Universidade de Brasília
Instituto de Ciências Biológicas
Programa de Pós-Graduação em Ecologia

# O comportamento reprodutivo do tiziu (*Volatinia jacarina*) sob risco de predação

**PEDRO ZURVAINO PALMEIRA MELO ROSA DE MORAES**

Orientadora: Regina H. F. Macedo

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Tese apresentada ao Programa de Pós-Graduação em Ecologia, do Departamento de Ecologia da Universidade de Brasília, como parte dos requisitos para a obtenção do grau de Doutor em Ecologia

Brasília, DF
2018

# AGRADECIMENTOS

Agradeço primeiramente aos meus pais, Sr. Wilson e Sra. Shirley de Moraes. Muitos são os percalços de um pós-graduando até a obtenção de seu título e muitas são as mãos e mentes que se disponibilizam a tornar este projeto viável até o seu fim. Mas sem a base erguida pelos meus pais, meu doutorado teria um fim antes mesmo de ter um começo. Agradeço por toda ajuda, por todo apoio, pela geladeira transbordando a cada visita e pela confiança em me deixar livre para seguir meu caminho. Amo vocês.

Agradeço à minha orientadora, Dra. Regina Macedo. Agradecer apenas pela orientação nesta tese seria muito pouco perto de como o meu universo se expandiu graças às suas atitudes e seus ensinamentos. Obrigado por entender que seus alunos são humanos antes de serem pós-graduandos. Obrigado por acreditar em mim e me fazer acreditar em mim. Obrigado por todas as portas abertas. E também por todas as janelas, abertas pra fora e pra dentro.

Agradeço imensamente ao meu colega de laboratório e amigo Dr. Pedro “Vovô” Diniz. Por estar me apoiando desde o começo do doutorado, ao ter a paciência de ensinar os métodos da ornitologia a um cara que nunca trabalhou com aves. Suas contribuições a este trabalho vão além da coautoria pelas análises estatísticas. Obrigado pelas amenidades, pelas seriedades e por abrir esse teu coração levemente autista a este seu xará. Obrigado por colaborar em um ambiente que nos pressiona a competir.

Agradeço a Dra. Lilian Manica, ao Dr. André Guaraldo, ao Dr. Eduardo Bessa e ao Dr. Miguel Marini por aceitarem ser membros da banca avaliadora desta tese. Acredito que a contribuição de vocês será muito importante para a publicação dos capítulos aqui presentes.

Agradeço ao Dr. Dida Mendes, ao Dr. Miguel Marini e ao Dr. Raphael Igor Dias por participarem da banca de qualificação do meu projeto de doutorado.

Agradeço à Coordenação de Aperfeiçoamento de Pessoal de Nível Superior e ao Conselho Nacional de Desenvolvimento Científico e Tecnológico pelas bolsas de estudo concedidas e pelo financiamento deste trabalho. Também sou grato à Fundação de Apoio à Pesquisa do Distrito Federal e ao Programa de Pós-Graduação em Ecologia da Universidade de Brasília pelos auxílios a viagens e congressos concedidos durante o doutorado. Muito obrigado a Animal Behavior Society pelos auxílios de pesquisa

concedidos através do *Student Research Grant* e do *Developing Nations Research Grant*, e pelos auxílios de viagem para os três encontros da sociedade que tive a oportunidade de participar durante o doutorado.

Agradeço ao Instituto Chico Mendes de Conservação da Biodiversidade e à Comissão de Ética no Uso Animal do Instituto de Ciência Biológicas da Universidade de Brasília pelas licenças para a realização deste trabalho.

Agradeço aos professores dos Departamentos de Zoologia e Ecologia da Universidade de Brasília pelos ensinamentos durante as disciplinas de meu curso de doutorado. Em especial, gostaria de agradecer às professoras Dra. Isabel Schmidt, Dra. Mercedes Bustamante e Dra. Rosana Tidon pelo exemplo de pesquisadoras e pela dedicação com que transmitem conhecimento aos seus alunos.

Agradeço aos meus colegas de laboratório (Carlos Biagolini, Guilherme Álvares, Ingrid Paneczko, João Victor Caetano, Julia Borges, Leonardo Castilho, Lia Kajiki, Milene Gaiotti, Paula Sicsu, Pedro Diniz, Pedro Ribeiro, Renata Alquezar, Rodrigo Borges) pela convivência ao longo destes cinco anos. Apesar da rotina maçante, ir ao laboratório sempre foi motivo de alegria por encontrar vocês, seja para discutir ciência, desabafar ou mesmo rir à toa. Vocês fazem falta.

A todas as pessoas que me ajudaram em campo ou no cuidado com os tizius em cativeiro. Eu não tenho uma memória boa e agradecer de um em um certamente resultaria na injustiça de esquecer um nome ou muitos. No entanto, alguns estagiários se destacaram pelo desempenho e dedicação a este trabalho. Meu muito obrigado aos estagiários e estagiárias Angélica Morais, Camila Bôa, Caren Sotto, Flávia Lisboa, Gianlucca Rech, Jhessica Couto e Lucas de Castro. Além de estagiários, muitos amigos se voluntariaram a ajudar em algumas saídas de campo, então aqui fica meu muito obrigado para: Carlos Biagolini, Desirée Ramos, Elba Militão, Gabriela Correia, Ingrid Paneczko, Julia Borges, Julia Machado, Lilian Manica, Pedro Diniz, Pedro Ribeiro, Renata Alquezar, Roberto Ricart, Rodrigo Borges e Thiago Filadelfo. Não é fácil acordar cedo todo dia para estar no meio do mato antes do sol nascer, mas todos vocês adicionaram leveza ao trabalho de campo e, por isso, sou muito grato.

Agradeço ao Dr. Esteban Fernandez-Juricic por me acolher em seu laboratório na Universidade de Purdue durante o meu doutorado sanduíche. Minha estadia em Purdue foi um aprendizado intenso no âmbito profissional e no pessoal. Me tornei um cientista melhor sob a orientação do Esteban e espero que nossa colaboração continue por um bom tempo e renda muitos frutos. Sou muito grato ao pessoal do Laboratório de Ecologia Sensorial de Purdue por me acolher como um dos seus e me ajudar em diversos aspectos da minha adaptação ao novo ambiente. Um agradecimento especial a Patrice Baumhardt-Helm e ao Dr. Benny Goller, pela disposição infinita em me guiar pela nova rede acadêmica e pelo companheirismo do dia-a-dia. Agradeço também à Dra. Ximena Bernal por toda a ajuda ao longo da minha estadia em Purdue, por ser um grande exemplo de ser humano e pelo Barley. Não posso também deixar de agradecer à família Helm (Brandon, Patrice e Sydney) por me fazer se sentir em casa. Por fim, obrigado à Coordenação de Aperfeiçoamento de Pessoal de Nível Superior pelo financiamento de meu doutorado sanduíche na Universidade de Purdue. As diferentes perspectivas às quais fui submetido neste sanduíche me fizeram retornar ao Brasil com uma vontade ainda maior de contribuir com a ciência e educação de nosso país.

Foram muitas as amizades criadas e cultivadas em Brasília e eu sou muito grato a todas elas por fazerem desta cidade complicada um improvável lar. Aqui eu tenho que ser breve, pois o espaço pede agilidade, embora seja muita gratidão para poucas linhas disponíveis. Eu queria, no entanto, expressar gratidão para algumas pessoas específicas que são representantes de dezenas de outros corações e mentes, cada qual sendo muito importante em determinados momentos de minha estadia em Brasília. Um muito obrigado ao David Cho pelo acolhimento no meu primeiro mês nesta cidade e pela grande amizade. Obrigado à Candida Souza por me oferecer pontos de vista, por mostrar que não estamos sós e por me apresentar pessoas incríveis. Obrigado à Narjara Grossmann pela inconvencionalidade que pinta a minha vida. Obrigado à Samia Gomes pelos momentos de leveza nos corredores, no BDA e nos batuques. Obrigado à Thallita Grande pela velha amizade e pelo grupo de apoio aos surtados de comunidades e ecossistemas. Obrigado ao Victor de Souza por ser meu *hub* brasileiro em Purdue (achou que seria a Talita? Achou errado).

Por fim, gostaria de agradecer àqueles que tomam frente na luta pela ciência livre, sem a qual eu teria um acesso extremamente limitado à literatura que embasou esta tese. Muito obrigado a Aaron Swartz, Alexandra Elbakyan e tantos outros pela resistência.

