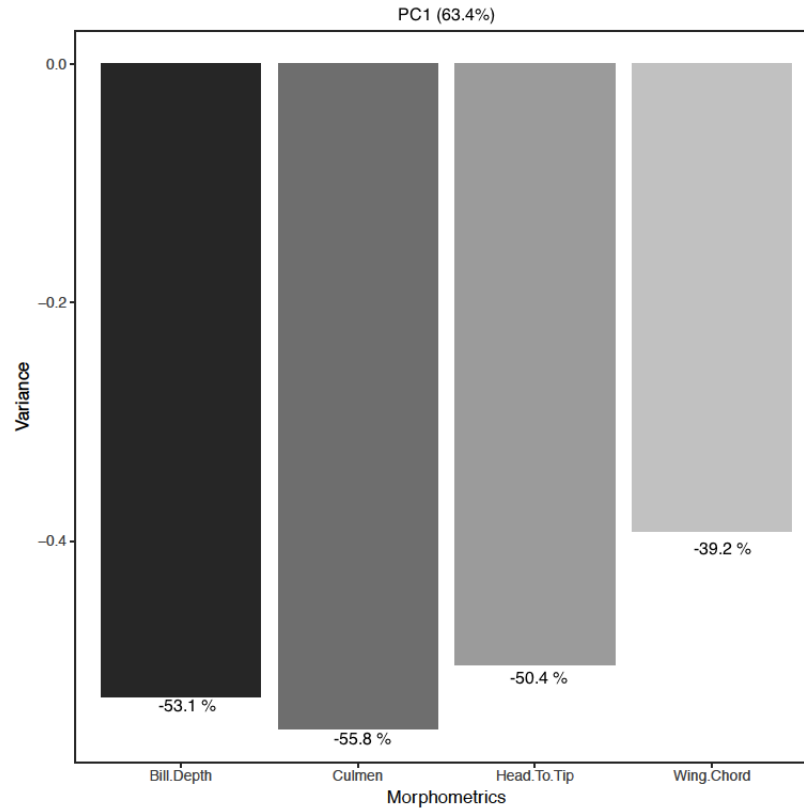


FIGURE B.1: Morphometric variables used for a principal component analysis for adult Smooth-billed Ani body size. Values below bars represent distribution of the variance for the first principal component (PC1) for each morphometric variable. PC1 explained 63.4% of the variance and was used to develop a condition index for each individual following an extraction of residuals from a regression with body mass.

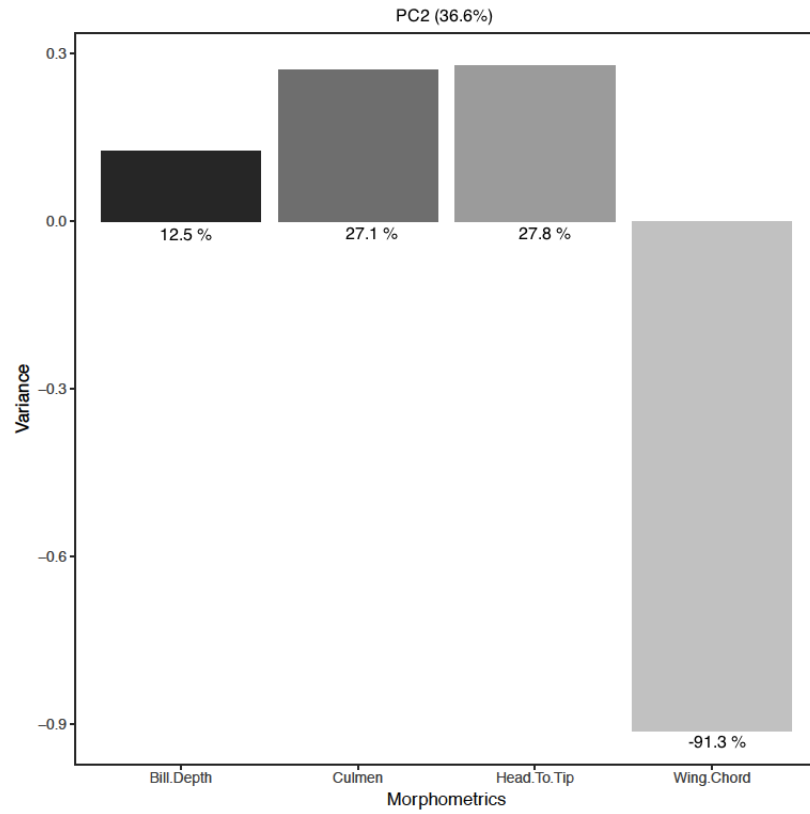


FIGURE B.2: Morphometric variables used for a principal component analysis for adult Smooth-billed Ani body size. Values below bars represent distribution of the variance for the second principal component (PC2) for each morphometric variable. PC2 explained 36.6% of the variance.

