

Seasonal Modulation of Testosterone during Breeding of the Rufous-Collared Sparrow (*Zonotrichia capensis australis*) in Southern Patagonia

Elizabeth A. Addis^{1,*}

Aaron D. Clark²

Rodrigo A. Vasquez³

John C. Wingfield⁴

¹Department of Biology, Gonzaga University, Spokane, Washington 99258; ²Department of Biology, University of Washington, Seattle, Washington 98195; ³Instituto de Ecología y Biodiversidad, Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Santiago 7800024, Chile; ⁴Department of Neurobiology, Physiology, and Behavior, University of California, Davis, California 95616

Accepted 7/30/2013; Electronically Published 10/30/2013

ABSTRACT

The roles of testosterone (T) during reproduction are often complex and vary among and within vertebrate taxa and locations, making general hypotheses relating T to breeding behavior and success difficult to integrate. In birds, T is thought to influence degrees of territoriality and associated aggression in males to maximize breeding success. Importantly, most work supporting these ideas has been conducted in the Northern Hemisphere. However, accumulating work on tropical species has shown divergent patterns of T in association with breeding behavior. The compilation of work from northern temperate and tropical species suggests that the function of T in relation to breeding behavior varies across latitude and environmental conditions. We investigate the patterns of T in relation to breeding behavior in a subspecies of the rufous-collared sparrow *Zonotrichia capensis australis* breeding at high latitude in the Southern Hemisphere (55°S). We then compare the T profiles and breeding behaviors of male *Z. c. australis* to conspecifics breeding in the tropics and congeners in North America to test the hypothesis that environments with breeding seasons of similar lengths will drive similar patterns of T in relation to breeding behavior. We found that *Z. c. australis* have high levels of T during the early-breeding periods when territories are being established and low levels of T during the parental phase of

breeding, similar to temperate and Arctic birds in the Northern Hemisphere but unlike tropical *Zonotrichia capensis costaricensis*. In contrast, we found that *Z. c. australis* also exhibit similar aggressive behaviors in early breeding and midbreeding, unlike many birds in the Northern Hemisphere.

Introduction

In vertebrates, testosterone (T) is a key hormone in male reproduction. T is necessary for sperm production and sexual behavior (Brown and Follett 1977; Balthazart 1983; Wingfield and Farner 1993; Wingfield et al. 2000; Hirschenhauser and Oliveira 2006). However, the exact role that T plays in reproduction varies not only across genera but also across environmental and breeding conditions (Hirschenhauser and Oliveira 2006; Demas et al. 2007). In birds, the varying roles of T in response to social interactions have been extensively investigated (for sample reviews, see Goymann et al. 2007; Goymann 2009). High levels of T can promote aggressive interactions between males during reproduction over territory disputes (Balthazart 1983; Wingfield et al. 1990) and the presence of receptive females (Moore 1983). However, elevated titers of T can indirectly increase risk of injury and death (Wingfield et al. 2001; Hau 2007), can suppress the immune system (Casto et al. 2001), and, in some species, can inhibit paternal behavior (Wingfield et al. 1990; for review, see Lynn 2008). As a result, having elevated levels of T incurs trade-offs between reproductive success and survival (Hau 2007).

One way that species have adapted to accommodate costs and benefits of T is to modulate circulating levels in response to environmental and social cues (Wingfield et al. 1990; Hau 2007). Theoretically, T levels and aggression are elevated in males during the early-breeding season, when males are establishing territories and recruiting mates (Wingfield et al. 1990). However, because high levels of T can be inhibitory to paternal care, T levels decrease during the parental phase of breeding (Wingfield and Farner 1978a, 1978b, 1993; Pinxten et al. 2007; Sasvári et al. 2009). Support of such modulation of T, known as seasonal modulation, is mixed but comes primarily from studies in the Northern Hemisphere (Wingfield and Wada 1989; Wingfield and Hahn 1994; Hunt et al. 1995; Romero et al. 1998; Lynn et al. 2002, 2005; Meddle et al. 2002; McGlothlin et al. 2008).

In many taxa that exhibit nonobligatory biparental care, such

* Corresponding author; e-mail: addis@gonzaga.edu.

Table 1: Life-history traits of subspecies of *Zonotrichia leucophrys* and *Zonotrichia capensis*

<i>Zonotrichia</i> species and subspecies	Breeding season length (mo)	Breeding altitude	Social modulation of T			References
			Observed?	STI	Baseline	
<i>Z. l. gambelli</i>	~2	Low	No	.5	.4	Wingfield and Farner 1978 <i>b</i> ; Chilton et al. 1995; Meddle et al. 2002
<i>Z. l. pugetensis</i>	~5	Low	Yes	.75	.4	Mewaldt et al. 1968; Wingfield and Hahn 1994; Chilton et al. 1995
<i>Z. l. nuttalli</i>	~5	Low	Yes	3.89	1.14	Mewaldt and King 1977; Chilton et al. 1995; E. A. Addis and J. C. Wingfield, unpublished data
<i>Z. c. costaricensis</i> Costa Rica ^a Ecuador	≥5	High	No	3.0–3 4.8	3.6–.4 3.9	Busch et al. 2009; Addis et al. 2010 Miller 1959; Miller and Miller 1968; Moore et al. 2004, 2006
<i>Z. c. chilensis</i> : Lowland	~5	Low	No	3.5	3.8	P. Sabat and R. Vasquez, personal communication; Addis et al. 2011
Highland	~3	High	No	1.9	1.8	P. Sabat and R. Vasquez, personal communication; Addis et al. 2011
<i>Z. c. australis</i>	~3	Low	No	1.1	1.2	This article

Note. All concentrations are given in nanograms per milliliter. All values listed are means. Testosterone (T) levels were measured during midbreeding unless otherwise noted. STI = simulated territorial intrusion.

^aStage of breeding not known.

as Puget Sound white-crowned sparrows *Zonotrichia leucophrys pugetensis* (Wingfield and Hahn 1994) and song sparrows *Melospiza melodia* (Wingfield and Wada 1989), males will temporarily increase T in response to male-male interactions and general social instability during the parental phase of breeding. This regulation is known as social modulation of T and can be tested experimentally using simulated territorial intrusions (Wingfield et al. 1990; Wingfield and Hahn 1994). However, in taxa such as *Zonotrichia leucophrys gambellii* (Hunt et al. 1995; Meddle et al. 2002) and Lapland longspurs (*Calcarius lapponicus*; Hunt et al. 1999) that exhibit obligatory male parental care, males do not socially modulate T during the parental phase (but see Romero et al. 1998).