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## RESUMO

Dentre as pressões seletivas que podem influenciar a evolução de diferentes estratégias comportamentais, o risco de predação pode ser considerado uma das mais importantes por potencialmente ceifar as oportunidades futuras de aptidão de um animal. Diversos estudos com os mais variados táxons já demonstraram a plasticidade comportamental que animais apresentam em suas estratégias reprodutivas sob risco de predação. Predadores podem influenciar, por exemplo: as estratégias de busca e atração de parceiros apresentadas por machos, o padrão de escolha de parceiros por fêmeas e o cuidado parental realizado por ambos. Embora estes efeitos do risco de predação na reprodução sejam bem conhecidos, não há muitos estudos que utilizem aves neotropicais como modelos. Além das plumagens e *displays* conspícuos exibidos por estas aves, elas vivem em ambientes com taxas de predação elevadas. Logo, é interessante investigar como estes organismos conseguem se reproduzir de maneira tão conspícua mesmo correndo o risco de atrair predadores e diminuir suas chances de sobrevivência. Os machos de tiziu (*Volatinia jacarina*), por exemplo, apresentam um *display* sexual composto por saltos, canto e exibição de plumagem ornamentada. Além disso, os ninhos dessa espécie sofrem altas taxas de predação e estudos passados com o tiziu já demonstraram que a exibição sexual de machos pode atrair predadores para o ninho. Mas nenhum trabalho até o momento investigou se e como machos e fêmeas de tiziu modificam suas estratégias reprodutivas de modo a contornar o risco de predação iminente. Esta tese, portanto, objetivou responder perguntas acerca da reprodução do tiziu sob risco de predação. Especificamente, investiguei como predadores influenciam: o comportamento sexual dos machos; a escolha de parceiros pelas fêmeas; e o cuidado parental dedicado aos ninhegos. Experimentos foram executados em condições naturais e em cativeiro onde utilizei *playbacks* acústicos de aves simpátricas ao tiziu para simular diferentes níveis de risco de predação: predador de adultos, predador de ninhos e controle sem risco. Quanto à execução de *displays* sexuais, eu observei que machos modificam sua performance de acordo com o risco de predação, mas este interage com a condição individual. Machos mais parasitados e menos ornamentados aumentam a intensidade de seus *displays* com o aumento do risco de predação, o que pode ser considerado uma estratégia de investimento terminal. Quanto à escolha de parceiros, eu observei que fêmeas são indiferentes aos estímulos dos machos apresentados e este padrão de ausência de escolha foi independente do risco de predação. Aqui, eu discuto como esse resultado pode significar um padrão de seleção indireta de parceiros nesta espécie, através da disputa de machos por territórios. Por fim, eu observei que tizius modificam o cuidado biparental de acordo com o risco de predação. A resposta parental, no entanto, pode ter sido influenciada pelo dicromatismo sexual da espécie: machos, mais conspícuos, diminuem suas atividades no ninho, enquanto fêmeas, mais crípticas, aumentam a duração dos turnos de incubação. Estas estratégias dos sexos podem ter o objetivo comum de diminuir a detectabilidade visual e acústica do ninho por predadores. Estes resultados demonstram as adaptações comportamentais de uma espécie que sobrevive e reproduz em um ambiente com altas taxas de predação, como é o caso da região Neotropical.

Palavras-chave: *display* sexual; escolha de parceiros; cuidado parental; hipótese do investimento terminal; seleção de parceiros indireta; dicromatismo sexual.

## ABSTRACT

Predation risk is a major evolutionary pressure that influences the evolution of different behavioral strategies, given that a predation event will result in the animal losing all future fitness. Several studies with diverse taxa have demonstrated the behavioral plasticity that animals present in their reproductive strategies under predation risk. For instance, predators may influence: male mate searching strategies and mate attraction, female partner choice and biparental care. Although predation risk effects on reproduction are well known, there is a shortage of research using Neotropical birds as study models. In addition to the conspicuous plumages and displays exhibited by these birds, they live in high predation rate environments. Therefore, it is worthwhile to investigate how these organisms are able to reproduce conspicuously even under the risk of attracting predators and, hence, reducing survival probability. Males of the Neotropical songbird blue-black grassquit (*Volatinia jacarina*), for instance, perform sexual displays composed of conspicuous leaps and songs and have ornamented plumages. Further, there is a high nest predation rate for this species and past studies showed that male sexual displays attract predators to the nest. But no study until this moment has investigated how grassquit males and females modify their reproductive strategies to decrease predation risk. Therefore, the objective of this thesis was to answer questions related to grassquit reproduction under predation risk. Specifically, I investigated how predators influence: male sexual behavior, female partner choice, and biparental care. I performed experiments in field and captive conditions and used acoustic playback of sympatric birds to simulate different levels of perceived predation risk: predator of adults, predator of nests, and no-risk control. Regarding sexual display performance, I observed that males modify their displays according to predation risk, but this effect interacts with individual condition. Less parasitized and more ornamented males increase display intensity under high predation risk, which may be considered a terminal investment strategy.

Regarding mate choice, I found that females are irresponsive to male stimuli and this lack of choice pattern was independent of predation risk. Here, I discuss how this result possibly means that grassquit females perform an indirect mate choice based on male competition for territories. Lastly, I observed that grassquits modify biparental care according to predation risk. Parental responses, however, may be influenced by the sexual dichromatism presented by grassquits: conspicuous males reduce nest activities, while cryptic females increase the duration of brooding bouts. These sex-related responses may be strategies with the common goal to reduce nest visual and acoustic detectability by predators. These results highlight the behavioral adaptations of a species that survives and breeds in high predation rate environments such as the Neotropics.

Keywords: sexual display; mate choice; parental care; terminal investment hypothesis; indirect mate choice; sexual dichromatism.

# INTRODUÇÃO GERAL

A partir das premissas da teoria evolutiva e da ecologia comportamental, espera-se que um animal administre seu orçamento temporal diário em atividades como forrageio, socialização e reprodução, de modo a garantir sua sobrevivência e aptidão (Alcock, 2009; Darwin, 1859; Krebs & Davies, 1993). Embora a busca por alimentos e parceiros reprodutivos exerça pressões seletivas relevantes na evolução de traços que propiciem a otimização destas atividades, um animal que não se alimente em um dia ou que não consiga acasalar em uma estação reprodutiva pode ter oportunidades futuras para cumprir estas necessidades. O mesmo não acontece no caso de o animal ser predado, quando suas perspectivas de aptidão futura são anuladas. O risco de predação, portanto, é considerado uma das pressões seletivas mais influentes na evolução de traços morfológicos e comportamentais em animais (Lima & Dill, 1990).

São muitos os exemplos de comportamentos de animais de grupos diferentes que podem ter suas origens ligadas a um risco de predação iminente. A alta mortalidade da prole de aves por ataques de predadores pode ter selecionado traços ligados à construção de ninhos e ao cuidado parental (Fontaine et al., 2007). A própria vida em grupo em muitos invertebrados e vertebrados pode ter surgido como uma estratégia de efeito de diluição ou para aumentar as chances de defesa contra predadores (Hamilton, 1971). Portanto, a investigação da influência que predadores podem exercer sobre suas presas pode auxiliar o melhor entendimento de muitos dos comportamentos apresentados por animais.

Por exemplo, por muito tempo se pensou que a taxa de alimentação seria o principal fator de variação nas estratégias reprodutivas de aves, uma vez que a quantidade de alimento consumido pode influenciar a fecundidade de um animal (Martin, 1987). Contudo, ao incluir o efeito do risco de predação de ninhos nas taxas de fecundidade de pássaros norte-americanos, Martin (1995) observou que a pressão seletiva exercida por predadores apresentava uma maior influência na reprodução e sobrevivência destas aves, maior até do que a exercida pela necessidade de alimentação. Desde então, muitos estudos têm demonstrado o efeito do risco de predação sobre diversos aspectos comportamentais de pássaros, como a escolha dos sítios de nidificação (Eggers et al., 2006), o tamanho da ninhada (Martin et al., 2000a), a taxa de entrega de alimento aos filhotes no ninho (Ghalambor & Martin, 2000), e o período de incubação (Massaro et al., 2008).

O efeito do risco de predação na reprodução não se limita à criação da prole, mas também pode ocorrer antes mesmo da fecundação. Na maioria das espécies animais, os machos precisam se engajar ativamente em estratégias voltadas para a atração de fêmeas e oportunidade de cópulas (Darwin, 1871). Muitas dessas estratégias envolvem a apresentação de ornamentos, seja de partes chamativas do corpo ou de objetos encontrados no ambiente, e/ou a realização de comportamentos ritualizados e conspícuos (i.e. *displays*). Embora o foco principal destes comportamentos seja a atração de fêmeas, os machos também estão sujeitos à detecção por predadores quando realizam seus *displays* sexuais (Stuart-Fox et al., 2003; Zuk & Kolluru, 1998). Portanto, a evolução de caracteres sexuais em machos pode ser resultante de um *trade-off* entre a capacidade de atração de fêmeas e a necessidade de evitar-se a predação. É válido notar que fêmeas de aves, normalmente não sujeitas às pressões mais intensas da seleção sexual, apresentam colorações crípticas que as camuflam no ambiente, provavelmente em resposta ao risco de predação (Caro, 2005).

A presença de predadores no ambiente também pode afetar a escolha das fêmeas por parceiros para o acasalamento. Uma vez que o maior risco de predação pode aumentar os custos relacionados à reprodução, fêmeas podem se comportar de maneira oportunista e não discriminar os machos de acordo com a qualidade demonstrada por seus *displays* sexuais (Godin & Briggs 1996). Contudo, a evolução de ornamentos sexuais em machos a partir da escolha das fêmeas pode ter relação com a maior exposição do sexo masculino a predadores. Segundo esta hipótese (Zahavi, 1975), em um ambiente com alto risco de predação, fêmeas podem preferir machos mais ornamentados por estes demonstrarem sua melhor qualidade, seja na capacidade de prover e defender a prole ou mesmo na herança genética de melhores habilidades de evite de predação.

Dada a influência do risco de predação sobre os diversos comportamentos reprodutivos, fica claro que predadores não são apenas agentes da seleção natural que afetam diretamente a sobrevivência de uma presa, mas também podem exercer pressão evolutiva nas tomadas de decisão referentes a seleção intra e intersexual.

### *Displays sexuais sob risco de predação*

Diferentemente da seleção natural, que promove a evolução de traços ligados à maior sobrevivência e/ou reprodução de uma estirpe, a seleção sexual atua sobre traços que aumentem o potencial reprodutivo de um organismo, mesmo que sua capacidade de sobrevivência seja diminuída no processo (Darwin, 1871). Em muitos casos, *trade-offs* são observados na ontogenia e no comportamento de um mesmo indivíduo, onde o investimento na atração de parceiros, na maior fertilização de gametas e/ou na criação de uma maior prole resulta em um decréscimo significativo do tempo de vida do mesmo, e vice-e-versa (Brooks, 2000; Hunt et al., 2004). Em termos práticos, um organismo bem adaptado ao seu ambiente será aquele que consegue otimizar suas atividades, de modo a se manter vivo por tempo suficiente para garantir sua representatividade genética na geração seguinte (Andersson, 1994). É uma fórmula aparentemente simples, que se complica com a introdução da dinâmica presa-predador.

