JOÃO PAULO GUSMÃO TEIXEIRA

DO MACRO AO MICRO: O PAPEL DO FOGO E DA TESTOSTERONA NA ECOLOGIA DE *Geositta poeciloptera*, AVE AMEAÇADA DO CERRADO BRASILEIRO

Dissertação apresentada à Universidade Federal de Viçosa como parte das exigências do Programa de Pós-Graduação em Biologia Animal, para obtenção do título de *Magister Scientiae*.

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Guilherme Henrique Silva de Freitas

Nerilson Terra Santos

Leonardo Esteves Lopes
(Orientador)

Aos professores, que mesmo diante de tanta dificuldade, resistem na arte de educar.

“Pois as asas de um coração sonhador ninguém irá roubar"

Pegasus Fantasy - Angra

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SUMÁRIO

RESUMO

TEIXEIRA, João Paulo Gusmão, M.Sc., Universidade Federal de Viçosa, novembro de 2016. **Do macro ao micro: o papel do fogo e da testosterona na ecologia de *Geositta poeciloptera*, ave ameaçada do Cerrado brasileiro**. Orientador: Leonardo Esteves Lopes. Coorientadores: José Marinaldo Gleriani e Rômulo Ribon.

Os campos naturais compreendem apenas quatro por cento da área total do Cerrado, o que causa preocupação quanto à conservação deste habitat. Muitas áreas de campos naturais foram perdidas por causa da exagerada proteção contra o fogo natural ou práticas antrópicas culturalmente adotadas. Neste estudo, buscamos compreender a ecologia e biologia de *Geositta poeciloptera*, uma ave ameaçada e endêmica dos campos naturais do Cerrado. Um primeiro objetivo foi avaliar a presença de *G. poeciloptera* em função do fogo. Para isso, plotados pontos de ocorrência da ave plotados em uma matriz de tempo desde a última queimada em uma área dos Campos Naturais do Alto Rio Grande. Esta matriz foi construída utilizando imagens de 32 anos (1982-2015) e classificando-as em áreas queimadas e não queimadas. A relação foi acessada utilizando um modelo linear generalizado (GLM) com distribuição Poisson, corrigida pela binomial negativa e os resultados indicam que a presença da ave é relacionada a um tempo desde a última queimada de cinco anos ou menos. *G. poeciloptera* é uma ave que defende território durante o período reprodutivo, porém a intensidade deste comportamento varia ao longo da estação. Um segundo objetivo do estudo foi acessar a relação entre o comportamento territorial da ave e sua relação com o hormônio testosterona (T), que influencia o comportamento territorial, o esforço parental e outras características sexuais secundárias de diversas espécies. Em regiões tropicais, os efeitos da T no comportamento das aves precisam ser mais bem estudados. Buscou-se compreender a relação entre a dosagem de T sérica e vários fatores da biologia da ave, como a diferença da concentração entre machos no período reprodutivo e não reprodutivo, a diferença da concentração entre machos e fêmeas, a influência da T na agressividade e a relação entre a dosagem de T dos machos e o dia de postura do ovo pela fêmea. Utilizou-se um teste Mann-Whitney unilateral para duas médias independentes para fazer a relação testosterona x temporada (para machos e fêmeas) e testosterona x sexo. Para relação testosterona x agressividade, utilizou-se um GLM com distribuição binomial. Por fim, para a relação entre dosagem de testosterona e o dia de postura da ave, utilizou-se um modelo exponencial, no objetivo de testar a hipótese de

que antes do dia de postura a ave teria maior quantidade de testosterona e essa quantidade iria progressivamente cair após a postura. Os resultados indicam que a ave possui maior quantidade de testosterona no período reprodutivo e nos machos do que no período não reprodutivo e nas fêmeas, respectivamente. A concentração de testosterona dos machos e fêmeas foi comparativamente elevada (respectivamente, média de 3,20 ng/ml e 1.93 ng/ml), padrão similar ao que ocorre em aves da região temperada. Além disto, a dosagem de testosterona teve relação com o dia da postura do ovo. Antes os machos das aves apresentam maiores níveis, visto que estão em busca ou protegendo territórios, depois da postura e com o início do cuidado parental, os níveis de testosterona baixam, implicando em menor agressividade.

ABSTRACT

TEIXEIRA, João Paulo Gusmão, M.Sc., Universidade Federal de Viçosa, November, 2016. **From macro to micro: the role of fire and testosterone on the ecology of *Geositta poeciloptera*, an endangered bird from the Brazilian Cerrado**. Advisor: Leonardo Esteves Lopes. Co-advisors: José Marinaldo Gleriani and Rômulo Ribon.

Natural grasslands comprise only four percent of the total area of the Cerrado, which causes concern about the conservation of this habitat. Many areas of natural grasslands have been lost because of excessive protection against natural fire or anthropogenic practices culturally used. In this study, we aimed to understand the ecology and biology of Campo Miner (*Geositta poeciloptera*), an endangered and endemic bird of natural Cerrado fields. A first objective was to evaluate the presence of Campo Miner in function of fire regime. For this, we plotted bird occurrence points in a matrix of time since the last fire of an area of the Upper Rio Grande Grassland to access the bird presence in time. The relationship was accessed using a generalized linear model (GLM) with a negative binomial distribution and the results indicate that the bird presence is related to a last time to burn of five or less years. Campo Miner defends territory during the breeding season; however, the intensity of the territorial behavior varies within the station. A second objective of the study was to assess the relationship between the testosterone (T) and ecological traits of the species. It is known that T influences the territorial behavior, parental care and other secondary sexual characteristics. In tropical regions, the effects of T on the behavior of birds must be better studied. We seek to understand the relationship between the serum T levels and various factors of the bird biology, as the difference in male concentration between breeding and non-breeding season, the difference in concentration between males and females, the influence of testosterone on the bird aggressiveness and the relationship between male testosterone levels and the egg-laying day. We use a Mann-Whitney U-test for two independent means to access the relationship male testosterone x season and testosterone x sex. For the aggressiveness, we used a GLM with binomial distribution. Finally, for the relation between male testosterone level and the egg-laying day, we used an exponential regression model, as we believe that before this day the bird would have a greater amount of testosterone and this amount would gradually dropping after laying. Our results indicate that the male birds have higher testosterone levels in the breeding

season than non-breeding season, as well as males have higher T levels than and females. The levels of testosterone was high (male: mean of 3.20 ng/ml; female: mean of 1.93 ng/ml), similar to the pattern that occurs in temperate birds. Furthermore, the male testosterone level was related to the day of egg laying. Before the bird have higher levels, as they are searching or protection territories. After laying the egg and parental care starts, T levels get lower, which makes the bird less aggressive.

1. INTRODUÇÃO GERAL

O termo em inglês *grassland* gera confusão em sua tradução para o português, pois é conceitualmente amplo e engloba diversas formações não-florestais. De forma simplificada, *grasslands* são ambientes caracterizados pela dominância de gramíneas (Poaceae) e pela ausência ou baixa frequência de espécies lenhosas (Gibson 2009). *Grasslands* são importantes fornecedores e reservas de recursos agrícolas e genéticos, respectivamente (World Resources 2000-2001). Ainda mais, os *grasslands*, por sua fitofisionomia característica, são comumente explorados para atividades de agricultura e pecuária, por sua fácil transformação em área de cultivo ou pelo uso do próprio habitat como área de pastejo (Jepson 2005; Andrade et al. 2015). Além de sua importância antrópica, são ambientes com alta diversidade biológica e endemismo de fauna e flora (White et al. 2000).

Os *grasslands* exibem grande dinamismo tanto no espaço, quanto no tempo, sendo o fogo uma das principais forças modeladoras desses ecossistemas (Murphy & Bowman 2012). O fogo tem influência na biota terrestre desde muito antes do homem, fazendo, por exemplo, parte da origem e distribuição dos biomas da Austrália, sendo também responsável pelas diferenças desses em relação aos biomas da África e América do Sul (Bond & Keeley 2005). Mais recentemente, o manejo antrópico de queima e pastoreio tem favorecido a expansão dos *grasslands* pelo mundo (Bond et al. 2005), o que acaba por modificar o regime de queimadas, com efeitos ainda não bem conhecidos sobre o ambiente e sua biota associada (Ramos-Neto & Pivello, 2000; Bond et al., 2010). De qualquer forma, é consenso entre pesquisadores que a frequência, a intensidade e a extensão do fogo devem ser analisadas e que tanto a ausência quanto a presença intensa (e.g. regime anual de queimadas) podem ter consequências negativas para os ecossistemas (Ramos-Neto & Pivello 2000; Fuhlendorf et al. 2006; Taylor et al. 2012; Sitters et al. 2014; Griffiths et al. 2015).

A supressão do fogo em ecossistemas abertos geralmente resulta em mudanças significativas na paisagem, as quais podem ser negativas para algumas espécies da flora. Nos *grasslands* da África Austral, duas classes de plantas foram encontradas, as resistentes ao fogo e as não-resistentes. Para promover alta diversidade alfa e beta, o ideal seria manter um regime de fogo em mosaicos, com algumas áreas queimadas em intervalos não maiores que cinco anos, enquanto que outras áreas permaneceriam protegidas dele por pelo menos dez anos (Uys et al. 2004). Por outro lado, regimes anuais do fogo causam perdas significativas da qualidade do solo, o que afeta a flora local (Pivello & Coutinho 1992). Na região Neotropical são poucos os estudos sobre a ecologia do fogo, o que gera a necessidade de se realizar mais pesquisas que objetivem predizer o regime ideal do fogo, uma vez que ele afeta diferentemente as variadas espécies em uma comunidade (Hopmans 2003; Uys et al. 2004; Overbeck et al. 2005; Brotons et al. 2013).