Recent studies on tropical birds, including white-bellied antbirds (*Myrmeciza longipes*; Fedy and Stutchbury 2006), spotted antbirds (*Hylophylax naevioides*; Hau and Beebe 2011), song wrens (*Cyphorhinus phaeocephalus*; Busch et al. 2008), and tropical rufous-collared sparrows (*Zonotrichia capensis costaricensis*; Moore et al. 2004; Addis et al. 2010), do not reveal the same patterns of social modulation of T during the breeding season found in Northern Hemisphere species. Neither Ecuadorian nor Costa Rican *Z. c. costaricensis* exhibit social modulation of T (Moore et al. 2004; Addis et al. 2010); Ecuadorian *Z. c. costaricensis* were investigated during early breeding and midbreeding, whereas in Costa Rica breeding is asynchronous, so birds in all breeding and nonbreeding substages were included.

However, of potential significance, tropical environments tend to be more aseasonal, and birds breeding in these environments show greater variation in length of breeding season than those in temperate and Arctic habitats. In a comprehensive, phylogenetically controlled meta-analysis, Garamszegi et al. (2008) showed that breeding season length and latitude were significant factors affecting the role of T as a mediator of breeding trade-offs.

To begin to analyze the role of environment separately from that of phylogeny, we investigated both seasonal and social modulation of T and aggression in *Z. c. australis*, the southernmost subspecies of *Z. capensis* and of the genus *Zonotrichia*. We hypothesize that the limited breeding season of high-latitude environments in the Southern Hemisphere will affect how *Z. c. australis* modulates T, both seasonally and socially. Because seasonality at southern latitudes is the mirror image of that at the north, we compare modulation of T and aggression across the breeding season and in response to social stimuli among related species in the Northern and Southern Hemispheres (see table 1 for life-history characteristics). These predictions are based on the patterns of T modulation exhibited by *Z. l. gambellii*, the congener to *Z. capensis* that breeds in an environment with a breeding season similar in length to that of *Z. c. australis*. Specifically, we predicted that male *Z. c. australis* would have high baseline levels of T in the beginning of the breeding season, when territories are being established and mates are being re-

cruited. Then, as males and females transition into nesting and caring for young, we predicted that this subspecies would exhibit attenuated baseline levels of T during the parental phase of breeding, similar to *Z. l. gambelii*. In response to social stimuli, we predicted that males would not increase T during early breeding or midbreeding.

Methods

Study Species

Zonotrichia capensis is distributed across more than 6,000 km in Central and South America, breeding from sea level to 4,500 m across a variety of habitats (Chapman 1940) and is thought to be ancestral to the congener *Zonotrichia leucophrys* (Zink and Blackwell 1996). Early reports suggest that *Zonotrichia capensis australis* are long-distance migrants, breeding in the region of Tierra del Fuego and wintering in Bolivia (Chapman 1940; King 1974). The breeding season of *Z. c. australis* is short, lasting roughly 3 mo (King 1974). *Zonotrichia c. australis* are socially monogamous, and both males and females provide parental care (Chapman 1940).

Sample Collection

All samples for this study were collected along the north coast of Isla Navarino, Chile (54°56'S, 67°27'W), an island located directly south of the island of Tierra del Fuego. Isla Navarino has a maritime climate with cool, wet summers and mild, wet winters. Snow can fall late in the spring, and temperatures rarely exceed 13°C in the summer or drop below 1.5°C in the winter (Carrasco et al. 2002). We captured and sampled males during early breeding, October 15–19, 2005, and midbreeding, December 7–13, 2007. Because samples for early breeding and midbreeding were collected in two separate years, we realize there could be an effect of year for which we cannot control.

We determined sex by the presence or absence of a swollen cloacal protuberance (CP) in males and a brood patch in females. We then defined breeding substage by observation and morphological characteristics. We defined early breeding as when males were establishing territories and recruiting mates and midbreeding as when males and females were feeding young. For an associated study, we caught females ($N = 23$). The specific categorization of the female breeding condition was based on a brood patch index by Mewaldt and King (1977). During early breeding, females had brood patches of two or three (defeathering and development of edema) and, during midbreeding, four (refeathering). More exact categorizations of male breeding substage were not made. All birds we captured were in breeding condition, and, therefore, we were able to determine sex by these morphological characteristics. No birds were molting in either study period. All birds were handled in accordance with the guidelines set out by the University of Washington's Institute of Animal Care and Handling Committee (protocols 2212-24 and 2212-46).

We used either potter traps or mist nets to catch birds. Potter traps were seeded with crushed corn and used to catch birds

passively for baseline T levels. Birds were never in a trap for longer than 10 min. Birds were lured into mist nets using song playback (songs used are explained below). Birds used for baseline T samples and caught via mist net were never exposed to more than 10 min of playback because concentrations remain at baseline levels through that time period (Wingfield and Hahn 1994; Moore et al. 2002; Addis et al. 2011). The baseline samples were also used for examination of seasonal modulation of T.

In 2005, we recorded songs from four *Z. c. australis* individuals on Isla Navarino to be used for playback for both baseline captures and simulated territorial intrusions. Each song was looped, so the track would be repeated every 10 s. For each playback, we randomly selected one of the four songs to use, in an effort to minimize pseudoreplication (Kroodsma et al. 2001). By using locally recorded songs, we reduced the likelihood of disparate reactions to a novel dialect. These songs were also used for all captures (simulated territorial intrusion and baseline) that involved playback.

Blood samples of 250 μ L or less were taken from each bird for hormone analysis. Blood was collected from the alar wing vein and stored on ice until the end of the day, at which time it was centrifuged to separate the red blood cells from the plasma. The red blood cells and plasma were stored separately and frozen at -20°C . Samples were transported on dry ice from Chile and then stored again at -20°C until assayed at the University of Washington and at the University of California, Davis.