Do ponto de vista de uma presa que precisa se reproduzir, encontrar um parceiro reprodutivo em um ambiente com alto risco de predação pode ser uma tarefa difícil. Buscar ativa e furtivamente por um parceiro pode demandar um tempo e exigir uma quantidade de energia que um animal não tem condições de investir, tendo em vista que sua manutenção também depende de outras atividades, como forrageio (Krebs & Davies, 1993) ou mesmo tempo para descanso. Um modo de economizar tempo é alertar a sua posição a potenciais parceiros reprodutivos, emitindo sinais em uma ou mais modalidades sensoriais no ambiente e alcançar receptores atentos a estes sinais (Bradbury & Vehrencamp, 2011). Contudo, não apenas parceiros reprodutivos podem captar esta sinalização; predadores também podem estar entre os receptores (Stuart-Fox et al., 2003; Zuk & Kolluru, 1998).

Como machos geralmente competem pelo acesso às fêmeas, muitas vezes por exibições visuais e acústicas chamativas, é este sexo que costuma enfrentar o *trade-off* entre atração de parceiras e de predadores (Godin, 1995). Para lidar com predadores interceptadores de sinalizações, machos dos mais diversos grupos taxonômicos apresentam estratégias reprodutivas que variam de acordo com o contexto predatório. Por exemplo, machos da esperança Neotropical *Docidocercus gigliotosi* atraem parceiras emitindo sinais acústicos no ar (Römer et al., 2010). No entanto, estes sinais são interceptados pelo morcego *Lophostoma silvicolum*, que utiliza a sinalização dos machos para detectá-los e depois predá-los. Em noites de lua cheia, quando a maior iluminação no ambiente permite uma localização visual mais eficiente por parte dos morcegos, os machos de *D. gigliotosi* aumentam a frequência de sinalizações por estridulação no substrato. Esta modalidade sensorial é mais custosa que a emissão de vibrações no ar e alcança apenas receptores que compartilham o mesmo substrato que o emissor. Logo, machos desta espécie optam por estratégias menos eficientes e que demandam mais energia na atração de fêmeas em contextos de maior risco de predação, de modo a evitar o alto custo de ser predado ao empregar estratégias menos custosas, porém mais conspícuas (Römer et al., 2010).

A literatura está repleta de exemplos de como presas modificam seus comportamentos, reprodutivos ou não, quando confrontadas com um maior risco de predação. Contudo, a maior parte dos estudos focados em reprodução tem utilizado artrópodes e vertebrados como peixes, anfíbios e lagartos como modelos experimentais (Lima & Dill 1990, Magnhagen 1991) e apenas recentemente alguma atenção tem sido dada às aves (e.g. Grunst et al. 2015). Tendo em vista a conspicuidade visual e acústica que aves, especialmente machos em estágio reprodutivo, podem apresentar, é interessante que estudos sejam realizados para se averiguar como organismos tão chamativos realizam suas atividades reprodutivas ao mesmo tempo em que lidam com o custo decorrente da atração de predadores.

*Escolhendo parceiros sob risco de predação*

Segundo os preceitos da seleção sexual (Darwin 1871), o sexo que fornece o menor investimento médio na reprodução (normalmente os machos) deve competir entre si para ter acesso a oportunidades reprodutivas com parceiros do sexo que apresenta o maior investimento em reprodução (normalmente fêmeas). Enquanto machos interagem agressivamente ou executam exibições contínuas, vigorosas e conspícuas, às fêmeas é atribuída a escolha de qual macho será o genitor de sua prole. Essa tomada de decisão da fêmea pode ser influenciada por fatores intrínsecos, como genótipo, hormônios e capacidades sensoriais (Brooks & Endler, 2001; Lynch et al., 2006; Ronald et al., 2012), e por fatores externos, como o contexto social em que as fêmeas estão inseridas e as variáveis ecológicas do ambiente (Hunt et al., 2005; Madden & Whiteside, 2013).

Um possível fator a influenciar a escolha das fêmeas é o risco de predação. Ao realizar seus *displays* sexuais ou ostentar estruturas morfológicas chamativas, machos se expõem a um maior risco de predação, o que é caracterizado como o “princípio da desvantagem” (Zahavi, 1975). Segundo esta proposta, um macho que consegue arcar com o custo da alta exposição a predadores e sobreviver em um ambiente hostil, mesmo apresentando estruturas ou comportamentos conspícuos e desvantajosos, é um macho de boa qualidade e, portanto, deve ser escolhido como parceiro reprodutivo pelas fêmeas. Por exemplo, fêmeas do peixe espada (*Xiphophorus helleri*) preferem machos que possuem nadadeiras caudais longas (MacLaren, 2017). Machos que apresentam esta característica, no entanto, são mais visados por predadores, que direcionam mais ataques a machos com caudas longas do que a machos que tiveram suas caudas removidas experimentalmente (Hernandez-Jimenez & Rios-Cardenas, 2012). Ao escolher um parceiro ornamentado em um ambiente com alto risco de predação, as fêmeas estariam garantindo, por exemplo, um cuidado parental mais eficiente, tanto na coleta de recursos como na defesa da prole por parte dos machos. Ainda segundo essa hipótese, em casos em que as fêmeas não recebem benefícios diretos dos machos, os filhotes podem herdar os caracteres genéticos responsáveis pelas características antipredatórias do macho.

Por outro lado, o risco de predação pode ter um efeito contrário ao esperado pelo princípio da desvantagem de Zahavi (1975). Por exemplo, Godin & Briggs (1996) observaram que fêmeas de *guppy* (*Poecilia reticulata*) diminuem sua discriminação entre machos e passam a escolher parceiros aleatoriamente quando confrontadas com predadores. Estes autores observaram também que as fêmeas consideram o padrão de coloração dos machos, preferindo machos mais coloridos, quando o risco de predação é baixo. Outro importante exemplo é o estudo de Johnson & Basolo (2003) com o peixe *Xiphophorus helleri*. Nestes peixes, as fêmeas não apenas alteram sua preferência pelos machos, como passam a evitar aqueles mais conspícuos e preferir os mais crípticos. Um caso extremo foi constatado por Willis et al. (2012), onde fêmeas de *X. birchmanni* não preferiam machos da própria espécie quando podiam escolher entre coespecíficos em ambientes de maior risco de predação e heteroespecíficos (*X. malinche*) em ambientes de menor risco, potencialmente aceitando o custo de gerar híbridos em troca do aumento na sobrevivência. Estes três estudos demonstram que o risco de predação pode influenciar a escolha de parceiros por fêmeas ao inserir um custo na sobrevivência destas caso se associem a machos conspícuos, o que potencialmente pode reduzir a seleção pelo sinal exagerado dos machos e frear a evolução do mesmo. Dada a diversidade de estruturas e comportamentos conspícuos utilizados na atração de parceiros (Darwin, 1871), é interessante questionar se fêmeas de diferentes espécies modificam ou não seu padrão de escolha de parceiros em diferentes níveis de risco de predação.

*Cuidando da prole sob risco de predação*

O cuidado parental é definido como todo e qualquer comportamento executado pelos pais que aumenta as chances de sobrevivência da prole atual, mas diminui a capacidade parental de investir em proles futuras (Trivers, 1972; Clutton-Brock, 1991). Conforme o nível de dependência da prole, o investimento parental pode ocorrer antes mesmo do nascimento do filhote, com fêmeas alocando recursos durante a formação do ovo (e.g. Coslovsky & Richner, 2011), até depois da maturação sexual da prole, quando uma vida longa permite que os filhos continuem em contato com os pais (e.g. Hawkes et al., 1997). O nível de cuidado alocado a uma prole específica pode variar com fatores ambientais, como a disponibilidade de alimento (Dewey & Kennedy, 2001) e o clima (Öberg et al., 2014). Assim, pais podem frequentemente enfrentar o *trade-off* entre criar uma ninhada que atualmente apresenta menores chances de sobrevivência, ou abandoná-la e reservar recursos para gerar uma nova prole quando as condições ambientais forem melhores (Clutton-Brock, 1991).

Um dos fatores que pode influenciar na decisão entre cuidar ou abandonar uma prole é o risco de predação, sendo este um dos principais motivos para o insucesso de ninhos em aves (Martin, 1995). Uma vez que predadores podem usar o comportamento dos pais para encontrar o ninho (Martin et al., 2000b), estes se veem obrigados a modificar o cuidado com a prole para garantir a sua sobrevivência. O cuidado parental em aves é reconhecidamente plástico em situações de maior risco de predação, com pais alterando nessas condições, por exemplo, o tamanho da ninhada (Martin et al., 2000a), a taxa de provisionamento aos filhotes (Ghalambor & Martin, 2000) e o período de incubação (Massaro et al., 2008).

Um fator que pode interagir com o risco de predação e influenciar o cuidado parental é a coloração apresentada pelos pais. Em espécies de aves sexualmente dicromáticas, fêmeas comumente apresentam cores mais pálidas, enquanto machos são bastante conspícuos (Andersson, 1994). Como indivíduos conspícuos são mais facilmente detectados por predadores visualmente orientados (Endler, 1992), o cuidado biparental pode ser diferenciado de acordo com o sexo em espécies dicromáticas (Colombelli-Négrel & Kleindorfer, 2010; Ekanayake et al., 2015; Krištofková et al., 2011). Por exemplo, machos da batuíra-de-capuz-vermelho (*Charadrius ruficapillus*) incubam durante a noite, enquanto fêmeas ficam responsáveis pela incubação durante o dia (Ekanayake et al., 2015). Esta espécie apresenta dicromatismo sexual na plumagem da cabeça, onde machos apresentam penas mais avermelhadas que fêmeas. Predadores ativos durante o dia e que utilizam a visão para encontrar suas presas predam machos em maior frequência que fêmeas; no entanto, não há diferença na taxa de predação entre os sexos durante a noite, quando predadores visuais estão inativos (Ekanayake et al., 2015). Assim, dadas as diferenças sexuais de coloração e detectabilidade por predadores, comportamentos parentais gênero-específicos podem evoluir em resposta ao risco de predação.