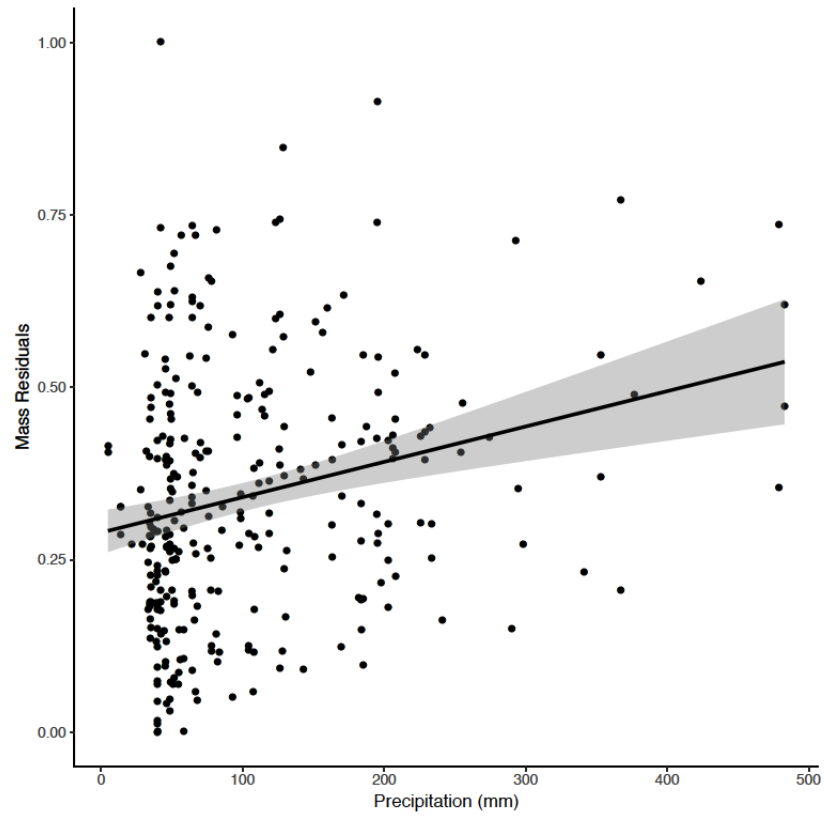


FIGURE B.3: Scatterplot of mass residuals (condition) as a function of precipitation (defined as the cumulative sum of rainfall one month prior to date of capture). Sample size includes 305 adults (147 Females, 158 Males). Shaded area represents 95% confidence intervals of regression line.

TABLE B.1: Linear mixed effect model examining if precipitation (defined as one month of cumulative rainfall prior to date of capture) influences mass residuals (condition) of Smooth-billed Ani adults. Mass residuals (condition) were the response variable and precipitation as the sole fixed effect. Territory and year were included as a random intercepts. Confidence intervals generated via parametric bootstrapping. Model sample size includes 305 adults (147 Females, 158 Males)

Fixed effect	Estimate	95% CI	<i>p</i> -value
Intercept	0.303	-0.141, 0.261, 0.349	<0.01
Precipitation	0.000392	0.000117, 0.000650	0.0113

B.2 Growth Analysis

To test differences in growth rates between sexes of nestlings, we utilized a data set containing various measurements of chick morphology (i.e. exposed culmen, tarsus, mass and head to tip). For the majority of the chicks, measurements were taken every other day but not all measurements were taken over a similar length of time (min = 2 days, max = 11 days). Mass scaled linearly with all other metrics making log transformations unnecessary prior to statistical analyses. Our sample size included a total of 66 males and 67 females.