Assim como para a flora, o fogo pode possuir relações benéficas ou não para a fauna, com cada espécie exibindo uma resposta muito particular. Na Austrália, um experimento demonstrou que o manejo por queimada pode trazer consequências negativas para a riqueza de aves (Taylor et al. 2012), assim como para a sobrevivência de pequenos mamíferos (Griffiths et al. 2015). Já nas pradarias do Estados Unidos, regiões queimadas possuíram maior diversidade local de aves (Fuhlendorf et al. 2006). De maneira similar, várias outras espécies são afetadas positivamente pelo fogo, como por exemplo, *Rhinoptilus chalcopterus*, ave da África do Sul (Dean 1974) e algumas espécies de grandes mamíferos da África e da América do Sul (Prada 2001; Riginos & Grace 2008). O fogo também pode alterar paisagens inteiras, como observado na África do Sul, onde incêndios abrindo novas áreas, que foram utilizadas por macacos *Chlorocebus aethiops*, típicos de savana, para se dispersar e estabelecer novos territórios (Herzog et al. 2014).

Grasslands podem ser encontrados em diversas províncias biogeográficas da América do Sul, merecendo destaque, em território nacional, os Pampas e o Cerrado (Morrone 2014). Esta última província biogeográfica abrange tanto formações savânicas quanto campestres. Ambientes campestres, objeto deste estudo, podem ser definidos como sendo "áreas dominadas por gramíneas e herbáceas, com geralmente poucos arbustos e árvores espaçadas, de até 4 m de altura" (Vickery et al. 1999). De acordo com esta definição, apenas o "campo limpo" e o "campo sujo" podem ser considerados como campos naturais, sendo que esses compreendem apenas ~4% da área total do Cerrado (Sano et al. 2010).

Os ambientes campestres do Cerrado são particularmente mal estudados, o que dificulta o seu manejo ecológico e conservação, pois, conforme discutido acima, este é um ecossistema sensível e com alto dinamismo. Além do mais, a percepção errônea de que os campos naturais são áreas desmatadas intensifica o problema da conservação desse ecossistema (Veldman et al. 2015). Por exemplo, na Estação Ecológica de Assis, em São Paulo, áreas de fitofisionomias campestres sofreram uma redução de 23% da área total da reserva para menos de 1% devido à proteção exagerada contra o fogo (Pinheiro & Durigan 2009). A dramática perda de hábitat, associada ao manejo inadequado das áreas de campo natural protegidas, trazem consequências negativas para as aves endêmicas desse tipo de ambiente, que, em sua maioria, estão em declínio populacional e ameaçadas de extinção (Stotz et al. 1996; Lopes et al. 2010; Azpiroz et al. 2012). Várias espécies de aves dependem de áreas de campo natural pelo menos em parte do seu ciclo de vida, sendo, por isso, chamadas de aves campestres. Na região Neotropical, 25% das 298 espécies campestres são restritas a este ecossistema, sendo que na América do Sul, 124 espécies são totalmente dependentes, portanto classificada como campestres obrigatórias (Vickery et al. 1999). As aves campestres obrigatórias carecem de estudos sobre sua biologia básica, sendo difícil planejar e avaliar como as diferentes estratégias de manejo da paisagem podem afetar esse grupo, que possui várias espécies altamente seletivas e exigentes quanto às características do hábitat (Stotz et al. 1996; Vickery et al. 1999; Lopes et al. 2010).

Geositta poeciloptera (andarilho) é uma espécie campestre obrigatória e endêmica dos campos naturais do Cerrado, sendo considerada ameaçada de extinção em nível global e nacional (Birdlife International 2012; MMA 2014). Essa ave rara se encontra amplamente distribuída, mas de maneira local, pela porção centro-sul do Cerrado (Lopes et al. 2010). Embora pouco se conheça sobre a biologia e os requerimentos ecológicos da espécie, estudos recentes indicam que ela pode ser utilizada como modelo para o entendimento da história natural de espécies tropicais campestres ameaçadas (Silva 2015).

Geositta poeciloptera possui alguma relação ainda não bem compreendida com áreas recentemente queimadas (Braz 2008; Silva e Silva 2008). Estudos recentes demonstram que, terminada a estação reprodutiva da espécie, alguns casais permanecem na mesma área de vida durante o período não reprodutivo, enquanto que outros se dispersam, aparentemente dentro da mesma região (Silva, 2015). Os requerimentos ecológicos e as características do habitat que levam os indivíduos a permanecerem na sua área de vida ou a selecionarem novas áreas ainda não são conhecidos, mas existem indícios de que o histórico de queima do local e, consequentemente, a estrutura da vegetação, são fatores decisivos (Braz 2008; Peixoto 2014).

Silva (2015) relata que a espécie é territorialista, mas que a intensidade desse comportamento varia acentuadamente ao longo do ano, bem como entre indivíduos. De uma maneira geral, as aves exibem marcado comportamento territorial durante o início da estação reprodutiva, defendendo territórios exclusivos e de uso múltiplo. Entretanto, durante o período de alimentação dos ninhegos, a intensidade do comportamento territorial é diminuída (Silva 2015). Tamanha variação na intensidade do comportamento territorial pode estar relacionada com a mudança dos níveis séricos de testosterona, conforme demonstrado por estudos anteriores (Ketterson & Nolan 1994; Owen-Ashley et al. 2004; Hau 2007). Uma alta concentração de testosterona pode indicar maior agressividade, a qual normalmente vem acompanhada de melhora no sucesso reprodutivo (Archer 2006). Todavia, altas concentrações do hormônio podem deixar o indivíduo mais suscetível à predação, por esse se concentrar mais em encontrar fêmeas e defender território do que em se proteger de predadores (Marler & Moore 1988). Além disto, altas concentrações também afetam a imunocompetência, deixando as aves mais suscetíveis a doenças e ectoparasitas (Owen-Ashley et al. 2004; Stutchbury & Morton 2008). Por outro lado, baixas concentrações do hormônio estão comumente relacionadas a um aumento do cuidado parental (Hirschenhauser & Oliveira 2006). Deve-se ressaltar que o conhecimento do efeito hormonal sob o comportamento das aves é melhor compreendido em região temperada, e que somente nos últimos anos espécies tropicais foram alvo de estudos (Chiver et al. 2014).

Objetivando entender a influência do regime de queimadas e dos níveis hormonais sobre a o comportamento de *G. poeciloptera*, realizou-se este estudo de caráter inovador e integrador. Isso porque as técnicas empregadas nessa dissertação visam compreender a história natural de uma ave em duas escalas distintas: uma de paisagem, utilizando o sensoriamento remoto para acessar a relação da espécie com áreas queimadas, e outra na escala de organismo, utilizando a fisiologia para entender a influência da testosterona sobre o comportamento de *G. poeciloptera*.

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2. **CHAPTER 1:** João P.G. Teixeira, Ricardo C. Meireles, José M. Gleriani, Gerson R. Santos, Tamara L.S.S. Machado, Wesley Davies, Leonardo E. Lopes. Occurrence of the Campo Miner in response to fire history: an applied conservation study with a threatened Neotropical grassland bird.

Chapter 1: Occurrence of the Campo Miner in response to fire history: an applied conservation study with a threatened Neotropical grassland bird

Abstract: *Open grassland habitats require adequate landscape management. We studied the influence of fire in the occurrence of the threatened Campo Miner (*Geositta poeciloptera*), a terrestrial and insectivorous passerine endemic to the open grasslands of the South American Cerrado. The study was conducted in Upper Rio Grande Grassland, southern state of Minas Gerais, Brazil. The bird occurrence points were collected by global position device over three years and the occurrence point were plotted on a map of Time Since Fire (TsF) and Times Of Fire (ToF; i.e how many times one pixels was burned), build with satellite images from 1982 to 2015, and it was counted the amount of points that fell on each class of TsF. We used a negative binomial model to predict the occurrence of bird in function of TsF. ToF measurement was used to access the fire regime in sense of percentage of burned area in the study area. The likelihood of the bird occurrence was greater into low classes of TsF (i.e. recent burned areas; equal or less than five years) and decreases in higher class of TsF, by the negative binomial regression model. We conclude that the bird is fire dependent. The study area has an intense fire regime, as showed by the average of eight percent of the total area being burned every year, and this could be one of the primary reasons for the maintenance of the species in the region. It is imperative that the area be protected, and, within that, the protection must consider the importance of the current fire ecology and management traits. We recommend more research with others grassland birds to reach an ideal fire regime to the study region. Studies about Campo Miner prey abundance and foraging behavior may bring new robust evidences about the species dependence of burned areas.*

Keywords: Fire regime, Time since fire, *Geositta poeciloptera*, open-habitats, Neotropics

Introduction

Fire is a natural disturbance in many ecosystems (Bond & Keeley 2005; Bowman et al. 2009; Bond & Parr 2010), but modern human activity dramatically changed fire patterns worldwide (Hardesty et al. 2005; Bowman et al. 2009; Driscoll et al. 2010; Taylor et al. 2012; Watson et al. 2012). Fire can radically change the plant species composition, promoting marked differences in habitat structure and resources availability for animals (Cheal 2010). Grassland habitats are particularly shaped by fire and, consequently, fire regime is an important conservation issue, with a mosaic of unburned and burned areas at different times being the optimal choice, since it promotes landscape heterogeneity, under the assumption that "pyrodiversity begets biodiversity" (Martin & Sapsis 1992; Fuhlendorf et al. 2006; Duchardt et al. 2016).