Além da presença ou ausência momentânea de predadores nas proximidades do ninho, o cuidado parental em aves também está relacionado ao risco de predação histórico ao qual uma espécie e seus ancestrais foram submetidos (Ghalambor et al. 2013). Em um estudo comparativo entre espécies de zonas temperadas e de zonas subtropicais, as quais sofrem historicamente diferentes taxas de predação (Ricklefs 1969, Skutch 1985), Ghalambor et al. (2013) observaram que todas as espécies estudadas apresentaram uma mudança significativa no comportamento de cuidado parental com o aumento do risco de predação percebido. Contudo, espécies que vivem em ambientes com maiores taxas de predação (i.e. subtropicais) tiveram respostas mais intensas às pistas dos predadores, reduzindo o risco de predação no ninho com o custo concomitante de reduzir a taxa de provisionamento dos filhotes. Embora espécies tropicais sejam mais suscetíveis a predadores, a maior parte dos estudos sobre predação de ninhos investigou espécies de zonas temperadas e pouco se sabe sobre o comportamento parental de aves Neotropicais sob risco de predação (Ibáñez-Álamo et al. 2015).

### *Espécie de estudo*

Tendo em vista a importância do risco de predação como pressão seletiva a influenciar diversas etapas de vida de um indivíduo, eu utilizei o tiziu (*Volatinia jacarina*) como modelo animal para responder perguntas referentes a este tema. O tiziu se mostra como um modelo promissor para este estudo por que, além de ser uma espécie Neotropical, esta ave também apresenta uma série de traços morfológicos e comportamentais que podem teoricamente se relacionar com a ação de predadores. Primeiro, durante a estação reprodutiva (novembro a abril; Sick, 1997), os machos desta espécie passam por uma muda e substituem o padrão de plumagem amarronzado críptico, também apresentado por fêmeas e juvenis, para uma coloração negro-azulada iridescente (Maia & Macedo, 2011). Não obstante a conspicuidade desse caractere sexual secundário, os machos desta espécie ainda defendem pequenos territórios onde realizam *displays* aéreos provavelmente ligados à defesa de territórios e à atração de oportunidades reprodutivas (Manica et al., 2013, 2017). Este *display* consiste em séries de saltos verticais, executados em pequenos poleiros na vegetação e repetidos intensivamente ao longo do dia, especialmente em dias ensolarados (Sicsú et al., 2013). Ao saltar, o macho bate as asas atrás da cabeça e exibe manchas de plumagem branca embaixo das asas que contrastam com a coloração preto-azulada do restante do corpo (Manica et al., 2013). No ponto mais alto do salto, o macho emite uma vocalização característica que se junta ao som mecânico produzido pela batida de asas. Experimentos anteriores demonstraram que este *display* multimodal é dependente da condição individual e, portanto, pode sinalizar condições inerentes do macho (Aguilar et al., 2007).

Com a atração de uma parceira, ambos os sexos formam um par reprodutivo socialmente monogâmico com cuidado biparental (Almeida & Macedo, 2001). O casal constrói um ninho em forma de taça utilizando grama e arbustos como substrato, ficando a alturas de 10 a 50 cm do chão (Almeida & Macedo 2001; Carvalho et al., 2007). O tamanho da ninhada varia de 2 a 3 ovos, que demoram cerca de 10 dias para eclodir, enquanto os ninhegos levam mais 10 dias para deixar o ninho (Carvalho et al., 2007). Mesmo com ninhos ativos em seus territórios, machos continuam executando *displays* aéreos como uma possível estratégia para atrair oportunidades de cópulas extra-par com fêmeas de territórios vizinhos (Manica et al., 2016). As fertilizações extra-par são elevadas nesta espécie, onde até 50% dos ninhegos podem não ser aparentados a um dos pais sociais (Manica et al. 2016). No entanto, a pressão por cópulas extra-par é um fator problemático, tendo em vista que predadores de ninhos podem ser atraídos ao território de machos que continuam executando *displays* (Dias et al. 2010). Além disso, as taxas de predação de ninho nesta espécie são elevadas e até 80% dos ninhos são predados em algumas estações (Macedo et al. 2012; Diniz et al. 2015).

Tizius, portanto, apresentam um sofisticado sistema social centrado no conflito entre seleção natural e sexual, onde indivíduos sofrem uma forte pressão competitiva para conseguir cópulas extra-par, enquanto uma alta mortalidade de ninhos pela ação de predadores reprime as tentativas reprodutivas. Sendo assim, a investigação de como o risco de predação influencia comportamentos reprodutivos do tiziu pode ajudar a entender como esta espécie está adaptada a ambientes de alto risco, além de servir de modelo para outras aves que sobrevivem e se reproduzem em ambientes hostis como o Neotrópico.

## *Objetivos da tese*

Com esta tese, eu objetivei investigar o possível efeito do risco de predação sobre comportamentos reprodutivos do tiziu (*Volatinia jacarina*). Dado o conflito adaptativo entre sobrevivência e reprodução, eu levantei e testei diversas hipóteses para tentar prever como o tiziu soluciona este *trade-off*. Eu executei três experimentos, tanto em ambiente natural quanto em cativeiro, utilizando *playbacks* acústicos de predadores e de aves que não oferecem risco para simular diferentes níveis de risco de predação. Em cada experimento, uma etapa diferente do comportamento reprodutivo do tiziu foi explorada.

No primeiro capítulo, eu investiguei como tizius machos modificam seus *displays* aéreos (um comportamento potencialmente ligado à competição entre machos e atração de fêmeas) em diferentes níveis de risco de predação. Três cenários hipotéticos foram testados. O primeiro deles previa que tizius machos devem priorizar a sobrevivência e reduzir a intensidade de seus *displays* em condições de maior risco de predação, quando comparados a situações controle de baixo risco (hipótese focada na sobrevivência; Lima & Dill, 1990; Magnhagen, 1991). O segundo cenário previa que comportamentos de risco seriam sexualmente selecionados e, portanto, machos deveriam aumentar a intensidade de seus *displays* quando o risco de predação fosse maior (hipótese do princípio da desvantagem; Zahavi, 1975). O último cenário previa que a resposta de um macho ao risco de predação seria dependente do seu potencial reprodutivo residual. Ou seja, machos com maior probabilidade de se reproduzir no futuro (i.e. machos de maior qualidade) devem reduzir a intensidade dos seus *displays* quando o risco de predação é alto (hipótese da proteção de recursos; Clark, 1994). Este cenário também prevê que machos com menor potencial reprodutivo residual (i.e. machos de menor qualidade) devem aumentar o investimento reprodutivo atual quando há um maior risco de morte extrínseca (hipótese do investimento terminal, Pianka & Parker, 1975; Trivers, 1972; Williams, 1966). Portanto, espera-se que machos de menor qualidade aumentem a conspicuidade de seus *displays* quando o risco de predação for maior. Para testar estas hipóteses, eu avaliei as alterações de *displays* de machos em ambiente natural frente a diferentes níveis de risco de predação simulado.

No segundo capítulo, eu investiguei como fêmeas de tiziu modificam seu padrão de escolha de parceiros frente a diferentes níveis de risco de predação. Desta vez, duas hipóteses foram testadas. A primeira delas previa que fêmeas deveriam preferir machos que aumentassem a intensidade de seus *displays* com o aumento do risco de predação. Esta predição foi baseada no princípio da desvantagem (Zahavi, 1975), onde machos com características exageradas são selecionados pela sua qualidade inerente em sobreviver em ambientes hostis mesmo apresentando traços conspícuos a predadores. A segunda hipótese previa um padrão contrário, com fêmeas escolhendo machos ao acaso ou mesmo preferindo machos com *displays* de intensidade reduzida. Isso porque, ao se associar com machos chamativos, fêmeas podem aumentar o risco de predação sofrido por elas mesmas e por sua prole (Dias et al., 2010; Marzal et al., 2016). Para testar estas duas hipóteses, eu realizei um experimento em cativeiro onde fêmeas foram colocadas em uma arena experimental com dois estímulos de machos de intensidades distintas (*playbacks* de canto) disponibilizados em cada lateral da arena. O posicionamento das fêmeas na arena, utilizado como um indicador da preferência pelo estímulo de um ou outro macho sendo simulado, foi registrado em diferentes níveis de risco de predação, também simulados através de *playbacks*.

No terceiro capítulo, eu avaliei como o cuidado biparental no tiziu é modificado de acordo com o risco de predação. Para tanto, ninhos de tiziu em campo foram observados durante a apresentação de diferentes níveis de predação simulados por *playbacks*. Desta vez, previmos que machos e fêmeas se comportariam de modo a reduzir a conspicuidade visual e acústica do ninho quando o risco de predação fosse maior. Contudo, esta redução da detectabilidade do ninho seria proporcionada diferentemente entre os sexos dado o dicromatismo sexual presente nesta espécie. Ou seja, machos de tiziu, que apresentam plumagem nupcial chamativa, devem reduzir suas visitas ao ninho e o tempo em que passam no mesmo, enquanto fêmeas, de plumagem mais críptica, devem passar mais tempo no ninho incubando e camuflando os filhotes. Dada a redução das atividades no ninho em resposta ao maior risco de predação, também foi previsto que machos e fêmeas devem aumentar o fornecimento de comida aos filhotes para evitar que os mesmos sofram um maior risco de inanição. Por fim, eu observei se outros comportamentos relacionados ao ninho também são afetados pelo risco de predação, como os movimentos de entrada e saída no mesmo e a execução de *displays* por machos nos seus arredores.