To assess differences in growth rate between sexes we looked at growth during two time frames: 1) time period between first and second measurement, 2) time period between first and last measurement. Firstly, for each time point of interest, we loaded measurements of tarsus (mm), head-to-tip (i.e. back of the skull to tip of bill; mm), tarsus (mm), exposed culmen (mm), and mass (g) into a PCA. The principal component that explained the greatest amount of variance was selected to assign each individual a unique body score. All components were scaled (0-1) and centered.

Using the assigned body scores for each chick at each time point, we then calculated the rate of growth (i.e. slope) between the two time frames aforementioned. We did this by taking the difference between body scores (i.e. for chick growth rate) divided by the number of days elapsed (e.g. Body score at day 5 – Body score at Day 1 / 4 days). Each chick was then assigned a growth rate for the two timeframes.

Using linear mixed effects models (LMM), we tested for a difference in growth rates between sexes. We fitted linear models as our data set only provides resolution for the linear phase of growth (Max last day of measurement = 11 days). This phase of linear growth varies according to species (Rosivall et al. 2010, Jennings et al. 2016) and has been observed to fall between 4 to 11 days in other cuckoo species (Geltsch et al. 2012). In the first model, growth rate was the response variable. Year and territory were included as random intercepts for both models. An interaction between sex and hatch order was the sole fixed effect in the initial models to test for differences in growth rate in relation to hatch order. In the initial model, the interaction was non-significant and removed. The final models includes sex and hatch order as fixed effects.

To examine initial size differences that may exist at hatching between sexes, we also used another LMM. In this model, body scores of chicks at hatching (day 1) were the response variable while Sex and Hatching Order were fixed effects. Year and Territory were included as random intercepts. Likewise, the interaction between sex and hatch order was included in the initial model but removed due to non-significance. The final model includes sex and hatch order as fixed effects.

B.2.1 Results

No significant differences in growth between sexes were observed across both time frames (Table A.1, Figure A.1). No significant difference in size at hatching was observed (Table A.2, Figure A.2).

B.2.2 Literature Cited

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155-163.

B.2.3 Figures and Tables

TABLE B.2: Linear mixed effect models examining if sex influences growth rates of Smooth-billed Ani chicks over two time frames – between 1st and 2nd measurements and between 1st and last measurements. Growth rates were the response variable while sex and hatch order was fixed effects. The effect of sex is reported for males relative to females. Territory and year were included as a random intercepts. Confidence intervals generated via parametric bootstrapping. Model sample size includes 133 chicks (67 Females, 66 Males).

Fixed Effect	Estimate	95% CI	<i>p</i> - value
<i>Model 1- Growth rate between 1st and 2nd Measurement</i>			
Intercept	0.0990	0.0770, 0.121	<0.001
Sex	0.000790	-0.0131, 0.0141	0.909
Hatch Order	0.000357	-0.00503, 0.00568	0.890
<i>Model 2: Growth rate between 1st and Last Measurement</i>			
Intercept	0.0991	0.0720, 0.121	<0.001
Sex	0.00227	-0.00995, 0.0131	0.721
Hatch Order	- 0.000154	-0.00481, 0.00506	0.949