Simulated Territorial Intrusions

We conducted simulated territorial intrusions (STIs) to investigate whether *Z. c. australis* socially modulate T and behavior in response to a territorial invasion by a conspecific (Wingfield and Hahn 1994). We chose birds for STIs by selecting a location where we heard birds singing. We then broadcast recorded song, and if a bird responded, we set up a furred mist net and placed a conspecific male caged decoy adjacent to it. For 10 min, we played the selected song and recorded observations of four behaviors from the focal bird: (1) number of flights across the territory, (2) number of songs, (3) closest approach to the decoy, and (4) time spent within 5 m of the decoy. We limited the STIs to 10 min in duration because studies in temperate *Z. leucophrys* (Wingfield and Hahn 1994) showed that 10 min was sufficient to trigger increases in T, and *Z. c. costaricensis* showed no difference in T levels among birds exposed to STIs that were 10, 30, or 60 min in duration (Moore et al. 2004). At the end of 10 min, we opened the mist net and lured the bird into it. Upon capture of the bird, we took a blood sample as soon as possible. No maximum limit was given to the amount of playback for birds caught after an STI, but all STI birds were exposed to a minimum of 10 min of playback (range: 10–42 min). We conducted STIs in the early-breeding (2005) and midbreeding (2007) periods to determine whether breeding substage affects the hormonal and behavioral responses to a simulated invasion. In both 2005 and 2007, we conducted eight STIs for a total of 16. In both 2005 and 2007, we used two

decoys, for a total of four. As hormonal controls for the STIs, we also caught nine birds in 2005 and seven birds in 2007 for baseline T samples using the same playback recordings as the STIs.

Hormone Assays

All blood samples were analyzed by radioimmunoassay to measure hormone concentrations. Each sample was analyzed in duplicate and followed the protocol of Wingfield et al. (1991). For the 2005 (early breeding) samples, we separated androgens 5 α -dihydrotestosterone (DHT) from T using column chromatography. We used a direct assay that binds all androgens (both T and DHT) for the 2007 samples after concluding that the patterns of DHT paralleled those of T. Therefore, note that any differences between the 2005 and 2007 samples are conservative. We ran two assays in 2005 and one in 2008. The limits of detection for all assays ranged from 0.07 to 0.11 ng/mL, depending on plasma volume (40–100 μ L). Intra-assay and interassay variation were 5.7% and 14.3%, respectively.

Statistical Analysis

All statistical analyses were performed using JMP (SAS Institute 2010). The α level of significance was set at 0.05. The T and DHT data were log transformed to meet normality assumptions. First, we compared baseline androgen levels between individuals caught with a trap and those caught with a net and playback using a Student's *t*-test. Then we regressed time to capture against androgen concentration for baseline and those exposed to the STI treatment to determine whether time of playback exposure affected androgen levels. Because it did not (see "Results"), we used an ANOVA to test the effects of breeding substage (early breeding and midbreeding) and STI treatment on T levels. As DHT levels were examined only in early breeding, we used a one-way ANOVA to compare baseline and STI means.

We compared individual behavioral measurements between early breeding and midbreeding. Because individual behavioral measurements were not normally distributed, we log transformed number of flights and number of songs and compared them using *t*-tests. Log transformation did not normalize the closest approach and time within 5-m data, so we analyzed them using nonparametric Wilcoxon tests. Finally, we regressed the behavioral measurements against log-transformed androgens to determine whether a correlation existed between the behaviors and androgen levels.

Results

Androgens

The method of capture (net with playback or trap) did not affect baseline androgen levels ($t_{32} = -0.72$, $P = 0.479$). Time to capture did not significantly affect androgen levels for baseline or STI samples, although there was a trend for an interaction between time to capture and season for the baseline

samples (baseline: $F_{3,15} = 7.89$, model $P = 0.004$, season $P = 0.01$, time to capture $P = 0.541$, season \times time to capture $P = 0.06$; STI: $F_{3,15} = 9.75$, model $P = 0.002$, season $P = 0.004$, time to capture $P = 0.99$, season \times time to capture $P = 0.35$; fig. 1A). Both baseline and STI androgen levels of males were significantly higher in the early-breeding stage than in the midbreeding stage (ANOVA, model: $F_{3,31} = 13.55$, $P < 0.0001$; season: $t_{1,31} = 2.05$, $P < 0.001$; fig. 1B). Note that early-breeding values are of T only, while midbreeding values are of T and DHT combined. Additionally, the STI experimental treatment had no effect on birds' androgen levels in either the early-breeding or midbreeding stages (ANOVA, model: $F_{3,31} = 13.55$, $P < 0.0001$; STI: $t_{1,31} = 2.05$, $P = 0.49$; fig. 1B), and there was no interaction between season and treatment (ANOVA, model: $F_{3,31} = 13.55$, $P < 0.0001$; treatment \times season $t_{1,31} = 2.05$, $P = 0.35$). In the birds' early-breeding season, STIs had no effect on DHT levels (ANOVA, model: $F_{1,15} = 0.84$, $P = 0.37$; fig. 2). Because we separated T from DHT in our assays of the early-breeding samples, the T levels we found are conservative in comparison to values we would have found from direct assays that do not separate DHT and T levels, such as those used for our midbreeding samples.

Behavior

Both early-breeding and midbreeding males showed aggressive behaviors during an STI. The individual behavioral measurements of number of songs ($t_{14} = -1.05$, $P = 0.310$), closest approach to decoy (Wilcoxon: $Z = 0.88$, $P = 0.382$), and time spent within 5 m of the decoy (Wilcoxon: $Z = -1.21$, $P = 0.227$) were not different between the early-breeding and midbreeding stages (fig. 3). However, males flew significantly more times during the STI in midbreeding than in early breeding ($t_{14} = 2.38$, $P = 0.032$; fig. 3). Androgen levels were not correlated with any of the behaviors (number of flights: $F_{1,14} = 1.274$, $P = 0.278$; number of songs: $F_{1,14} = 0.041$, $P = 0.842$; closest approach: $F_{1,14} = 2.787$, $P = 0.117$; time within 5 m: $F_{1,14} = 1.307$, $P = 0.272$).