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# CAPÍTULO 1

# Flirting with danger: predation risk affects male aerial display in a Neotropical songbird

# ABSTRACT

Sexual signalling co-evolves with the sensory systems of intended receivers; however, predators may be unintended receivers of these signals. Conspicuous aerial displays in some species may place males at high risk of predation from eavesdropping predators. Here, we used blue-black grassquits (*Volatinia jacarina*) to test whether males modify leap displays under different levels of predation risk. Grassquit males develop an iridescent nuptial plumage and spend considerable time emitting a multimodal signal: while leaping from a perch, males clap their wings above their heads and emit a high-pitched short song. We exposed males to predator and non-predator playbacks while video recording their displays. Males in worse body condition exhibited more vigorous displays, regardless of predation risk. Additionally, predation risk influenced displays as a function of ectoparasite infestation and proportion of nuptial plumage coverage. Less parasitized males and those with higher proportion of nuptial plumage showed no response to predation risk. Alternatively, more parasitized males and those with lower proportion of nuptial plumage increased the vigour of displays under predation risk. In other words, males with low residual reproductive value increased reproductive effort when there was a high risk of extrinsic death. Our study provides empirical support for the terminal investment hypothesis.

Keywords: blue-black grassquit; sexual display; sexual selection; handicap principle; asset protection; terminal investment.

# INTRODUCTION

Male displays may have a high degree of conspicuousness to attract females, but these highly salient signals may also attract unintended receivers (Endler, 1992). Conspecific competitors, parasites, and predators may eavesdrop on the signal (Otte, 1974; Zuk & Kolluru, 1998). Therefore, selection is expected to favour those signallers that can accurately transmit information without suffering a drastic reduction in survival and fitness due to eavesdropping, ultimately affecting the evolution of male signals (Lewis & Cratsley, 2008; Zuk & Kolluru, 1998). Studies examining the implications of signalling conspicuousness within predator-risk environments are scarce and have rarely been conducted in the wild, often producing inconsistent or inconclusive results, casting doubt upon evolutionary explanations for signal evolution. Several hypotheses, with different predictions, have been raised in the literature, but lack empirical substantiation.

Intuitively, we expect that under high predation risk, natural selection should favour males that reduce the conspicuousness of their displays at the cost of decreasing their appeal to females, resulting in lower mating success. Here, we refer to this decision of adaptively reducing courtship to avoid predation as the survival focused hypothesis (predation avoidance behaviours; Lima & Dill, 1990; Magnhagen, 1991). This potential trade-off between survival and reproduction is often solved with males choosing survival and waiting for a future breeding opportunity (Godin, 1995; Römer et al., 2010), especially if they have high residual reproductive value (Williams, 1966). Alternatively, some males may tip the scale in favour of reproduction, especially if risk-taking is a sexually selected feature (handicap principle hypothesis; Zahavi, 1975). In this context, males should continue their display performance (or even enhance it) under high predation risk. Under these conditions, we would expect that only males of superior inherent quality (e.g., healthier, more elaborate ornaments) would be able to escape predator attacks while signalling, and successfully attracting females and mating. The reproductive outcome of such behaviour should compensate for the higher predation risk costs.

However, the behavioural rules of thumb regarding choice between survival and reproduction may not be as stereotyped as postulated by these more conventional hypotheses (Lima & Dill, 1990; Zahavi, 1975). Males within the same population may respond differently to predation risk, since individual attributes are variable and can influence risk-taking behaviours based upon the individual´s current and future reproductive prospects. The asset protection hypothesis suggests that the larger the current reproductive value (i.e., expected future lifetime reproduction; Fisher, 1930), the stronger the need to protect it (Clark, 1994). In other words, animals with high potential for future breeding prospects should take fewer risks relative to predators. The asset protection hypothesis is akin to the terminal investment hypothesis, a longstanding concept suggesting that iteroparous animals should increase reproductive effort when their residual reproductive value decreases, that is, when their prospects of survival and reproduction decline due to age (Pianka & Parker, 1975; Trivers, 1972; Williams, 1966). The asset protection and the terminal investment hypotheses can be combined in the context of predation risk to predict that animals that vary in their chances of immediate death from predation, and contingent upon their residual reproductive value, should adjust the conspicuousness of their courtship behaviour.

We investigated the trade-off between survival and reproduction by assessing how males of a Neotropical songbird, the blue-black grassquit (*Volatinia jacarina*), coped with predation risk while attempting to attract females. The blue-black grassquit is uniquely suited for testing this trade-off because of three specific reasons. First, males have a striking iridescent nuptial plumage and conduct elaborate and highly conspicuous aerial displays (detailed below). Second, reproductive opportunities for this migratory granivorous bird are limited because they have a short breeding period limited to the last three months of the rainy season in central Brazil (January to March; Sick, 1997). Finally, both adults and nests are subjected to very high levels of predation typical of tropical latitudes. At the start of the breeding period, males moult from a cryptic brownish plumage to an iridescent blue-black nuptial plumage (Maia & Macedo, 2011). Males then start defending small, clustered territories and perform their typical aerial displays (Manica et al., 2013, 2017), which consist of a stereotyped sequence (electronic supplementary material, Videos S1 and S2). First, the male leaps vertically from a perch and claps its wings at high speed behind its head several times. During the leap, the male exposes white underwing plumage patches that sharply contrast to the blue-black coloration. Second, the male emits a high-pitched strident vocalization at the peak of the leap, which adds to the mechanical sound produced by the wing beats, while rotating its body axis and pointing its beak to the ground. Finally, the male returns to the initial perch and most likely leaps again a few seconds later. Blue-black grassquit males may perform this display for hours, especially during sunny days (Sicsú et al., 2013). Experiments with artificial nests showed that this highly conspicuous male display can attract predators (Dias et al., 2010). After pairing, both sexes engage in a social monogamy with biparental care (Almeida and Macedo, 2001). However, males continue performing their sexual displays even with active nests in their territories, possibly to seek extra-pair copulations, since genetic studies revealed extra-pair fertilization rates from 8 to 50% in the species (Carvalho et al., 2006; Manica et al., 2016).

We used predator simulation experiments to assess whether males modulated their mate attraction performances under different predation risk regimes. Specifically, we tested the three theoretical scenarios introduced above, each one predicting a different outcome. First, the survival focused hypothesis (Lima & Dill, 1990; Magnhagen, 1991) predicts that males should prioritize survival and reduce display rate and/or performance attributes (e.g. leap duration) in situations of high predation risk and independent of male condition. The second scenario, based on the handicap principle hypothesis (Zahavi, 1975), predicts an interaction between male condition and predation risk by which males in better physical condition should increase display rate and/or performance attributes under high predation risk, in comparison to males in worse condition. Lastly, the asset protection hypothesis (Clark, 1994) combined with the terminal investment hypothesis (Pianka & Parker, 1975; Trivers, 1972;

Williams, 1966) together predict a different pattern for the interaction between male condition and predation risk. In this case, males with high residual reproductive value (described above as males in better physical condition) should reduce display rate and/or performance attributes under high predation risk, while under these conditions males with low residual reproductive value should present the opposite pattern, that is, increase display rate and/or performance attributes.

## METHODS

### *Study area and subjects*

This study took place within savanna vegetation patches in the University of Brasilia campus, Brazil (15°44'S, 47°52'W), during two breeding seasons (November to March) in 2015/2016 and 2016/2017. We captured male blue-black grassquits with mist nets (2.5 m x 12 m, 10 mm mesh) and banded them with unique combinations of four plastic colour bands. We took different measures to assess their overall body condition and their degree of ornamentation, and afterwards released them in the same locations where they were captured. We measured their body mass to the nearest 0.2 g with a scale and the length of the left tarsus with callipers (accuracy: ± 0.01 mm). With these data, we developed a male body condition index (body mass divided by tarsus length; Magalhães et al., 2014; Santos et al., 2009). As a proxy of condition, we also visually counted the number of ectoparasites (feather lice) on both wings (data pooled).

We estimated an index of moulting by measuring the proportion of the male body covered by nuptial plumage corrected by the time interval from the beginning of the breeding season (Maia & Macedo, 2011; Manica et al., 2014), since these two variables are positively correlated (see below). The index consisted of the residuals of a linear regression (nuptial plumage coverage vs. scaled time since the beginning of the breeding season; $\beta = 0.43 \pm 0.06$, *df* = 309, $p < 0.0001$), using male data from a larger dataset compiled by previous studies (2013 to the 2017 breeding seasons; mean ± SD: 78.75 ± 30.18 males/season, N = 315; based on Diniz et al., 2015). Thus, males captured for the current study were assessed relative to average nuptial plumage coverage of the population from previous breeding seasons. High moulting index values indicate a high proportion of nuptial plumage coverage, whereas low values, a lower nuptial plumage coverage.

We used binoculars to monitor the banded males that performed aerial displays in the area and marked the trees and bushes that were used as display perches. Experiments started after we identified display sites for at least 20 banded males. All animal procedures were in agreement with the Universidade de Brasília ethical standards for animal welfare (UnBDoC #92808/2014) and we captured animals with the endorsement of the Brazilian Ministry of the Environment (permit #42365-3).