The largest fire-dependent biogeographic province in South America is the Cerrado, which covers almost all of central Brazil, eastern Paraguay, and eastern Bolivia, with several types of pure grasslands and savannas occurring there (Oliveira & Marquis 2002; Pivello 2011). There are records of naturally ignited fires in the Cerrado since at least 32,000 Years Before Present (YBP) (Ledru 2002), with indigenous people using fire as a tool for land management since at least 4000 to 5000 YBP (Fiedel 1992; for more about indigenous practices see Pivello 2011).

Fire has both undesirable and desirable outcomes on biodiversity conservation. For example, fire suppression in a Cerrado conservation unit in southeastern Brazil has led to a dramatic change in vegetation physiognomies, with a reduction of open grasslands from 23% to less than 1% of the area studied, thus reducing landscape heterogeneity (Pinheiro & Durigan 2009). On the other hand, frequent fires (e.g. every 2 years) in Cerrado can reduce the overall plant species richness, change the overall physiognomy, and hamper the complete recovery of soil nutrients, thus affecting the whole biota (Pivello & Coutinho 1992; Franco & Haridasan 2008; Diniz et al. 2011).

Fire impact is a function of the fire regime, which describes fire size, frequency, severity and seasonality; therefore, it is essential to analyze these variables to build good management plans for grassland ecosystem (Bond & Keeley 2005). One measurement that can offer valuable information for the development of fire management strategies in fire-prone habitats is the correlation between Time since Fire (TsF) and the occurrence of species (Driscoll et al. 2010). Fire response curves indicate the extent to which a species depends on a particular post-fire age, also allowing the identification of thresholds necessary to ensure required habitat resources (Keith et al. 2001; Driscoll et al. 2010).

Studies of the impact of fire on tropical grassland species is greater for flora than fauna (Cavalcanti & Alves 1997; Parker & Willis 1997; Briani et al. 2004). To date, there is no specie-specific study on the influence of fire on the Cerrado endangered fauna, even though the Cerrado is a hotspot of biodiversity (Myers et al. 2000), with 30 endemic bird species, 16 of them non-forest (Silva & Bates 2002), seven of which are threatened and five near-threatened (Birdlife International 2012; MMA 2014).

The Campo Miner (*Geositta poeciloptera*) is a terrestrial and insectivore passerine endemic to the Cerrado, considered endangered in Brazil and vulnerable at global level (Birdlife International 2012; MMA 2014). The primary threat to Campo Miner is habitat loss, largely due to changes in land-use patterns, especially the increment of agriculture, forestry and invasive exotic grasses (Silva e Silva 2008; Lopes et al. 2010). Although poorly known, recent studies showed that the Campo Miner can be used as a model species for the understanding of the life history of Neotropical grassland birds (Peixoto 2014; Silva 2015). This species is frequently recorded at recently burned sites (Braz 2008; Silva e Silva 2008; Silva 2015), but its relationships with fire is not yet understood. This study aims to investigate the influences of fire in the occurrence of the Campo Miner. We hypothesize that the species is fire-dependent, so it has higher probability of occurrence in recently burned areas.

Methods

Study area

The study took place in the northern portion of the Upper Rio Grande Grasslands (URGG), in the municipalities of São João del-Rei and Tiradentes (figure 1), state of Minas Gerais, southeastern Brazil. The URGG is an extensive mountainous region (~21º29'–21º32'S and 43º50'–44º55'W) with altitudes ranging mainly from 900 to 1400 m. The landscape is transitional, with Cerrado areas at the mountain slopes and tops, where the soil is poor and shallow, and Atlantic Forest along the valleys, where the soil is deeper and richer (Azevedo 1962; Oliveira-Filho & Fluminhan-Filho 1999). The region has a humid subtropical climate with dry winter and temperate wet summer, Cwb in Köppen's climate classification system (Sá Júnior et al. 2012). The mean temperature during the winter is 14.3ºC and during summer is 17ºC (Sá Júnior et al. 2012). Mean annual rainfall is ~1500 mm, with marked dry (May to August) and wet (September to April) seasons.

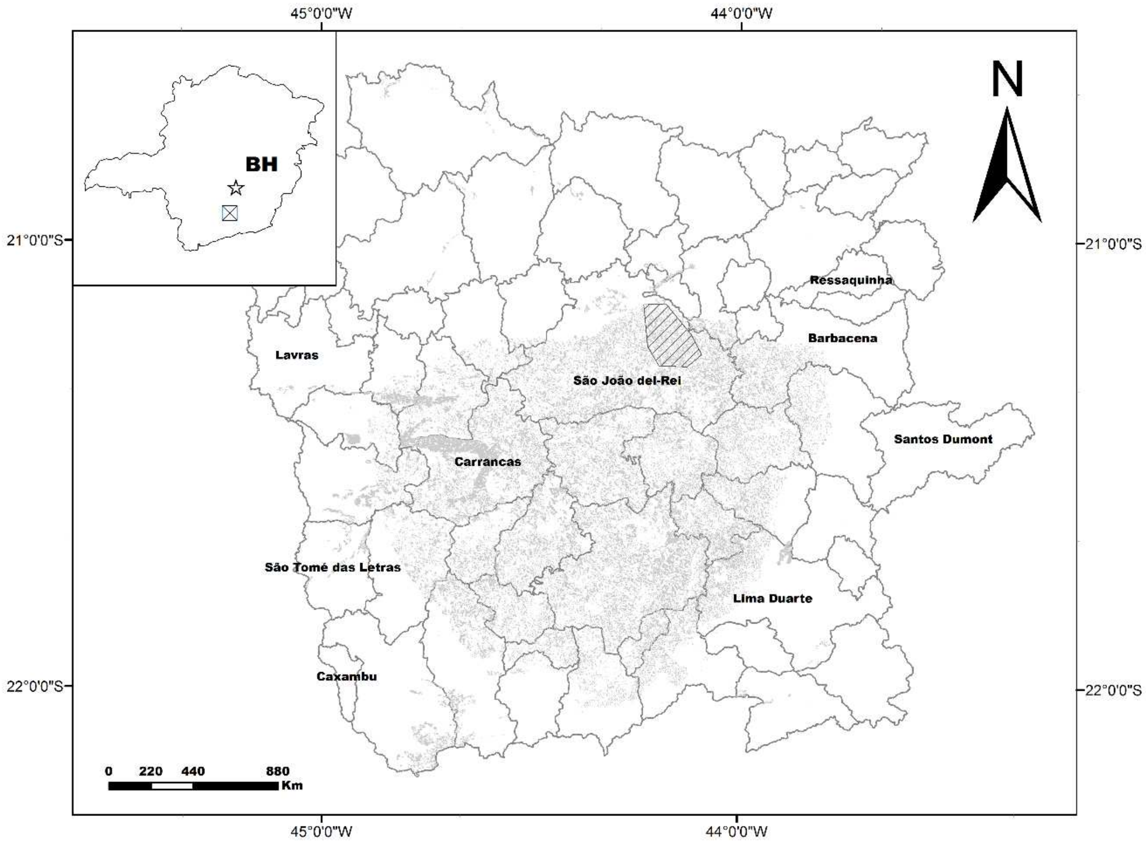

Figure 1: The Upper Rio Grande Grasslands, southern state of Minas Gerais, southeastern Brazil. Gray shadow indicates grassland habitat. Hatched indicates the study area. In the small window, the lined box indicates the location of the study region in Minas Gerais. The star represents Belo Horizonte (BH), the capital of the state.

The study area is accessible by the BR265 road, between the Km 244 and Km 253, comprising ~15 ha. Two types of grasslands form the bulk of the vegetation found in the study area: 1) *Campo limpo*, which is a pure grassland, and 2) *Campo sujo*¸ which also have some few shrubs and small trees (Leite & Klein 1990). The main economic activity in the region is cattle grazing, but eucalyptus plantations for charcoal production and crops (predominantly corn and bean) are also found (IBGE 2014). Cattle raisers traditionally use fire in pasture management.

Fire history

We obtained the burning scars areas at the end of dry season from 1984 until 2015 after data from TM/Landsat5, ETM+/Landsat7 and OLI/Landsat8. We used the composition 3(B)NDVI(G)5(R) for the two first sensors and 4(B)NDVI(G)6(R) for OLI sensor, all of them in the scene path/row 218/75, UTM/WGS-84. We used photointerpretation after MaxVer supervised classification to correct errors due to topography and cloud shadows. Only one LISS3-IRS/P6 scene 333/93 path/row with 2(B)NDVI(G)4(R) composition was used (16 September 2012) due to cloud cover in Landsat data, totaling thirty-one (31) scenes. To confirm burning scars classification, we compared the fire position to the fire database of the Brazilian Institute of Geography and Statistics (IBGE). This database utilizes 29 satellites to monitoring fire in Brazil <http://queimadas.cptec.inpe.br>. At the end, we obtained 32 matrices, each of them with pixels classified as burnt or unburnt (0= unburnt, 1= burnt). We also classified and clipped off forest patches within the study area, since it is a grassland habitat species.