Discussion

In this study, we found that *Zonotrichia capensis australis* exhibits high baseline levels of androgens during early breeding and low levels during midbreeding, like those of *Zonotrichia leucophrys gambelii* breeding in the Arctic of North America, supporting our hypothesis that seasonal conditions, such as breeding season length, are a factor in patterns of T expression. Furthermore, this pattern is different from that exhibited by Ecuadorian *Zonotrichia capensis costaricensis* (Moore et al. 2004), even though *Z. c. australis* is more closely related to *Z. c. costaricensis* than *Z. l. gambelii* (Zink and Blackwell 1996). We also found that in *Z. c. australis*, most aggressive behaviors measured were not different between early breeding and midbreeding, contrary to our prediction that aggression would be higher in early breeding than midbreeding, as seen in *Z. l. gambelii* (Meddle et al. 2002).

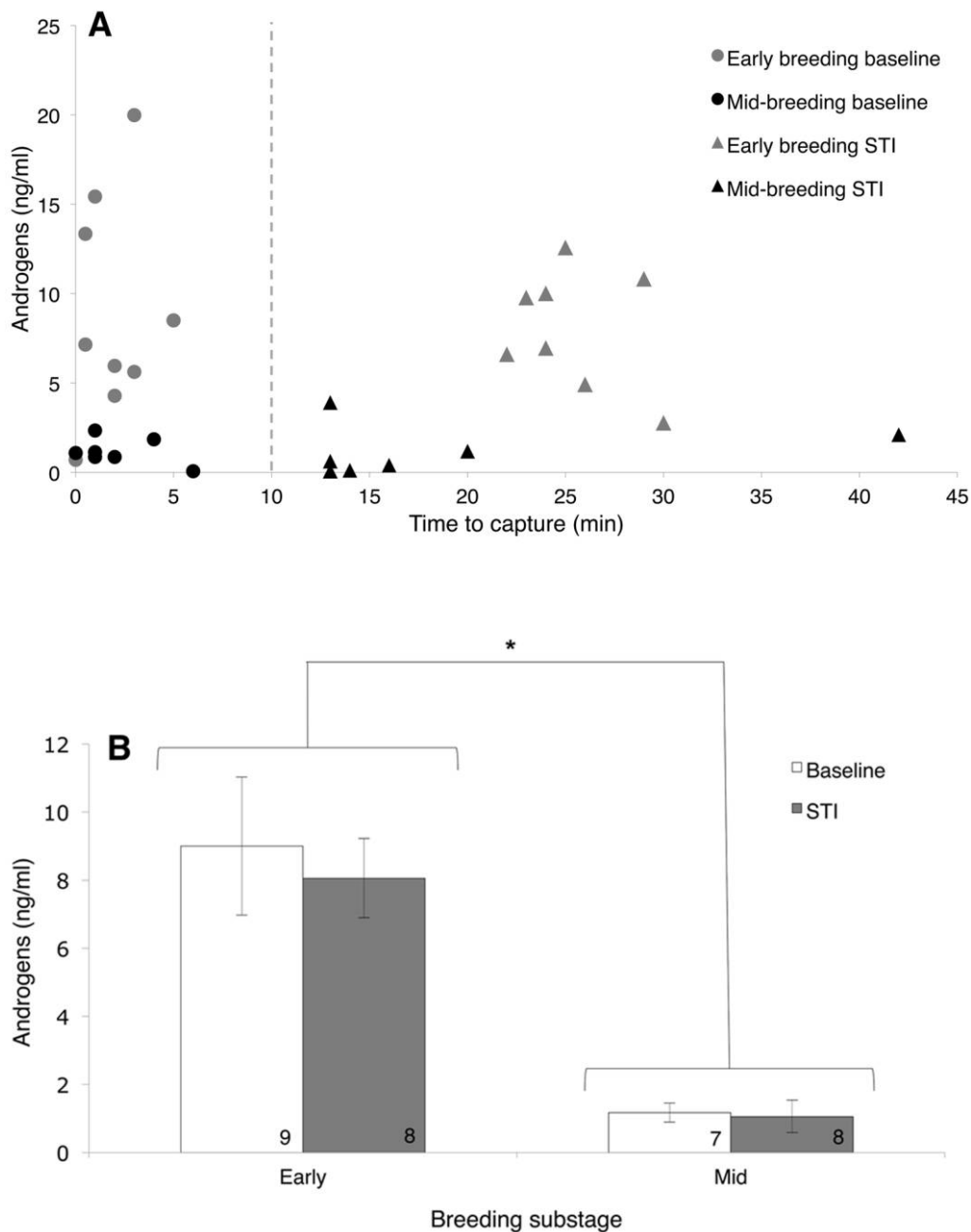


Figure 1. *A*, Duration of playback exposure did not significantly affect baseline androgen levels irrespective of treatment. The dashed gray line denotes the 10-min mark; birds caught before 10 min and without the presence of a decoy were classified as baseline samples. *B*, Baseline and post-STI androgen levels in early-breeding and midbreeding stages. STIs did not have a significant effect on testosterone levels. Androgen levels were lower in the midbreeding season than in the early-breeding season. Note that early-breeding values are of testosterone only, while midbreeding values are of total androgens. The asterisk denotes significant difference. Sample sizes are given in the lower right corner of each column. Bars are means \pm SE. STI = simulated territorial intrusion.