### Experimental design

Playback trials used vocalizations of two bird species that occurred in the study area. For the predation treatment, we used vocalizations of the aplomado falcon (*Falco femoralis*), which preys on adult birds (Bó, 1999; Hector, 1985). For the control treatment, we used the vocalizations of the sayaca tanager (*Tangara sayaca*), a sympatric species that does not engage in aggressive interactions with grassquits. We used three different playback stimuli for each treatment and we sorted out which one would be presented before each trial. All vocalizations were taken from an online library of avian songs (Xeno-Canto Foundation©; https://www.xeno-canto.org/). Playbacks were broadcast with portable sound-speakers (Kayue KY-907), which were calibrated before each trial with a decibel meter (SEW® 2310 SL) positioned at a distance of 1 m from the speakers to a standard of 69 dB (based on the amplitude of the grassquits' breeding song; de Moraes et al. *in prep*). We performed two playback trials per day when the birds were more active (Almeida & Macedo, 2001): the first trial started one hour after sunrise, and the second trial started two hours before sunset. We presented a different experimental treatment to the subjects in each playback trial, and the order of stimuli presentation and the period of the day that they were presented varied randomly. There was a habituation period of one hour before each trial (i.e. habituation started at sunrise or three hours before sunset), so birds could adjust to the presence of the observer. If a male did not perform any sexual displays during the one-hour habituation period, the trial was cancelled, and a new attempt was made in the next trial period.

Treatment exposure consisted of: 5 min period of playback presentation; 30 min period of observations; 5 min period of the same playback presentation; 30 min period of new observations. During the playback presentations, we held the sound-speaker 1.5 m above ground facing the focal male display arenas (3-5 m distance). We video recorded male aerial displays during the two observation periods with a single video camera (Casio HD digital camera EX-FH25) set on a tripod for stability, at two frame rates: 30 and 240 frames per second (FPS; regular-speed and high-speed videos, respectively). We used 30 and 240 FPSs to allow the measure of both relative display rate and leap duration. We recorded at 240 FPS to obtain the number of wing beats per leap (see below). We switched FPSs during the video recording within each observation period to achieve a balance between the numbers of leaps recorded for each FPS rate. We controlled for FPS and the order of the leap display in a leaping bout in our models (see below). After the treatment ended, we classified weather conditions in one of four categories: 0 = sunny, 1 = partly cloudy, 2 = mostly cloudy, 3 = cloudy. Trials were not run under the rain. We used a measuring tape to estimate the height of the perches used during the male displays.

We filmed 33 males during the execution of 840 leaps (details in appendices, table A1). Grassquit males displayed in long bouts usually starting and ending at the same perch. We estimated the number of leaps per bout for 91% of the leaps recorded. We used Windows® Movie Maker v. 2012 (Microsoft Corporation 2012) to analyse all recorded leaps. For each leap, we measured: (1) the duration of the leap (using both the regular-speed and high-speed videos) (33 males, 830 leaps); and (2) the number of wing beats performed during the leap (using only the high-speed videos) (29 males, 386 leaps). Previous studies indicate that the height of the male leaps is positively correlated to leap duration and number of wing beats (Manica et al., 2017), so we did not measure leap height. We then calculated (3) relative display rate, computed as the number of leaps performed during a display bout divided by the duration of the display bout.

### *Data analysis*

We analysed data using R version 3.4.1 (R Core Team, 2017). First, we used a mixed model (package lme4; Bates et al., 2015) to evaluate the relationship between duration of the leap (response variable) and the number of wing beats per leap (predictor), controlling for individual identity (random factor). These two variables were highly correlated (Pseudo-R-Squared = 0.64, $p < 0.0001$, N = 384 leaps from 28 males), thus only leap duration was used in the statistical analyses.

We used a Linear Mixed Model (LMM; Zuur et al., 2013) to test the effect of predation risk treatment, body condition index, nuptial plumage coverage (moulting index), and ectoparasite count (main effects) on relative display rate and leap duration. Sample sizes to run the models were the number of display bouts (for relative display rate) and the number of leap displays (for leap duration), while controlling for individual identity (appendices, table A1).

To test the handicap principle and the asset protection hypotheses, we added the following interactions (and associated main effects): predation risk treatment * nuptial plumage coverage, predation risk treatment * ectoparasite count, and predation risk treatment * body condition index. The handicap principle and the asset protection hypotheses predict divergent interactions between predation risk and these variables, which taken together, could be interpreted as reflecting some aspect of male quality.

We also included possible confounding variables as predictor factors: date, breeding season (2015/2016 or 2016/2017), weather category, day time (morning or afternoon), FPS (30 or 240; only for leap duration), perch height, and sequence number (i.e. the order) of the leap within the display bout (only for leap duration). Finally, we added the identity of males, the identity of playback stimuli, and experimental trial as random factors in all models to avoid pseudo-replication.

We performed backward stepwise model selection based on likelihood ratio tests (and Wald tests for main effects of variables with significant interaction terms) to simplify the models and test for predictor effects (Zuur et al., 2013). We used the “stepwise-reintroduction for parameter estimation” (SRPE) method, which consists in adding removed terms one by one for the final model and checked their fitting and effect sizes (Hegyi & Garamszegi, 2011). Although stepwise modelling has some drawbacks (Whittingham et al., 2006), the SRPE method mitigates the main issue of extreme parameter estimation bias, and even increases estimation accuracy compared with full models (Hegyi & Laczi, 2015). Results were checked using an AIC model selection procedure (dredge function), and results remained qualitatively unchanged. All continuous variables were scaled before the analyses to obtain standardized (β) coefficients for predictors. In cases where we found an effect of an interaction between a continuous and a categorical variable, we reran the best-fitting model for each class of each categorical variable to help interpret the relationship between the response variable and the continuous variable involved in these interactions.

## RESULTS

Predation risk affected leap duration, but this effect varied with two male traits: ectoparasite load and moulting index (i.e. nuptial plumage coverage corrected by date). First, we found a significant interaction between wing ectoparasite count and predation risk explaining variation in leap duration (predation risk treatment * wing ectoparasite count; likelihood-ratio test: $\chi^2 = 7.30$, $df = 1$, $P < 0.01$; appendices, tables A2 and A3). Highly parasitized males increased leap duration when subjected to the predation treatment (figure 1). In contrast, leap duration of males with a low ectoparasite count did not vary with predation risk (figure 1).

[Place figure 1 here]

We also found that the interaction between moulting index and predation risk affected leap duration (likelihood-ratio test: $\chi^2 = 15.91$, $df = 1$, $P < 0.0001$; appendices, tables A2 and A3). Males with a low moulting index produced longer leaps in the predation treatment when compared with the control treatment (figure 2). In contrast, males with a high moulting index presented similar leap duration in both treatments (figure 2). In summary, lower quality males, with high ectoparasite count and low moulting index, increased leap duration under predation risk, whereas higher quality males did not modify leap duration in response to the predation treatment.

[Place figure 2 here]

We found a significant negative effect of body condition index on leap duration (likelihood-ratio test: $\chi^2 = 5.09$, $df = 1$, $P = 0.024$), which is independent of predation risk (predation risk treatment * body condition index; likelihood-ratio test: $\chi^2 = 0.007$, $df = 1$, $P = 0.93$). In other words, males with high body condition index values exhibited shorter leaps compared to males with a lower body condition index (figure 3). Finally, we found no direct effect of predation risk treatment on relative display rate (likelihood-ratio test: $\chi^2 = 0.62$, $df = 1$, $P = 0.43$; appendices, table A4).

[Place figure 3 here]

# DISCUSSION

We predicted three possible scenarios for how varying levels of predation risk would influence the sexual displays of blue-black grassquit males. Our findings partially supported the combined predictions of the asset protection and the terminal investment hypotheses (Clark, 1994; Pianka & Parker, 1975; Trivers, 1972; Williams, 1966). We found that lower quality males (i.e., those with high ectoparasite load and low moulting index) increased leap duration when predation risk was high. These males in poorer condition could have reduced chances of future breeding and therefore should be more willing to invest in risky behaviour to gain mating opportunities at a given breeding season. According to the asset protection hypothesis, higher quality males (i.e., those more highly ornamented and with a lower parasite load), should protect their reproductive assets, and decrease display performance under high predation risk because of greater chances of future breeding. However, our findings did not support this second prediction since higher quality males did not differ in display performance in high vs. low predation risk.

The traditional view of sexual signalling maintains that signals honestly reflect the health conditions of the signaller (i.e., only individuals in good conditions can display the signal; Andersson, 1994). However, our results challenge this view because low-quality males were able to sustain a similar intensity of sexual signals compared with high-quality males when exposed to high levels of predation risk. Our data agree with one of the key predictions of the terminal investment hypothesis (Pianka & Parker, 1975; Trivers, 1972; Williams, 1966). This hypothesis predicts that individuals with low residual reproductive value (i.e., reduced expectations of future reproduction) should increase their current reproductive effort when facing a high perceived risk of extrinsic mortality (Copeland & Fedorka, 2012; Nielsen & Holman, 2012; Velando et al., 2006, 2014).

The terminal investment hypothesis (Pianka & Parker, 1975; Trivers, 1972; Williams, 1966) may also account for two other findings: grassquit males with higher ectoparasite load and with less ornamentation performed longer leaps under high vs. low predation risk. First, parasites consume resources that are otherwise used for host development and maintenance (Sheldon & Verhulst, 1996), and also for investment in the expression of secondary sexual traits (Gustafsson et al., 1994; Magalhães et al., 2014). Depending on the costs of parasitism, infested males may not survive to the next breeding season (i.e., low residual reproductive value; Brown et al., 1995; Richner & Tripet, 1999), which should trigger a "terminal investment". Second, in the traditional view of intersexual selection, males may advertise a healthy and parasite-resistant profile by exhibiting fully developed sexual ornaments and performing dynamic sexual displays (Andersson, 1994; Hamilton & Zuk, 1982). The expression of these honest secondary sexual traits is typically associated with higher reproductive success (Sheldon et al., 1997), ultimately influencing a male´s residual reproductive value (Clark, 1994). However, less ornamented grassquit males (i.e. low residual reproductive value) can nevertheless perform "dishonest" sexual displays that are as intense as those performed by fully ornamented males in high predation risk contexts.