We calculated two variables using the classified maps. The first one was how many times each pixel was burned (i.e how many times fire occurred; ToF). The second was the TsF (i.e., areas burned in 2014 = 1 year since fire), which we obtained by counting each 0 until finding a 1. We used ToF variable to generate the mean percentage of burned area per year ($\overline{BA}$), using the equation 1. The TsF measure was used to relate the bird occurrence to the fire history, by plotting the occurrence points into the TsF map. Fire mapping was made in SPRING software (Câmara et al. 1996) and maps generated for TsF and ToF measurements were exported to ArcGIS software (ESRI, 2011).

$$\overline{BA} = \frac{\sum_{32}^{1} BA}{32.SA}.100$$

Equation 1: equation created to calculate the mean percentage of burned area per year. $\overline{BA}$: means percentage of burned area per year. BA: burned area. SA: total of study area. 32 because the database has 32 years.

Bird data

We used the Campo Miner presence data collected by us and collaborators (see acknowledgments) in the study area from October 2013 to February 2016 during a long-term study focusing on the natural history and reproductive success of threatened grassland birds. For each encounter, we obtained the geographical coordinates using a Global Position System device (GPS – Garmin). We used no bird survey protocol, since we had a robust point database with almost daily observations along three years, comprising an effort of ~ 300 days of fieldwork, distributed for four people. We believe that this high fieldwork effort will not be an embarrassment for statistical analyses. The point data were plotted in ArcGIS software (ESRI 2011) and then we counted in which class of TsF and ToF each point fell on. We also monitored every two or three days all nests found using a borescope.

Statistical analyses

We tested the Complete Spatial Randomness (CSR) of the occurrence points using *F* test. This test measures the cumulative distribution of the distance from a fixed point in space to the nearest point in our dataset. If an event is statistically clustered, the empirical *F* values must be smaller than the theoretical *F* (Baddeley 1998).

We used a generalized linear model (GLM) with Poisson distribution to access the dependence of burned areas by birds. The Poisson distribution is used when count data was available (Hastie & Pregibon 1992). We tested the model using χ^2 test. As we detected overdispersion (variances are much larger than means), we corrected the model by using Negative Binomial regression. We used R software, version 3.3.1, with the *maptools*, *MASS*, *moments*, *raster*, *rgdal*, *sp* and *spatstats* packages to run the statistical analyses (Venables & Ripley 2002; Baddeley & Turner 2005; Edzer et al. 2005; Bivand et al. 2013; Komsta & Novomestky 2015; Bivand & Lewin-Koh 2016; Hijmans 2016; R Core Team 2016).

Results

From the 119 Campo Miner occurrence points obtained, almost ninth percent fell into classes <5 years since the last fire (figure 2 and 3). Nearly twenty percent of the points fell into areas which were burned >10 times during the 32 years studied. ToF measure indicates that on average 9.5% of the study area is burned every year.

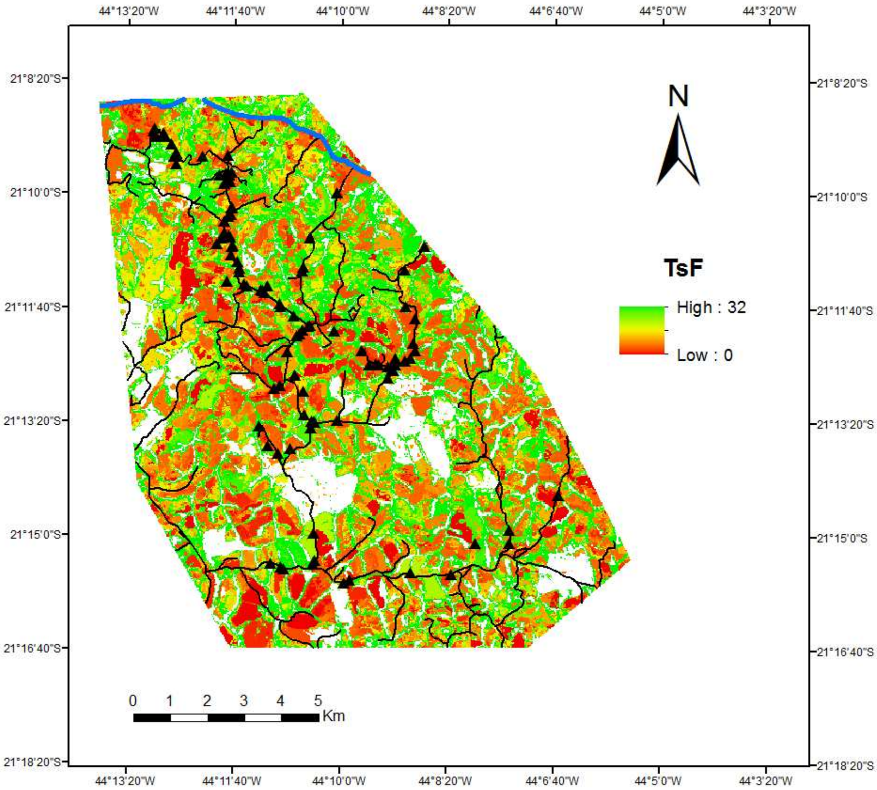

*Figure 2: Time since fire (TsF) map from the study area in the Upper Rio Grande Grasslands, São João del-Rei, Minas Gerais, Brazil. The Blue line represents the main highway, BR265, while black lines represent dirt roads within the study area. Lower TsF class indicates less times until the last fire. The Campo Miner (*Geositta poeciloptera*) occurrence points are indicated by black triangles.*

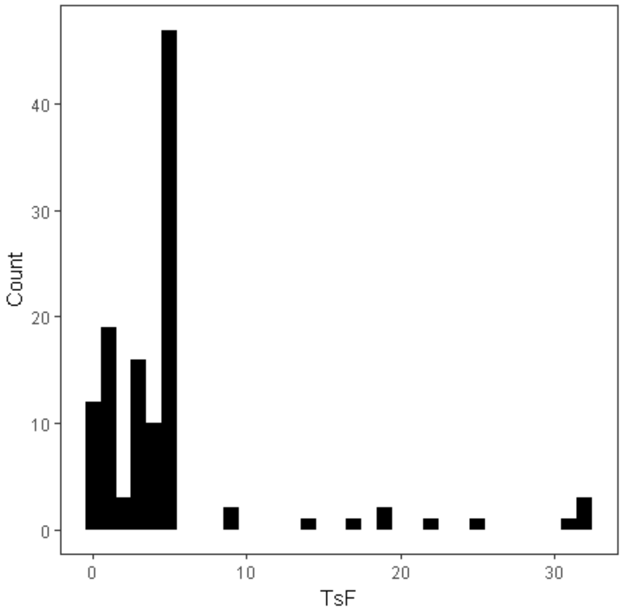

*Figure 3: Count of occurrence points of the Campo Miner (*Geositta poeciloptera*) within each class of Time since Fire (TsF) in the study area, Upper Rio Grande Grasslands, São João del-Rei, Minas Gerais, Brazil.*

The points are not in the CSR for F. All the estimated F were lower than the theoretical result (figure 4).

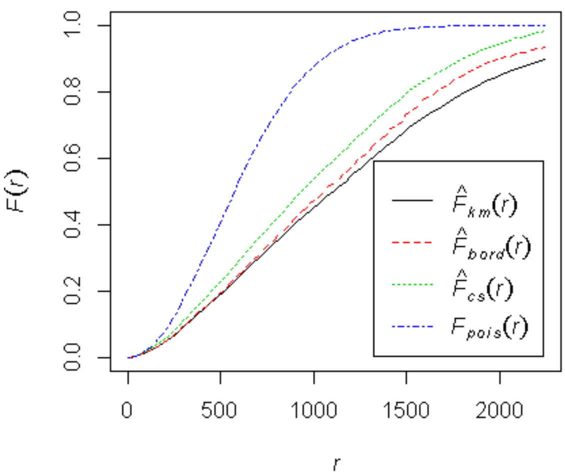

Figure 4: F function for testing the CSR. F(r): empty space function, the cumulative distribution function F of the distance from a fixed point in space to the nearest data point; r: distance argument; $\hat{F}_{km}(r)$: Kaplan-Meier estimate of F(r); $\hat{F}_{bord}(r)$: border corrected estimate of F(r); $\hat{F}_{cs}(r)$: Chiu-Stoyan estimate of F(r); $\hat{F}_{pois}(r)$: theoretical Poisson F(r). r: values in meters.

The NB model was statistically significant ($p < 0.001$, χ^2), which indicates a strong relationship between low classes of TsF (equal or less than five) and the bird occurrence. Thus, birds are more likely found within recently burnt areas (lower TsF values), as shown in the model predicted values for species occurrence in classes of TsF (Figure 5).