Zonotrichia c. australis demonstrates high levels of androgens in the early-breeding season and low levels of androgens during the midbreeding period. To note, early-breeding and midbreeding samples were collected during different years. We recognize that there could be a year effect because the effects of season and year cannot be separated. However, minimum daily tem-

peratures of the study periods in the nearby city of Ushuaia, Argentina, exhibited similar trends during the 2 yr of sampling (there are no temperature logs from Isla Navarino during this time period). In the early-breeding period, high androgen levels in males are concurrent with the establishment of territories and recruitment of mates. This period involves a great deal of

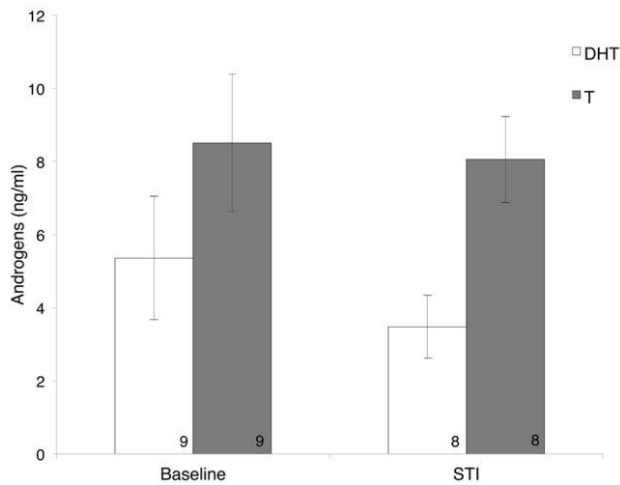


Figure 2. Baseline and post-STI T and DHT levels in the early-breeding season. Sample sizes are given in the lower right corner of each column. Bars are means \pm SE. DHT = 5 α -dihydrotestosterone; STI = simulated territorial intrusion; T = testosterone.

social instability that instigates male-male interactions over both territories and receptive females.

Because males do provide paternal care in *Z. capensis* (Miller and Miller 1968; Lynn et al. 2009), the drop in circulating T during midbreeding suggests that T may be inversely related to paternal care in this species, as in *Z. l. gambelii*. In Ecuadorian *Z. c. costaricensis*, Lynn et al. (2009) found that elevated T does decrease male feeding of offspring but not fledgling success. Unlike with the longer breeding season of Ecuador, the breeding season on Isla Navarino may be short enough that paternal care is essential for offspring survival. Young develop faster with more food; the suppressive effect of T on paternal care has been shown to retard offspring development (Silverin 1980; Hegner and Wingfield 1987; Ketterson et al. 1992; Schoech et al. 1998; Lynn et al. 2005). If male parental care is essential for offspring survival, it would be adaptive for male *Z. c. australis* to be insensitive to T during the parental phase of breeding, such as in *Z. l. gambelii* (Meddle et al. 2002).

Like other species with short breeding seasons, such as those found at high latitude in the Northern Hemisphere, *Z. c. australis* have very little time to establish a territory, find a mate, and raise young (Hunt et al. 1995, 1999; Romero et al. 1998; Meddle et al. 2002). Breeding within such populations tends to be highly synchronous (Goymann et al. 2007; Garamszegi et al. 2008). Because all males are vying for territories and females simultaneously, competition is acute, potentially leading to the extremely elevated levels of T found in high-latitude birds during early breeding (Hunt et al. 1995; Romero et al. 1998; Meddle et al. 2002). Additionally, work in dark-eyed juncos (*Junco hyemalis*) has shown that males with particularly high T levels have mates that are fertile (McGlothlin et al. 2008; Rosvall et al. 2012); the extremely high levels of androgens if the rufous-collared sparrow could partially be due to the presence of fertile females. During early breeding, most of the fe-

males we caught had brood patches of two or three. However, defining fertility based on development of brood patch alone comes with inherent error because we cannot determine when exactly the fertile period ends and the females are only incubating and not laying.

High synchronicity in a breeding population could decrease a reproductive advantage in males pursuing matings during the parental phase if there are no receptive females present. Males not only would not need to defend mates against other males during parenting but also would not be able to find receptive females with which to mate. As a result, even transient increases in T during parenting may be disadvantageous.

Using the technique of STIs allowed us to test how social (male-male) interactions affect both aggressive behavior and androgen levels, both of which we would expect to increase if males needed to defend mates and territories against rival males. We found that STIs did not trigger an increase in androgens in either the early-breeding period or the midbreeding period; therefore, we can conclude *Z. c. australis* does not socially modulate androgens in response to male-male interactions. It is important to note that we tested only androgen responses to

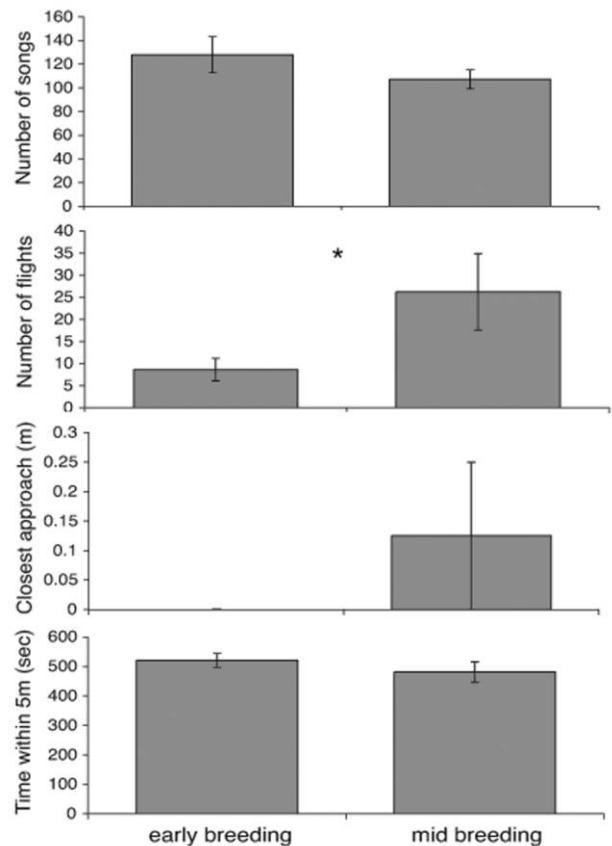


Figure 3. Behavioral responses of *Zonotrichia capensis australis* to simulated territorial intrusions during early-breeding and midbreeding stages. The asterisk denotes significant difference. Bars are means \pm SE. $N = 8$ for all behaviors in both early breeding and midbreeding.

interactions between males; we did not test whether the presence of a receptive female would stimulate an increase in androgens, as found by Moore (1983). Goymann et al. (2007) showed there are differences in male responses to interactions with males and those with receptive females.