A relevant question is why blue-black grassquit males with fewer ectoparasites and greater coverage of nuptial plumage were not affected by the predation treatment. Given that parasites may enhance host susceptibility to predator attacks (Gehman & Byers, 2016; Hudson et al., 1992), and that blue-black grassquit males with more ornaments have a lower ectoparasite load (Magalhães et al., 2014), we presume that less parasitized and more ornamented males would be less sensitive to variations in predation risk. Thus, although all males in the study faced the same predatory stimulus, males probably were intrinsically different relative to their perception of predation risk, with increased risk perception by low-quality males (leading to terminal investment strategies) and attenuated risk-perception by high-quality males (no response to predation treatment).

Presumably, males with a better body condition should perform sexual displays more vigorously (Hunt et al., 2004; Morales et al., 2003). However, we found that males with lower values for body condition actually performed more intense displays (i.e., longer leaps) relative to those with better body condition. Similarly, Santos et al. (2009) found that grassquit males with low body condition score were more likely to win aggressive interactions over food compared to males with better body condition. One possible explanation is that lighter males might be sexually selected since their aerial displays may be easier to perform because of the greater agility and ability to jump. These lighter males may signal better inherent quality to females (Barske et al., 2011). This is an interesting finding given that the typical pattern is for sexual selection to favour males with larger and heavier bodies as they usually prevail in intrasexual competition (Andersson, 1994).

In conclusion, our results suggest that predation risk affects blue-black grassquit sexual displays according to the male’s residual reproductive value. The fact that low-quality grassquit males were able to exhibit sexual signals as intensely as high-quality males, specifically in the context of high levels of predation risk, provides support for the idea of a terminal investment, based upon the perception of imminent death (Pianka & Parker, 1975; Trivers, 1972; Williams, 1966). Our results also suggest that the perception of predation risk varies between males, so that those in poorer condition have a more acute sensitivity to threatening situations.

Our study raises important questions associated with one of the major premises of sexual selection theory, which is that all sexual signals are honest displays of signaller health and parasite resistance. Our findings directly challenge this idea, since grassquit males in poorer conditions (less ornamented, more parasitized) were on a par with healthy males in terms of display intensity under high predation risk. Also, grassquit males with poorer body conditions conducted displays more vigorously than males that were supposedly in better conditions. Therefore, this tropical songbird deviates from classical evolutionary assumptions, and highlights the importance of considering the influence of ecological and life-history factors, such as predation risk and residual reproductive value, on signal evolution. One question remains: in such an evolutionary context, how did the blue-black grassquit sexual signal evolve? It is typically expected that high-quality males can monopolize most reproductive opportunities, and therefore any honest badge of quality would evolve through differential reproduction (Andersson, 1994). However, as we have shown, low-quality grassquit males are able to produce “dishonest” sexual signals, which hypothetically invalidates the premise of differential reproduction. A missing piece of the puzzle in this system is how females choose their mates under predation risk, when low-quality male behaviour reflects the terminal investment approach. Females that select a mate based on dishonest signals may have fitness losses due to pairing with lower quality males. Thus, we expect that female grassquits may have evolved capabilities to discriminate between high and low-quality males in contexts of high predation risk. In this case, we will be able to assume that blue-black grassquit sexual signalling evolved through intersexual selection even when dishonest signalling is embedded in the system. The next step in the study of grassquit signal evolution is to understand how females perform mate choice under different levels of predation risk.

## ACKNOWLEDGEMENTS

We thank Universidade de Brasilia for logistic support, and all students of the Laboratório de

Comportamento Animal (UnB) for field assistance. This study was funded by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Apoio à Pesquisa do Distrito Federal (FAP-DF), Developing Nations Research Grants of the Animal Behavior Society, and Programa de Pós-Graduação em Ecologia da Universidade de Brasília.

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## FIGURE LEGENDS

Figure 1. Interaction between predation risk (Control = no-risk control; Predator = predator simulation treatment) and wing ectoparasite count explains the variation in leap duration. We show predicted values (and 95% CI) by the linear mixed models (upper image) and the corresponding raw data (lower image, N = 578 leaps from 24 males; Control = 324 leaps, Predator = 254 leaps).

Figure 2. Interaction between predation risk (Control = no-risk control; Predator = predatory simulation treatment) and moulting index (nuptial plumage coverage corrected by date) explains the variation in leap duration. We show predicted values (and 95% CI) by the linear mixed models (upper image) and the corresponding raw data (lower image, N = 578 leaps from 24 males; Control = 324 leaps, Predator = 254 leaps).

Figure 3. Relation between body condition index (male body mass divided by tarsus length) and leap duration (N = 578 leaps from 24 males).

Figure 1.

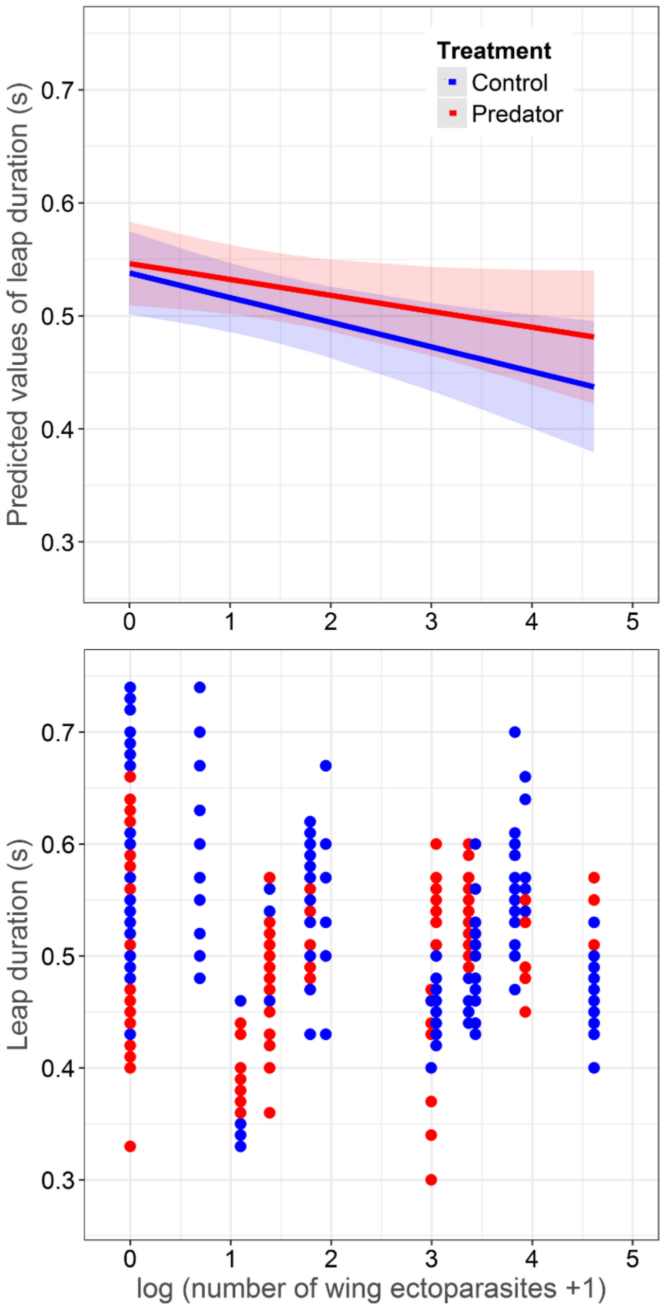

Treatment
Control
Predator
Predicted values of leap duration (s)
0.7
0.6
0.5
0.4
0.3
0
1
2
3
4
5
Leap duration (s)
0.7
0.6
0.5
0.4
0.3
0
1
2
3
4
5
log (number of wing ectoparasites +1)

Figure 2.

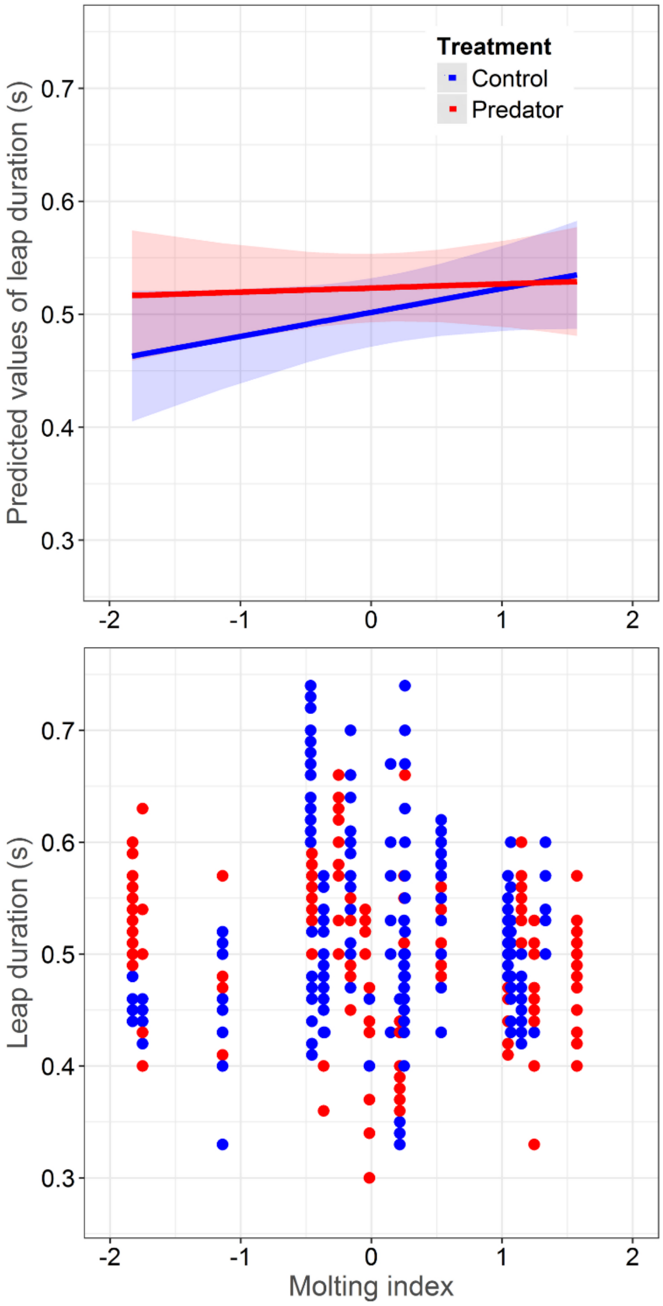
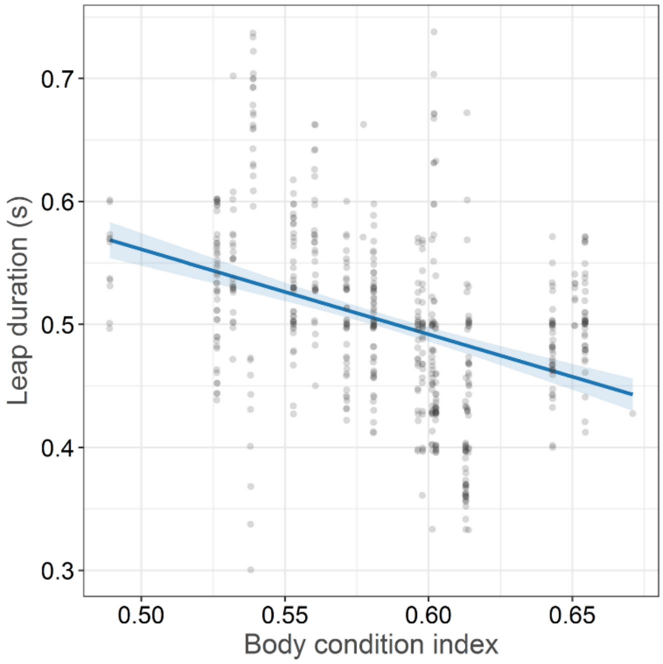