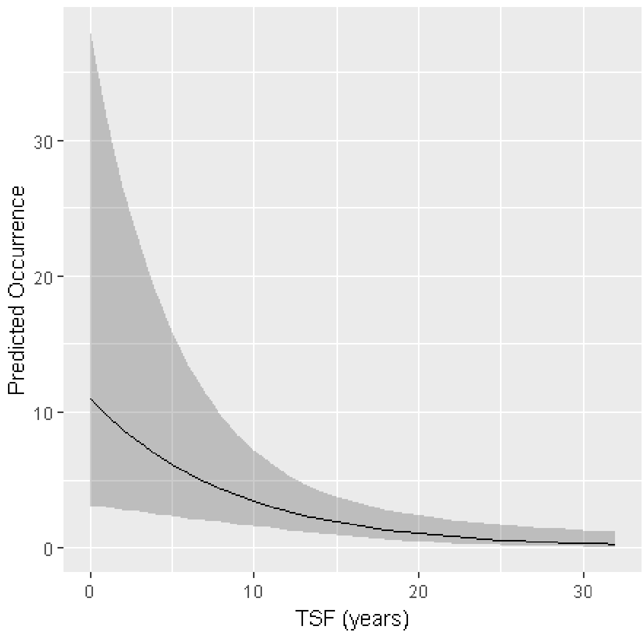

*Figure5: Negative binomial regression showing the predicted Campo Miner (*Geositta poeciloptera*) occurrence values for the classes of time since fire (TsF). The black line indicates the negative binomial model, while the grey shadow indicates a 95% confidence interval.*

Discussion

Our study supported a preference of the Campo Miner for recently burned areas, which has already been suggested by previous authors (Braz 2008; Silva e Silva 2008; Peixoto 2014; Silva 2015). The predicted occurrence of the Campo Miner showed a irruptive shaped curve (Response 1 of Watson et al. 2012), indicating that the probability of finding the species is greater in recently burned areas (<5 years since fire), rapidly declining afterward. Although the biology of the Campo Miner is not well-known, our field observations suggest that the foraging efficiency of the species is improved in areas with shorter and sparser grass cover, where it displays a random walk pattern of foraging (pers. obs.). When vegetation is denser, the Campo Miner typically forages along cattle trails. This foraging behavior is well documented for other grassland birds, with a decrease in grass height helping the bird to find its prey (Devereux et al. 2004; Whittingham & Evans 2004; Petry & Krüger 2010). The same foraging behavior and fire-influenced occurrence were found for the Saffron-cowled Blackbird (*Xanthopsar flavus*), a vulnerable species endemic to the Southern South America grasslands (BirdLife International 2013). Moreover, the Saffron-cowled Blackbird was not found inside the Aparados da Serra National Park, probably because of the lack of short-grass habitats in this protected area (Petry & Krüger 2010). Another trait that possibly links Campo Miner occurrence to fire events is the fact that in all the nests monitored (61) we found charcoal fragments lining the nest platform (Silva, 2015; Meireles et al., unpublished data). It is not known why the bird utilizes charcoal fragments in nest construction.

Parker and Willis (1997) suggested that the Campo Miner cannot survive in an annual fire regime. Our data cannot support or reject this hypothesis, but 20% of the occurrence points obtained were within 10 or more ToF, several of them in areas subject to annual fires. It is important to highlight that an annual fire regime causes damage to the fully nutrient recovery on soils (Pivello & Coutinho 1992), and it should be avoided for wider biodiversity conservation objectives, since different species have different responses to fire (Sitters et al. 2014), as the pyrodiversity can begets biodiversity (Parr & Andersen 2006; Taylor et al. 2012). Although, some fire regimes can be critical for some grassland species, as shown for the Strange-tailed Tyrant (*Alectrurus risora*) in north-east Argentina grasslands (Di Giacomo et al. 2011). Annual and biannual fires regimes reduced the reproductive success of this endangered bird (Birdlife International. 2012), since it uses tall grasses to breed. Only in the third breeding season after a prescribed fire the bird did not distinguished between burnt and unburnt patches (Di Giacomo et al. 2011). The current regime of ~10% percent of the study area (~24.000 ha) being burned annually could be a positive factor in the maintenance of the Campo Miner presence in the URGG, but this same regime might have negative effects for other threatened grassland birds found in the same region, as the example above. Species such as the Sharp-tailed Tyrant (*Culicivora caudacuta*) and the Brazilian related Cock-tailed Tyrant (*Alectrurus tricolor*) are closely tied to taller and denser grasslands, probably not tolerating frequent burns (Lopes et al. 2010). The ideal fire regime for grassland birds, therefore, seems to be one that maintain a mosaic of burned and unburned patches (Durigan & Ratter 2016).

There is a common public misunderstanding about the ecological role of fire, which is usually seen as harmful to the biodiversity in all kinds of ecosystems, and that fire suppression is a much necessary conservation measure (Veldman et al. 2015). This misunderstanding, if applied to fire-prone ecosystems, like the Cerrado, can negatively impact the local biodiversity, and that is why park managers must be aware of the best fire management practices available for the ecosystem under their care, which is only possible with effective research linking species trait to fire regimes (Overbeck et al. 2015; Veldman et al. 2015; Durigan & Ratter 2016). Severe fire regimes of the complete suppression of fire can both have dramatic impacts on biodiversity conservation (Pivello & Coutinho 1992; Durigan & Ratter 2016).

Protected areas in the Cerrado region, especially those with the more open grasslands, might lose area due to fire suppression policies. In few years, an area can switch from open grasslands to taller vegetation in absence of human management (Uresk & Juntti 2008; Pinheiro & Durigan 2009; Durigan & Ratter 2016). In this way, fire suppression policy can lead to the local extinction of open-grassland specialists, such as the Campo Miner, who has probably been extinct in Itirapina Ecological Station, São Paulo, due to fire suppression allied with others local problems (Willis 2004).

The URGG is an important region for bird conservation not only in the present, but also in the future. Marini et al (2009), using niche modeling tools in different climate scenarios, concluded that the ranges of several threatened and/or endemic grassland species found in the Cerrado will shrink, also shifting toward southeastern Brazil, in highly degraded areas lacking large conservation units. The URGG is one of the few regions in southeastern Brazil that harbor open Cerrado vegetation today and that will likely keep suitable climate conditions into the future, what makes it a region of high priority for nature conservation (Marini et al. 2009; Lopes et al. 2010). In addition to the present and future large-scale importance, the URGG harbor significant populations of at least seven threatened bird species, corroborating the idea that the region must be legally protected (Lopes et al. 2010; Peixoto 2014). Nevertheless, as stressed above, making part of the URGG a protected area must consider fire management as a necessary conservation tool (Petry & Krüger 2010). Otherwise, it can bring risk to the occurrence of Campo Miner and other open grassland birds that avoid denser and taller types of vegetation (Vickery et al. 1999; Silva e Silva 2008; Peixoto 2014).

Conclusion

Although fire ecology is a growing area of research in Brazil, currently there is no solid baseline for fire management in the Cerrado and, in fact, fire is often absent from many management plans, since park managers are reluctant to prescribe fire (Durigan & Ratter 2016). The evidence presented here, for the first time, demonstrate that an endangered bird species may be reliant on a fire regime in the Cerrado. We must highlight that our study is the first of its kind, with a single species in a single location, and that much work is needed before solid scientific bases can be established for fire management in the South American tropical grasslands.

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3. **CHAPTER 2:** João P.G. Teixeira, Ricardo C. Meireles, Wesley Davies, Leonardo E. Lopes. Seasonal variation in testosterone levels in the Campo Miner (*Geositta poeciloptera*), a threatened bird of the Brazilian Cerrado

Chapter 2: Seasonal variation in testosterone levels in the Campo Miner (*Geositta poeciloptera*), a threatened bird of the Brazilian Cerrado

Abstract: *Testosterone (T) is a sexual hormone which influences bird aggressiveness, reproductive success and other secondary sexual characteristics. The T traits on birds are better known in temperate zone, although recent studies have been made with tropical species, which commonly present low levels of T year-round. Most studies with tropical birds were made in environment with less marked seasonality. The Campo Miner (*Geositta poeciloptera*) is a threatened bird endemic to the open grasslands of the Brazilian Cerrado. In this study, we aimed to answer some basic questions about the bird biology. What is the species temporal T profile? Have male bird higher T levels than female, irrespective of season? Is the T related to the bird aggressiveness (measured with display behavior)? The time before and after egg-laying day influence T levels? We used a Mann-Whitney U-test for the first two questions, generalized linear models to third and an exponential regression to the last one. Our results for T traits in Campo Miner showed that the species presents a seasonal T profile that is more similar to temperate than tropical birds, since tropical ones usually present low levels of T year-round. The T levels pattern for Campo Miner can be related to the seasonality of the habitat, which can lead to a seasonality of territory defense. Moreover, male testosterone levels were higher than female ones. The testosterone levels were linked to the bird aggressiveness and the T levels tends to diminish before egg- laying date, which can be a result of the beginning of parental care.*

Keywords: Testosterone, Hormonal behavior, Tropical birds, female testosterone, aggressiveness

Introduction

It is well established that endocrine mechanisms mediate reproductive behavior, parental care, and territorial defense in global fauna (Wingfield et al. 1990; Archer 2006; Hau 2007). The trade-off between competitive behavior and maintenance behavior are regulated largely by the testosterone (T) hormone (Wingfield et al. 1987; Hau et al. 2010), which influences, for example, aggressive behavior, attractiveness, and immune function on birds (Wingfield et al. 1990; Folstad & Karter 1992; Roberts et al. 2004; Ketterson et al. 2005; Hau 2007). The relationship between aggression and T in birds is better known in the temperate zone (Goymann et al. 2004; Hau 2007; Stutchbury & Morton 2008), where environmental differences between breeding and non-breeding seasons are large, leading to marked differences in T serum levels, which usually peak when territories are being established (Wingfield et al. 1987; Goymann et al. 2004; Ketterson et al. 2005). On the other hand, tropical birds with year-round territories and/or long breeding seasons (> 6 months) usually exhibit low T levels all year-round, with the corticosterone hormone apparently playing the major role in sexual behavior (Stutchbury & Morton 2008; Chiver et al. 2014).