Additionally, our results may suggest that increasing androgens during midbreeding may not be advantageous. In other words, the lack of an increase in androgens in the midbreeding period is consistent with the hypothesis that paternal care is important enough to offspring survival that even transient increases in androgens in response to male-male encounters will hamper offspring survival. These patterns are congruent with those of *Z. l. gambelii* (Hunt et al. 1995; Meddle et al. 2002), which also has a restricted breeding season and does not socially modulate T. The lack of increase in androgens during the early-breeding season, when baseline androgens are already elevated, suggests that androgen levels are high enough to facilitate aggressive interactions (for reviews, see for reviews, see Hirschenhauser et al. 2003; Goymann et al. 2007; Garamszegi et al. 2008; Goymann 2009) or that changes occur in hormone and receptor expression in the brain (Soma et al. 2003).

With the exception of the number of flights, *Z. c. australis* males exhibited similar aggressive behaviors in both early breeding and midbreeding. These results raise the possibility that singing and interactions with the male decoy are independent of control by circulating androgens since androgen levels were significantly lower in midbreeding than the early-breeding season and there was no correlation between behaviors and androgen levels. For example, localized expression of androgen receptors and/or aromatase activity could be upregulated when circulating androgen levels decline.

However, the number of flights by the focal males during the STIs increased in midbreeding. Closer examination of the data reveals that two individuals are driving the high mean in midbreeding number of flights, suggesting further studies should explore this trend. This persistence of aggressive behaviors in midbreeding has been documented in *Z. l. gambelii* (Meddle et al. 2002) and fox sparrows *Passerella iliaca*, a species also breeding at high latitude with short breeding seasons (Wacker et al. 2010). The expression of aggressive behaviors during midbreeding could be to defend the male's territory rather than his mate; *Z. capensis* defend multipurpose territories (Miller and Miller 1968). Additionally, the expression of these behaviors during midbreeding raises the question of whether the males exhibiting these behaviors reduce parental behaviors. Future studies should explore this possible trade-off of behaviors. More hormonal and behavioral data should be collected in *Z. capensis* to further explore the relationship among aggressive behaviors, androgens, and breeding substage and the function of these aggressive behaviors.

The androgen profile of *Z. c. australis* is highly comparable to that of the congener *Z. l. gambelii* (Meddle et al. 2002) and the conspecific *Z. c. chilensis* (Addis et al. 2011). However, the fluctuations of androgens across the breeding season in *Z. c. australis* are very different from those of *Z. c. costaricensis*, which breeds in the tropics. *Zonotrichia c. australis* is conspecific to

Z. c. costaricensis but only congeneric to *Z. l. gambelii* (Zink and Blackwell 1996), suggesting that relatedness is not the cause of distinctions in androgen fluctuations between the species. However, latitude and seasonal conditions, such as breeding season length, among the breeding grounds of *Z. c. australis*, *Z. c. chilensis*, and *Z. l. gambelii* are much more similar than the seasonal conditions between the breeding areas of *Z. c. australis* and *Z. c. costaricensis*, suggesting that in the case of *Z. capensis* the breeding season environment plays a larger role in patterns of T levels than relatedness. This disparate use of T between subspecies of a single species is also seen in *Z. leucophrys*, although not to the same degree. All subspecies of *Z. leucophrys* show an increase in T at the beginning of the breeding season (see table 1 for comparisons). However, during the parental phase of breeding, both *Z. l. pugetensis* and *Z. l. nuttalli* will increase T in response to STIs. *Zonotrichia l. pugetensis* (Wingfield and Hahn 1994) and *Z. l. nuttalli* (J. C. Wingfield, unpublished data) both have longer breeding seasons than *Z. l. gambelii* and typically have multiple clutches each breeding season. The observed differences in T patterns in *Z. leucophrys* and *Z. capensis* suggest that flexibility exists within a species to respond optimally to seasonal environmental conditions, such as breeding season length. These results also suggest that selective pressures of divergent breeding environments can have a significant effect on the interaction among androgens, physiology, and behavior (Lynn et al. 2005; Goymann and Landys 2011).

In conclusion, our results support the prediction that *Z. c. australis* uses variations in androgens as a mediator of breeding trade-offs by having high levels of androgens during periods of social instability and mate recruitment at the beginning of the breeding season and low levels of androgens during parental care, similar to *Z. l. gambelii*. Our results provide evidence that the seasonal modulation of T is supported outside of the temperate regions of the Northern Hemisphere, specifically within the sub-Antarctic region of the Southern Hemisphere. Additionally, the results of this study also suggest that species can exhibit flexibility in response to selective factors of environmental conditions (i.e., tropical vs. sub-Antarctic), modifying the role of androgens as a mediator of trade-offs of reproduction.

Acknowledgments

We would like to thank Steve McGhee of Omora Ethnobotanical Park and the Omora Sub-Antarctic Research Alliance for logistical support as well as help in the field. Cristobal Venegas also provided valuable field assistance. Research was supervised by the Ethics Committee of the Faculty of Sciences, Universidad de Chile, under a permit issued by the Servicio Agrícola y Ganadero (permit 5193), Chile. This work was funded by National Science Foundation grant IBN-0317141 to J.C.W. and by Fondo Nacional de Desarrollo Científico y Tecnológico-Chile grant 1090794 and the Institute of Ecology and Biodiversity grants ICM-P05-002 and PFB-23-CONICYT to R.A.V.

A special thanks to Jim Kenagy, Josh Tewksbury's lab for providing helpful comments on the manuscript, and J. S. Addis for editorial help. Reviewers' comments substantially improved this manuscript.