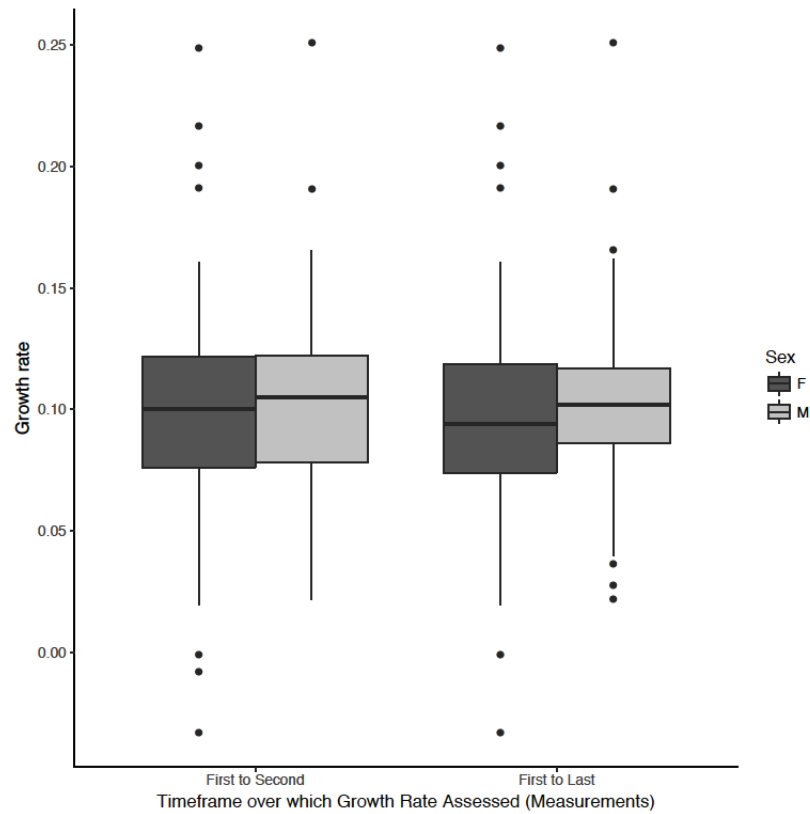


FIGURE B.4: Growth rates of male and female Smooth-billed Ani chicks (F = 67 Females, M = 66 Males). Growth rate was assessed over two time frames – 1) from the first measurements of a chick to the second measurements, 2) from the first measurements of a chick to the last measurement. Medians are represented by black lines through boxes. Whiskers represent ± 1.5 multiplied by the interquartile range (the distance between the first and third quartile).

TABLE B.3: Linear mixed effect models examining size differences between sexes at hatching. Confidence intervals generated via parametric bootstrapping. Model sample size includes 135 chicks (67 Females, 68 Males).

Fixed effect	Estimate	95% CI	<i>p</i> -value
<i>Model 2</i>			
Intercept	0.336	0.234, 0.129	<0.01
Sex	0.0166	-0.0343, 0.0720	0.545
Hatch Order	-0.00235	-0.0173, 0.0135	0.77

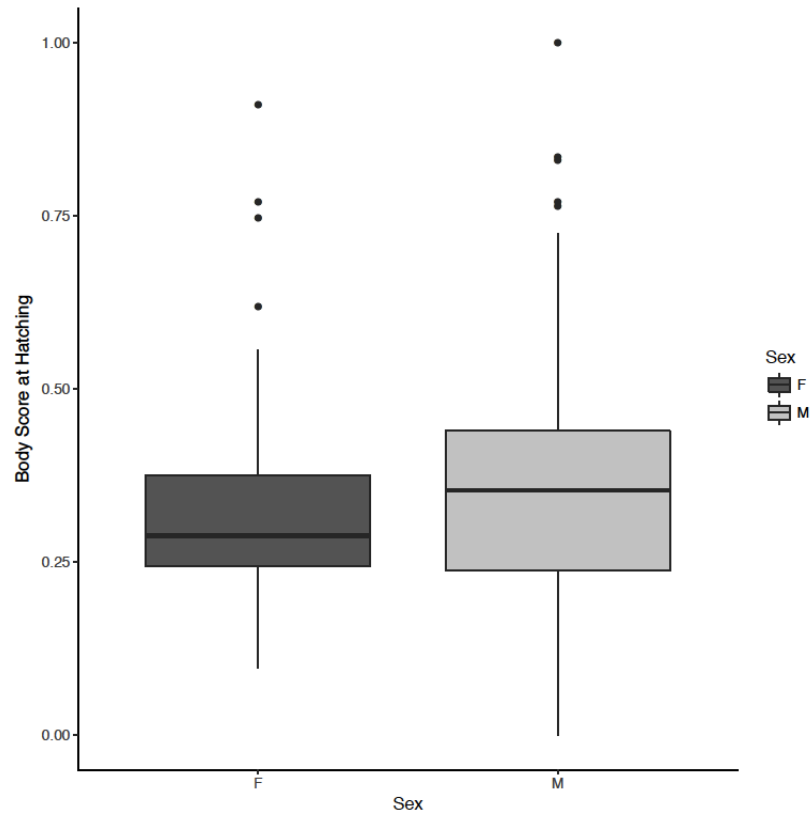


FIGURE B.5: Body size of male ($n = 68$) and female ($n = 67$) chicks at hatching based on body scores created using a PCA. Medians are represented by black lines through boxes. Whiskers represent ± 1.5 multiplied by the interquartile range (the distance between the first and third quartile).