Although T is commonly associated with males, the hormone also has influence in females' behavior. Females of many species have detectable levels of T, which in some cases may approach those levels observed in males (Wingfield et al. 2000; Ketterson et al. 2005). Females of species with less pronounced sexual dimorphism competing for territories or access to male paternal care usually has higher T levels, both in the tropics and in the temperate zone (Langmore et al. 2002).

Few studies have ever investigated T levels of Neotropical birds, most of them conducted in forested habitats in Central America (Hau et al. 2000, 2004; Busch et al. 2008; Addis et al. 2010; Chiver et al. 2014). Studies about tropical birds that inhabit grassland habitats, which are very dynamic in space and time (i.e unstable habitats) (Murphy & Bowman 2012), are particularly scarce (see below). Here we studied the seasonal variation in testosterone levels in the Campo Miner (*Geositta poeciloptera*, Scleruridae), a Neotropical grassland bird that exhibits strong territorial behavior during part of the year, defending exclusive territories during courtship and nest construction, but its aggressiveness decreases considerably during incubation and, especially, nestling care (Silva e Silva 2008; Silva 2015).

We first surveyed in which country and ecosystem the studies about T were made in the tropical region. Then, we hypothesized that the Campo Miner: 1) males show higher serum T levels during the breeding season than non-breeding season (we did the same test for females); 2) males have higher T levels throughout the year than females, but females show conspicuous T levels; 3) territorial aggression is related to T levels; and 4) T levels are higher before egg laying, decreasing thereafter.

Methods

Study area and species

We studied a population of the Campo Miner found in the Upper Rio Grande Grassland, an extensive mountainous region (~21º29'–21º32'S; 43º50'–44º55'W) with altitudes ranging mainly from 900 to 1400 m, encompassing over 10 municipalities in the state of Minas Gerais, Brazil. Our study area is within the municipalities of São João del-Rei and Tiradentes, and encompasses an area of ~15,000 ha. The region has a humid subtropical climate with dry winter and temperate wet summer, Cwb in Köppen's climate classification system (Sá Júnior et al. 2012). The mean temperature during the winter is 14.3 ºC and during summer is 17.0ºC (Sá Júnior et al. 2012). Mean annual rainfall is ~1500 mm, with marked dry (May to August) and wet (September to April) seasons (Sá Júnior et al. 2012).

The Campo Miner is an endangered passerine (Birdlife International 2012; MMA 2014) endemic to the open grasslands of the South American Cerrado biogeographic province (Silva & Bates 2002). It is a small, terrestrial and insectivorous bird that nests inside cavities dig on dirt banks and which breeding season extends from early August to early December (Silva 2015). Mean incubation time is 18 days and the mean nestling period is 16 days (Silva 2015). The bird often sings during hovering display flights to defend it territory (Silva, 2015). Even though the species does not shows sexual dimorphism, Silva (2015) demonstrated that bird behavior can be used as a proxy for sex identification, as the males are more active in territorial defense, frequently performing hovering display flights, and having high song output (Silva 2015).

Research survey

We surveyed the researches focusing on T in the tropical region using keywords as Testosterone, Tropical, Tropics, Hormonal, Sexual Hormones in databases as Google Scholar (https://scholar.google.com), Searchable Ornithological Research Archive (http://elibrary.unm/sora), and Web of Science (http://webofknowledge.com). In addition, we made a thorough investigation in the following journals: Hormones and Behavior, The Condor, and Ornitología Neotropical. We classified the articles found by country, ecosystem, species, and prevailing territorial system.

Blood sampling

We captured birds during part of the pre-breeding, breeding (July through mid-November) and non-breeding seasons (January) using one to three 12m long mist nets and playback of the bird songs and calls. Given that aggressive events, such as territorial intrusions, can lead to peaks of T (Wingfield et al. 1990; Gill et al. 2007), we used a standardized playback track consisting of one minute of bird songs followed by 30 sec of birdcalls with 15 sec of silence between songs and calls, and one minute between repetitions. The track was played until we capture the bird. We did that to standardize any eventual influence of the playback on the bird aggressive response and testosterone levels. After released from the net, the bird was accommodated in a cloth bag for five minutes before taking the blood sample (maximum of 100 µl) (Chiver, 2014). After that, the birds was color banded and released in the same place.. Blood samples were stored in natural climate conditions for a maximum of three hours and, in the lab, we ran the blood in a centrifuge (10,000 RPM for 10 min) and stored the serum at -20 ºC. Given that sexes of the species are alike, we send a small sample of blood of each captured specimen to a private laboratory for molecular sexing (BioTech Ciência e Tecnologia pelo DNA, Goiânia, Goiás, Brazil). Moreover, we monitored those nests found at every two or three days as part of a long-term study with this species.

Territorial aggressiveness

After a pilot study, we realized that incubating adult birds usually did not exhibit the hovering display flights or sing in response to the playback, so we used a binary evaluation scheme to indicate territorial aggressiveness. Birds that responded to the playback doing the hovering display were considered aggressive, and birds that did not were considered non-aggressive. We also counted how many birds were aggressive while incubating and compared the male T levels with the time from egg-laying date (standardized in 0). This last measure was possible only for those nests for which we had the exact date of egg laying, or when we could back-date the laying date using the mean incubation period of 18 days.

Testosterone analysis

We determined the total serum testosterone levels using a competitive enzyme immunoassay kit from Enzo Life (Catalogue number ADI-900-065; Farmingdale, NY). This is a competitive procedure Elisa that uses a monoclonal antibody to T to bind the hormone itself or others alkaline phosphatase molecule which has T covalently attached to it. The assay was performed following the instructions of manufacturer. We run the samples at a 1:20 dilution with 0.5% steroid displacement buffer to inhibit testosterone binding to proteins. The sensitivity of the assay was 5.67 pg/ml and average 2.37 ng/ml

Statistical analyses

We used Mann-Whitney U-test for unpaired data (Zar 2010) to compare the mean levels of male testosterone between seasons. It was not possible to recapture the same individuals in both seasons, so we used the mean testosterone levels in a one-tail test with the hypothesis that the T levels would be higher during the breeding season. We used the same test to compare T levels between sexes, predicting that T in males would be higher than in females.

We used a generalized linear model (GLM) with binomial distribution to test the association between aggressive behavior (0= no hovering display, 1= displaying). The binomial distribution is useful to compare dummy dependence data to continuous variable data (Hastie & Pregibon 1992). We also used an exponential regression (Hastie & Pregibon 1992) to access the relationship between T levels in relation to the egg-laying date. All tests were made in R software (R Core Team 2016) with an alpha of 5%.

Results

We found 31 articles about T conducted in the tropical region (figure 1), most of them from Panama (18), followed by Australia and Brazil (3). Almost all of these papers were conducted in forested habitats and with species holding year-round territories (appendix 1).

We obtained blood samples from 42 birds: 16 female, 23 males and 3 unsexed. Twenty-seven birds were captured during breeding and 15 during non-breeding season

(Figure 2). We could calculate egg-laying date for nests assisted by 14 males. Male T levels were significantly (*p*= 0.0077) higher in breeding [3.20(2.20) ng/ml, n = 16] than in the non-breeding season [1.82(0.43) ng/ml, n = 8]. Mean female T levels among breeding [1.93(0.66), n= 11] and non-breeding [1.81(0.28), n= 5] seasons did not differ significantly (*p*= 0.3669). For sex, irrespective of season, T levels were significantly (*p*= 0.048) higher in males [2.71(1.95), n= 23] than females [1.89(0.56), n= 16] (Figure 3). Male T peak was 10.70 ng/ml, but this was for an abnormally aggressive individual observed, which was the only bird that attacked the sound device during the playback. This peak occurred almost in the middle of the breeding season (late September, when this bird was not breeding). If we exclude this specimen, the highest T level recorded was 4.9 ng/ml for a male (early November, when it was establishing one territory which subsequently was used for nesting), while for female was 3.13 ng/ml in the late breeding season (middle November, when this bird was breeding).

The binomial GLM comparing the aggressiveness to T levels was significant (χ^2 = 11.01, df = 1, $p < 0.001$) (Figure 4). Only one of 16 females and one of 23 males displayed while incubating (Figure 5), approximate 5% in general (two of 39 individuals). The exponential regression revealed that the egg-laying date were inversely related to T levels (Figure 6; F test, $p = 0.003$), however, the model explains a low percent of the variance observed ($r^2 = 0.4889$).