Literature Cited

- Addis E.A., D.S. Busch, A.D. Clark, and J.C. Wingfield. 2010. Seasonal and social modulation of testosterone in Costa Rican rufous-collared sparrows (*Zonotrichia capensis costaricensis*). *Gen Comp Endocrinol* 166:581–589.
- Addis E.A., A.D. Clark, and J.C. Wingfield. 2011. Modulation of androgens in southern hemisphere temperate breeding sparrows (*Zonotrichia capensis*): an altitudinal comparison. *Horm Behav* 60:195–201.
- Balthazart J. 1983. Hormonal correlates of behavior. *Avian Biol* 7:221–365.
- Brown N. and B. Follett. 1977. Effects of androgen on the testis of intact and hypophysectomized Japanese quail. *Gen Comp Endocrinol* 33:267–277.
- Busch D.S., E.A. Addis, A.D. Clark, and J.C. Wingfield. 2009. Disentangling the effects of environment and life-history stage on corticosterone modulation in Costa Rican rufous collared sparrows, *Zonotrichia capensis costaricensis*. *Physiol Biochem Zool* 83:87–96.
- Busch D.S., T.R. Robinson, T.P. Hahn, and J.C. Wingfield. 2008. Sex hormones in the song wren: variation with time of year, molt, gonadotropin releasing hormone, and social challenge. *Condor* 110:125–133.
- Carrasco J., G. Casassa, and A. Rivera. 2002. Meteorological and climatological aspects of the Southern Patagonia Icefield. Pp. 29–42 in G. Casassa, F. Sepulvedu, and R. Sinclair, eds. *The Patagonian Icefields*. Kluwer Academic/Plenum, New York.
- Casto J., V.J. Nolan, and E. Ketterson. 2001. Steroid hormones and immune function: experimental studies in wild and captive dark-eyed juncos (*Junco hyemalis*). *Am Nat* 157:408–420.
- Chapman F. 1940. Post-glacial history of *Zonotrichia capensis*. *Bull Am Mus Nat Hist* 77:381–439.
- Chilton G., M. Baker, C. Barrentine, and M. Cunningham. 1995. White-crowned sparrow (*Zonotrichia leucophrys*). In A. Poole and F. Gill, eds. *The birds of North America*. Vol. 183. Academy of Natural Sciences, Philadelphia.
- Demas G.E., M.A. Cooper, H.E. Albers, and K.K. Soma. 2007. Novel mechanisms underlying neuroendocrine regulation of aggression: a synthesis of rodent, avian and primate studies. Pp. 337–372 in J.D. Blaustein, ed. *Behavioral neurochemistry and neuroendocrinology*. Vol. 21. Springer, Berlin.
- Fedy B.C. and B.J.M. Stutchbury. 2006. Testosterone does not increase in response to conspecific challenges in the white-bellied antbird (*Myrmeciza longipes*), a resident tropical passerine. *Auk* 123:61–66.
- Garamszegi L., K. Hirschenhauser, V. Bokony, M. Eens, S. Hurtez-Bousses, A. Moller, R. Oliveira, and J.C. Wingfield. 2008. Latitudinal distribution, migration, and testosterone levels in birds. *Am Nat* 172:534–546.
- Goymann W. 2009. Social modulation of androgens in male birds. *Gen Comp Endocrinol* 163:149–157.
- Goymann W. and M.M. Landys. 2011. Testosterone and year-round territoriality in tropical and non-tropical songbirds. *J Avian Biol* 42:485–489.
- Goymann W., M.M. Landys, and J.C. Wingfield. 2007. Distinguishing seasonal androgen responses from male-male androgen responsiveness—revisiting the challenge hypothesis. *Horm Behav* 51:463–476.
- Hau M. 2007. Regulation of male traits by testosterone: implications for the evolution of vertebrate life histories. *BioEssays* 29:133–144.
- Hau M. and K. Beebe. 2011. Plastic endocrine regulation of year-round territorial aggression in tropical male spotted antbirds. *Gen Com Endocrinol* 172:305–313.
- Hegner R. and J.C. Wingfield. 1987. Effects of experimental manipulation of testosterone levels on parental investment and breeding success in male house sparrows. *Auk* 104:462–469.
- Hirschenhauser K. and R.F. Oliveira. 2006. Social modulation of androgens in male vertebrates: meta-analysis of the challenge hypothesis. *Anim Behav* 71:265–277.
- Hirschenhauser K., H. Winkler, and R.F. Oliveira. 2003. Comparative analysis of male androgen responsiveness to social environment in birds: the effects of mating systems and paternal incubation. *Horm Behav* 43:508–519.
- Hunt K.E., T.P. Hahn, and J.C. Wingfield. 1999. Endocrine influences on parental care during a short breeding season: testosterone and male parental care in Lapland longspurs (*Calcarius lapponicus*). *Behav Ecol Sociobiol* 45:360–369.
- Hunt K.E., J.C. Wingfield, L. Astheimer, W. Buttemer, and T.P. Hahn. 1995. Temporal patterns of territorial behavior and circulating testosterone in the Lapland longspur and other Arctic passerines. *Am Zool* 35:274–284.
- Ketterson E., V.J. Nolan, L. Wolf, and C. Ziegenfus. 1992. Testosterone and avian life histories: effects of experimentally elevated testosterone on behavior and correlates of fitness in dark-eyed junco (*Junco hyemalis*). *Am Nat* 140:980–999.
- King J. 1974. Notes on geographical variation and the annual cycle in Patagonian populations of the rufous-collared sparrow *Zonotrichia capensis*. *Ibis* 116:74–83.
- Kroodsma D., B. Byers, E. Goodale, S. Johson, and W. Liu. 2001. Pseudoreplication in a playback experiments, revisited a decade later. *Anim Behav* 61:1029–1033.
- Lynn S.E. 2008. Behavioral insensitivity to testosterone: why and how does testosterone alter parental and aggressive behavior in some avian species but not others? *Gen Comp Endocrinol* 157:233–240.
- Lynn S.E., L.S. Hayward, Z.M. Benowitz-Fredericks, and J.C. Wingfield. 2002. Behavioural insensitivity to supplementary testosterone during the parental phase in the chestnut-collared longspur, *Calcarius ornatus*. *Anim Behav* 63:795–803.
- Lynn S.E., L.E. Prince, D.M. Schook, and I.T. Moore. 2009. Supplementary testosterone inhibits parental care in a tropically breeding sparrow, *Zonotrichia capensis*. *Physiol Biochem Zool* 82:699–708.
- Lynn S.E., B. Walker, and J. Wingfield. 2005. A phylogenetically