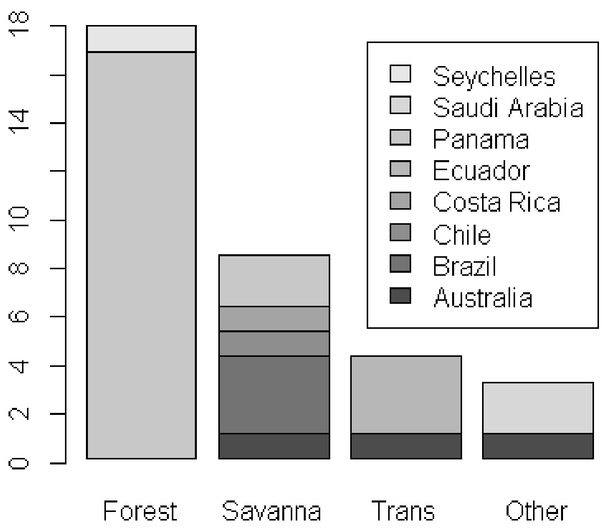

Figure 1: Survey of researches conducted in the tropical region ordered by habitat. Forest: comprises forest and forest edges. Savanna: comprises grasslands and woodlands. Trans: comprises the transition between forest and open habitats. Other: researches in aquatic or desert habitats

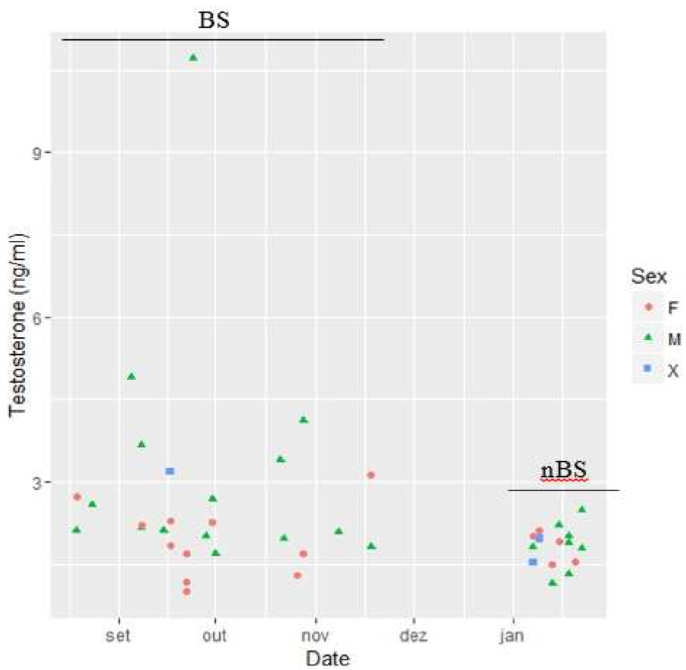

Figure 2. Total serum T levels samples by time (n = 42) for the Campo Miner

*(*Geositta poeciloptera*) in southeastern Brazil. No specimen was sampled in December, which marks the transition between breeding (BS) and non-breeding (nBS) seasons. F: females; M: males; X: unsexed bird.*

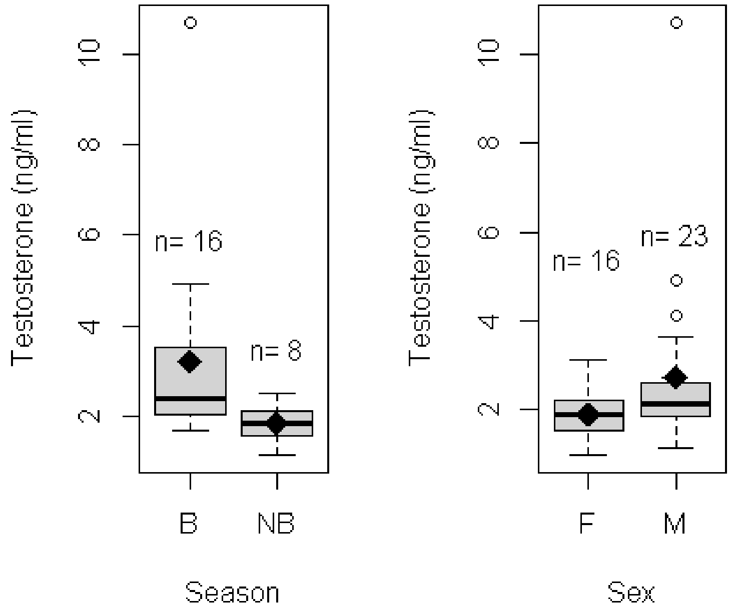

*Figure 3. Left. Boxplot of T levels of males of the Campo Miner (*Geositta poeciloptera*) among breeding (B) and non-breeding (NB) seasons. Right. Boxplot of T levels among females and males, irrespective of season. The box represents upper and lower quartiles and the thick line inside the box represents the median. Solid diamond represents the mean (outliers included). The bars represent the maximum and minimum values, excluding outliers. Empty points represent the outliers. In both cases, mean differences were significant according to the t-test.*

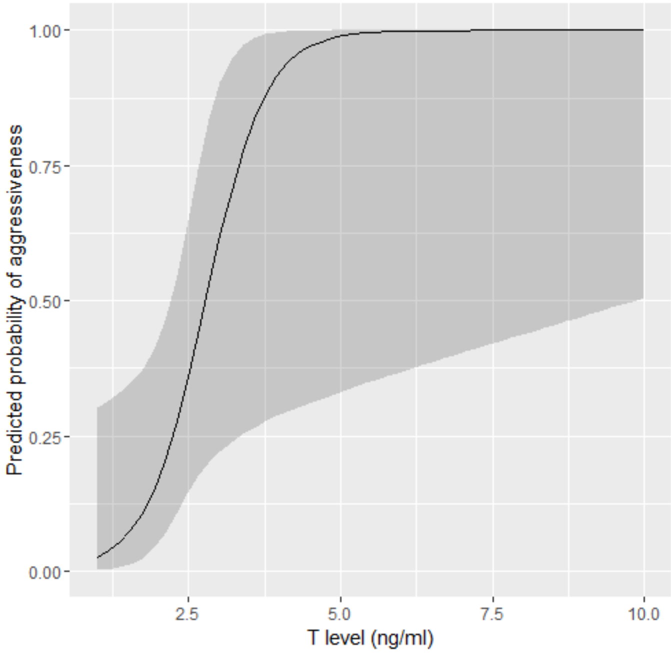

*Figure 4: Predicted probability of aggressiveness (1 = aggressive; 0 = non-aggressive) of Campo Miner (*Geositta poeciloptera*) related to testosterone levels, according to a binomial GLM model ($\chi^2 = 11.01$, $df = 1$, $p < 0.001$).*

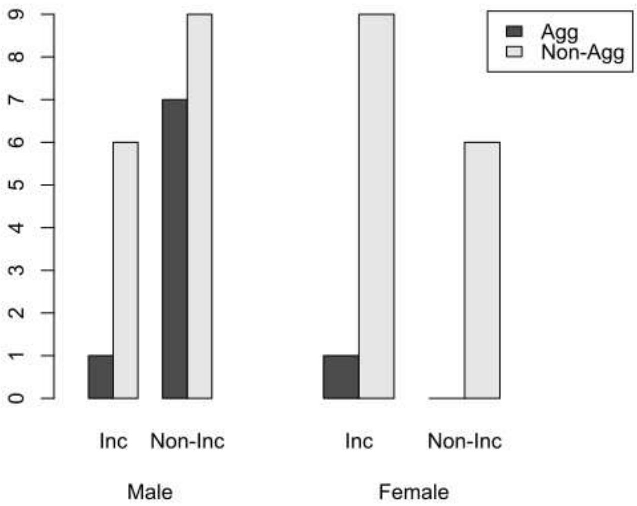

*Figure 5: Counting of male and female aggressive and/or incubating individuals of Campo Miner (*Geositta poeciloptera*). Inc: birds incubating. Non-Inc: Birds not incubating. Agg: aggressive birds (responded to playback with display behavior). Non-Agg: non-aggressive birds (no display behavior).*

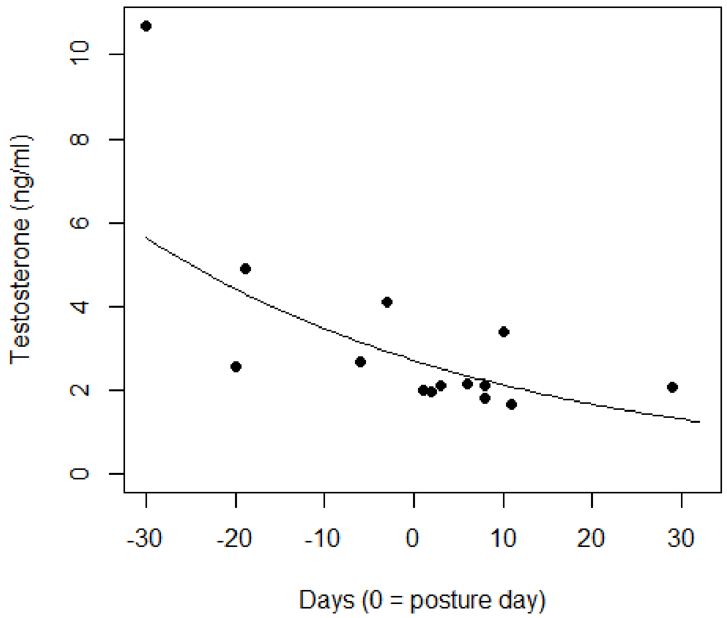

*Figure 6. Testosterone levels in relation to egg-laying date (standardized in 0) in the Campo Miner (*Geositta poeciloptera*). The bird T levels tend to decrease after laying the egg, according to an exponential regression, however the model explained a low percent of the variation observed (r^2= 0.4889).*