- controlled test of hypotheses for behavioral insensitivity to testosterone in birds. *Horm Behav* 47:170–177.
- McGlothlin J.W., J.M. Jawor, T.J. Grieves, J.M. Casto, J.L. Phillips, and E.D. Ketterson. 2008. Hormones and honest signals: males with larger ornaments elevate testosterone more when challenged. *J Evol Biol* 21:39–48.
- Meddle S.L., L.M. Romero, L.B. Astheimer, W.A. Buttemer, I.T. Moore, and J.C. Wingfield. 2002. Steroid hormone interrelationships with territorial aggression in an arctic-breeding songbird, Gambel's white-crowned sparrow, *Zonotrichia leucophrys gambelii*. *Horm Behav* 42:212–221.
- Mewaldt L.R., S.S. Kibby, and M.L. Morton. 1968. Comparative biology of Pacific coastal white-crowned sparrows. *Condor* 70:14–30.
- Mewaldt L.R. and J.R. King. 1977. The annual cycle of white-crowned sparrows (*Zonotrichia leucophrys nuttalli*) in coastal California. *Condor* 79:445–455.
- Miller A.H. 1959. Reproductive cycles in an equatorial sparrow. *Proc Natl Acad Sci USA* 45:1095–1100.
- Miller A.H. and V.D. Miller. 1968. The behavioral ecology and breeding biology of the Andean sparrow, *Zonotrichia capensis*. *Caldasia* 10:83–154.
- Moore I.T., G.E. Bentley, C. Wotus, and J.C. Wingfield. 2006. Photoperiod-independent changes in immunoreactive brain gonadotropin-releasing hormone (GnRH) in a free-living, tropical bird. *Brain Behav Evol* 68:37–44.
- Moore I.T., N. Perfito, H. Wada, T.S. Sperry, and J.C. Wingfield. 2002. Latitudinal variation in plasma testosterone levels in birds of the genus *Zonotrichia*. *Gen Comp Endocrinol* 129:13–19.
- Moore I.T., H. Wada, N. Perfito, D.S. Busch, T.P. Hahn, and J.C. Wingfield. 2004. Territoriality and testosterone in an equatorial population of rufous-collared sparrows, *Zonotrichia capensis*. *Anim Behav* 67:411–420.
- Moore M. 1983. Effect of female sexual displays on the endocrine physiology and behavior of male white-crowned sparrow, *Zonotrichia leucophrys*. *J Zool (Lond)* 199:137–148.
- Pinxten R., E. De Ridder, L. Archens, V.M. Darras, and M. Eens. 2007. Plasma testosterone levels of male European starlings (*Sturnus vulgaris*) during the breeding cycle and in relation to song and parental care. *Behaviour* 144:393–410.
- Romero L., K. Soma, K. O'Reilly, R. Suydam, and J.C. Wingfield. 1998. Hormones and territorial behavior during breeding in snow buntings (*Plectrophenax nivalis*): an arctic-breeding songbird. *Horm Behav* 33:40–47.
- Rosvall K.A., D.G. Reichard, S.M. Ferguson, D.J. Whittaker, and E.D. Ketterson. 2012. Robust behavioral effects of song playback in the absence of testosterone or corticosterone release. *Horm Behav* 62:418–425.
- SAS Institute. 2010. JMP. SAS Institute, Cary, NC.
- Sasvári L., P. Péczely, and Z. Hegyi. 2009. Plasma testosterone profile of male tawny owls *Strix aluco* in relation to breeding density, breeding experience, and offspring provision. *Acta Ornithol* 44:59–68.
- Schoech S., J. Buntin, P. Sharp, E. Ketterson, and V.J. Nolan. 1998. The effects of exogenous testosterone on parental behavior, plasma prolactin, and prolactin binding sites in dark-eyed juncos. *Horm Behav* 34:1–10.
- Silverin B. 1980. Effects of long-acting testosterone treatment on free-living pied flycatchers, *Ficedula hypoleuca*, during the breeding period. *Anim Behav* 28:906–912.
- Soma K.K., B.A. Schlinger, J.C. Wingfield, and C.J. Saldanha. 2003. Brain aromatase, 5 alpha-reductase, and 5 beta-reductase change seasonally in wild male song sparrows: relationship to aggressive and sexual behavior. *J Neurobiol* 56:209–221.
- Wacker D.W., A.J. Coverdill, C.M. Bauer, and J.C. Wingfield. 2010. Male territorial aggression and androgen modulation in high latitude populations of the sooty, *Passerella iliaca sinuosa*, and red fox sparrow, *Passerella iliaca zaboria*. *J Ornithol* 151:79–86.
- Wingfield J.C. and D.S. Farner. 1978a. The annual cycle in plasma irLH and steroid hormones in feral populations of the white-crowned sparrow, *Zonotrichia leucophrys gambelii*. *Biol Reprod* 19:1046–1056.
- . 1978b. The endocrinology of a naturally breeding population of white-crowned sparrow (*Zonotrichia leucophrys pugetensis*). *Physiol Zool* 51:188–205.
- . 1993. Endocrinology of reproduction in wild species. Pp. 163–327 in D. Farner, J. King, and K. Parkes, eds. *Avian biology*. Academic Press, New York.
- Wingfield J.C. and T.P. Hahn. 1994. Testosterone and territorial behavior in sedentary and migratory song sparrows. *Anim Behav* 47:77–89.
- Wingfield J.C., R.E. Hegner, A.M. Dufty, and G.F. Ball. 1990. The challenge hypothesis—theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. *Am Nat* 136:829–846.
- Wingfield J.C., R.E. Hegner, and D.M. Lewis. 1991. Circulating levels of luteinizing hormone and steroid hormones in relation to social status in the cooperatively breeding white-browed sparrow weaver, *Plocepasser mahali*. *J Zool (Lond)* 225:43–58.
- Wingfield J.C., J. Jacobs, A. Tramontin, N. Perfito, S. Meddle, D. Maney, and K. Soma. 2000. Toward an ecological basis of hormone-behavior interaction in reproduction of birds. Pp. 85–128 in K. Wallen and J. Schneider, eds. *Reproduction in context*. MIT Press, Cambridge, MA.
- Wingfield J.C., S.E. Lynn, and K. Soma. 2001. Avoiding the “costs” of testosterone: ecological bases of hormone-behavior interactions. *Brain Behav Evol* 57:239–251.
- Wingfield J.C. and M. Wada. 1989. Changes in plasma levels of testosterone during male-male interactions in the song sparrow, *Melospiza melodia*: time course and specificity of response. *J Comp Physiol A* 166:189–194.
- Zink R. and R. Blackwell. 1996. Patterns of allozyme mitochondrial DNA, and morphometric variation in four sparrow genera. *Auk* 113:59–67.