Discussion

Seasonal plasma T levels pattern

As mentioned by Hau and collaborators (2008), most of the studies made in the tropics were made in habitats with low seasonality a (table 1), leading to a bias in the overall knowledge of hormonal traits in that region. Here we hypothesize that tropical birds living in highly seasonal/unpredictable habitats and that, consequently, do not hold year-round territories, show marked seasonal differences in T levels, which peak when territories are being established, in a hormonal pattern similar to that exhibited by temperate zone birds. This distinct pattern can be attributed to the habitat of the Campo Miner, the grasslands, which are markedly seasonal and highly dynamic in space and time (Hau et al. 2008; Silva e Silva 2008; Murphy & Bowman 2012). Every year the bird can be forced to establish new territories (but it can use the same territory, if the environmental conditions do not change significantly from year to year), what could lead to frequent aggressive encounters. The species also presents comparatively short breeding season (4-5 months), what is often associated with higher T levels, possibly resulting from synchronous nesting and increased competition for extra-pair fertilization (Goymann et al. 2004). Maintenance of high T levels costs energy and indirectly inhibits immune function, increasing the susceptibility to parasites (Owen-Ashley et al. 2004; Mougeot et al. 2005). In this sense, species with short breeding seasons present a marked increase in T levels during the entire breeding season, because such increase sustain greater reproductive intensity, leading also to intense and frequent fights for territory establishment and defense. On the other hand, species with long breeding season avoid this costly strategy (Wingfield et al. 2001; Hau et al. 2010).

Testosterone in females

Although the males of Campo Miner have higher T levels than females, the comparatively high levels of T detected in females (mean 1.93 ng/ml, but the peak 3.12 ng/ml) corroborate with the observation of Ketterson et al. (2005) that females of species with a socially monogamous mating system express higher T levels than females of species with other mating systems (Ketterson et al. 2005). Females of the Campo Miner also showed peaks of T levels in the middle of the breeding season, what might indicate an aggressive event or a pre-laying peak. Aggressive events occurs mostly when territories are being established, or when females are disputing male paternal care (Ketterson et al. 2005). On the other hand, in laying hens, for example, the luteinizing hormone stimulates the thecal layer of the developing follicle within the ovary to produce T (Johnson 2014), which is after converted to an estrogen by adjacent granulose cells (Etches & Duke 1984; Gómez et al. 2001). Besides that, T also regulates the production of albumin by the oviduct (Yu & Marquardt 1973). As the peak was measured in a single female in the middle of breeding season and in the very onset of incubation (one day after the first egg was laid; the mean clutch for the species is two eggs; Silva 2015), the explanation for this peak is probably more related laying traits, than to aggressiveness.

Relationship between testosterone and behavior

According to the binomial GLM model (Figure 4), the Campo Miner aggressiveness is related to T, as both song output and T fluctuate together throughout the year. This same pattern occurs to other tropical and temperate birds, the European Nuthatch (*Sitta europaea*) showed stronger territorial behavior in spring, during the seasonal peak of T (Landys et al. 2010). The Campo Miner is aggressive mostly before egg-laying, afterward the bird show less aggressiveness, as demonstrated by the unresponsiveness to the playback of the majority of males while incubating or feeding their young (Figure 5). This evidence corroborates with Silva (2015), who observed that the bird becomes markedly less aggressive after egg laying, we also support the author when it says that male and female could be discriminated by behavior, since our data showed much more female than and male singing. The decrease in male aggressiveness could be related to the decrease of T in the beginning of paternal effort (Figure 6), as demonstrated by previous experiments with manipulated elevation of T and that resulted in a decrease in the paternal effort (Hegner & Wingfield 1987; Peters 2002; Lynn et al. 2009). Nevertheless, T levels seem not to influence paternal effort in all species, as demonstrated for the Chestnut-collared Longspur (*Calcarius ornatus*). Males of the species, even with an additional testosterone supplement, did not change its paternal effort during nestling care, even though the bird reduced sentry behavior and increased aerial display songs during the incubation phase (Lynn et al. 2002).

With the data available, the decrease of T levels after egg laying seems to continue throughout the rest of the breeding season, even in face of a subsequent breeding attempt (multiple breeding attempts in a single breeding season are common in the species; Silva 2015). Nonetheless, it is possible that a small increase in T levels briefly occurs in the interval between nest attempts, when the territory defense behavior back to happens, as suggested by a momentary increase in singing output (pers. obs.) in some birds (Wingfield et al. 1990). Secondary peaks in T levels between nest attempts is a possible explanation for the low percent of variance explained by the model (r^2=0.4889), but this hypothesis needs to be tested with a much large sample size.

Conclusion

Our findings show that the Campo Miner T levels shows a pattern that is similar to the patter reported for bird in temperate zones, probably because the length of breeding season (< 5 months) and the seasonal defense of territory (the species does not hold year-round territories). To date, most T studies for tropical birds where conducted with birds inhabiting humid forests, a mostly non-seasonal habitat. We endorse Hau et al. (2008) statement that, with more studies in markedly seasonal environments, more species of tropical birds will reveal T levels similar to temperate ones.

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Appendix 1: researches surveyed and respective references.

Species	Articles	Territorial defense	Number of articles
Review	Goymann & Landys, 2011; Goymann et al. 2004; Hau et al. 2008; Stutchbury & Morton, 2008		4
Acrocephalus sechellensis	van de Crommenacker et al. 2004	year-round	1
Chlamydotis undulata	Jalme et al. 1996	breeding season	1
Cyphorhinus phaeocephalus	Busch et al. 2008; Wikelski et al. 2003	year-round	2
Falco sparverius	Pereira, 2008	year-round	1
Gallirallus philippensis	Wiley & Goldizen, 2003	year-round	1
Gymnopithys bicolor	Wikelski et al. 2003	year-round	1
Habia fuscicauda	Chiver et al. 2014	year-round	1
Hylophylax naevioides	Hau et al. 2004; Hau et al. 2000, Wikelski et al. 1999; Wikelski et al. 2003	year-round	4
Malarus cyaneus	Peters, 2002	year-round	1
Manacus vitellinus	Schlinger et al. 2008; Day et al. 2007; Day et al. 2006; Wilkelski et al. 2003	breeding season	4
Myrmeciza longipes	Fedy & Stutchbury, 2006	year-round	1
Pipra mentalis	Wilkelski et al. 2003	breeding season	1
Thraupis episcopus	Wikelski et al. 2003	non-territorial	1
Thryothorus leucotis	Gill et al. 2008, Gill et al. 2007	year-round	2
Turdus grayii	Wikelski et al. 2003	breeding season	1
Vanellus chilensis	Pöerschke, 2011	year-round	1
Volatinia jacarina	Lacava, 2009	breeding season	1
Zonotrichia capensis	Moore et al. 2004, Moore et al. 2002, Lynn et al. 2009, Addis et al. 2011, Addis et al. 2010	breeding season	5

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4. CONCLUSÃO GERAL

O andarilho (*Geositta poeciloptera*) é uma ave vulnerável de extinção do Cerrado brasileiro que habita e é dependente dos campos naturais. Neste estudo, percebeu-se que a presença da ave possui relação com áreas queimadas, segundo um modelo em binomial negativa. A ocorrência da ave é maior em áreas queimadas antes de cinco anos, diminuindo subsequentemente. Futuros estudos sobre a quantidade e riqueza dos insetos da dieta da ave serão importantes para averiguar se há maior quantidade de insetos em áreas queimadas, ou apenas se a vegetação queimada facilita a visualização da presa pelo predador. O padrão dinâmico do fogo nos Campos do Alto Rio Grande parece abrir novas áreas de ocupação para *G. poeciloptera*. A espécie ocupa um território e o defende durante parte do ano, ficando agressiva neste período. A testosterona (T) esteve relacionada com o comportamento agressivo da ave, medido pelo canto. Além disto, a variação temporal de T em *G. poeciloptera* parece seguir os encontrados em aves de região temperada. Entretanto, a maior parte das pesquisas realizadas nos Neotrópicos foram executadas em áreas de floresta, onde as características do habitat são mais estáveis. Por outro lado, o Cerrado, por sua natureza, é um habitat de marcada sazonalidade (como são as regiões temperadas). É provável que, com mais pesquisas em habitat campestre/savânico, mais espécies com padrão temperado serão encontradas. *G. poeciloptera* carece de estudos sobre proporção de cópula extrapar e comportamento agressivo, pois estes fatores também influenciam a variação temporal de T nas aves, portanto, para se chegarem a conclusões mais sólidas a respeito da biologia hormonal da espécie, estes estudos são imprescindíveis.