Treatment
Control
Predator
Predicted values of leap duration (s)
0.3
0.4
0.5
0.6
0.7
-2
-1
0
1
2
Leap duration (s)
0.3
0.4
0.5
0.6
0.7
-2
-1
0
1
2
Molting index

Figure 3.


Leap duration (s)
0.7
0.6
0.5
0.4
0.3
0.50
0.55
0.60
0.65
Body condition index

## APPENDICES

Table A1. Sample sizes and descriptive statistics for leap displays recorded for blue-black grassquit males.

| Treatment | Number of males | Number of display bouts | Number of leap displays | Mean (± SD) number of leap displays/display bout (range) | Mean (± SD) number of leap displays/ male (range) |
|---|---|---|---|---|---|
| Control | 29 | 54 | 462 | 7.89 ± 3.14 (3-14) | 15.93 ± 7.55 (1-29) |
| Predator | 27 | 42 | 379 | 8.31 ± 3.02 (3-12) | 14.54 ± 7.20 (2-22) |

Table A2. Backward stepwise model selection with SRPE method for explaining variation in leap duration. Variables highlighted in bold were kept in the final model. All models contain the identities of males, playback stimuli and experimental trial as random effects. Main effects for variables involved in meaningful interactions were assessed by Wald tests.

| | Likelihood ratio test (df = 1) | *P* |
|---|---|---|
| Date | 0.77 | 0.38 |
| Order of the leap on the leap bout | 0.0002 | 0.99 |
| Playback treatment * body condition index | 0.007 | 0.93 |
| Breeding season | 2.87 | 0.09 |
| **Playback treatment * wing ectoparasite count (log)** | 7.30 | 0.007 |
| **Playback treatment * nuptial plumage coverage index** | 15.91 | < 0.0001 |
| **Playback treatment** | 12.10 | 0.0005 |
| **Wing ectoparasite count (log)** | 8.65 | 0.003 |
| **Nuptial plumage coverage index** | 1.61 | 0.20 |
| **Body condition index** | 5.09 | 0.024 |
| **FPS** | 9.38 | 0.002 |
| **Weather** | 11.00 | 0.0009 |
| **Perch height during leaping (log)** | 13.39 | 0.0003 |
| **Daytime** | 14.65 | 0.0001 |

Table A3. Beta coefficients for predictors to explain variation in leap duration. Variables highlighted in bold were kept in the final model.

| | β ± se |
|---|---|
| **Playback treatment (Predator)** | 0.29 ± 0.08 |
| **Nuptial plumage coverage index** | 0.28 ± 0.14 |
| **Wing ectoparasite count (log)** | -0.48 ± 0.14 |
| **Body condition index** | -0.31 ± 0.13 |
| **Weather** | 0.23 ± 0.05 |
| **Perch height during leaping (log)** | -0.16 ± 0.04 |
| **Daytime (1600 – 1710)** | -0.31 ± 0.08 |
| **FPS (240)** | -0.17 ± 0.05 |
| **Playback treatment (Predator) * Nuptial plumage coverage index** | -0.24 ± 0.06 |
| **Playback treatment (Predator) * Wing ectoparasite count (log)** | 0.17 ± 0.06 |
| Date | 0.17 ± 0.19 |
| Order of the leap on the leap bout | 0.00 ± 0.03 |
| Playback treatment * body condition index | 0.007 ± 0.08 |
| Breeding season (2016/2017) | -0.72 ± 0.39 |

Table A4. Backward stepwise model selection with SRPE method for explaining variation in leap rate. Variables highlighted in bold were kept in the final model. All models contain the identities of males, playback stimuli and experimental trial as random effects.

| | Likelihood ratio test (*df* = 1) | *P* |
|---|---|---|
| Perch height during leaping (log) | 0.49 | 0.48 |
| Daytime | 0.97 | 0.32 |
| Playback treatment * body condition index | 0.84 | 0.66 |
| Date | 0.09 | 0.76 |
| Breeding season | 0.61 | 0.43 |
| Playback treatment * wing ectoparasite count (log) | 0.40 | 0.82 |
| Wing ectoparasite count (log) | 0.21 | 0.64 |
| Body condition index | 0.82 | 0.36 |
| Playback treatment * nuptial plumage coverage | 0.93 | 0.63 |
| Playback treatment | 0.62 | 0.43 |
| Nuptial plumage coverage index | 0.13 | 0.72 |
| Weather | 2.77 | 0.10 |

# CAPÍTULO 2

# Dangerous love? Predation risk does not affect female partner choice in blue-black grassquits

## ABSTRACT

Mate choice, the intersexual component of sexual selection, is typically conducted by females. Several intrinsic and extrinsic factors affect female mate choice, and predation risk may be a major influence in this context. Some theoretical predictions suggest that females may choose extravagantly ornamented males that are able to survive in high predation risk environments. However, this decision could be different if choosing a conspicuous male under high predation risk is costly for females or reduces offspring survival. In such contexts, females could become indifferent to male quality or even prefer less conspicuous males. We tested these hypotheses experimentally using blue-black grassquits (*Volatinia jacarina*) as the study object. Females were placed in an experimental arena where speakers on opposite sides emitted male courtship songs. One of the speakers emitted vocalizations at a high rate (proxy for a conspicuous male), while the other speaker broadcast lower rate vocalizations (proxy for a less conspicuous male). Simultaneously, while the female made a choice between the two types of male vocalizations, a third speaker emitted vocalizations characterizing three risk-level treatments: adult predator, nest predator and no-risk control. Females showed no preference for either male stimuli across the three predation risk treatments. Given that male grassquit sexual displays signal status to other males when engaging in competition for nesting territories, we propose that female blue-black grassquits exercise indirect mate choice. In this scenario, females choose their mates based on male ability to establish and defend a territory and rely only secondarily upon male phenotype attributes.

Key Words: handicap principle; survival cost; multimodal display; indirect mate choice; passive mate choice.

## INTRODUCTION

The intersexual component of sexual selection commonly ascribes to females the role of choosing among males the one that will sire their offspring (Darwin, 1871). Female mate choice may depend on myriad intrinsic factors, such as genotype (Brooks & Endler, 2001), hormones (Lynch et al., 2006), sensory capabilities (Ronald et al., 2012), physical conditions (Hunt et al., 2005), as well as environmental factors including the social environment (Madden & Whiteside, 2013) and ecological variables (Conrad et al., 2017). Predation risk also influences female choice in basically two distinct ways. First, in risky environments, females may prefer males that present elaborate ornaments and conspicuous displays, since these males are able to do so even at the risk of drawing predator attention to themselves. This is known as the handicap principle (Zahavi, 1975), a concept that proposes that males who survive in a risky environment with detrimental but striking ornaments probably has high quality genes. Thus, females that choose conspicuous males to sire their offspring may increase survival probability by indirectly selecting the antipredator genes. Some studies have found evidence supporting the handicap principle. For instance, female green swordfish (*Xiphophorus helleri*) prefer males with elongated caudal fins (i.e. swords; MacLaren, 2017), but this trait also increases male predation risk as predators direct more attacks towards individuals with intact swords than those that had their sword experimentally removed (Hernandez-Jimenez & Rios-Cardenas, 2012).

A different scenario predicts that females may suffer a high survival cost by associating with conspicuous males (Marzal et al., 2016). Female guppies (*Poecilia reticulata*), for example, have a strong preference for conspicuous ornaments displayed by males in risk-free environments, but they decrease sexual activity and choose their mate randomly under high predation risk (Godin & Briggs, 1996). In other fish species of the genus *Xiphophorus*, females go even further and completely avoid conspicuous males in risky scenarios, preferring less ornamented mates (Johnson & Basolo, 2003). Females from this species can also show no preference between conspecific males in risky environments and heterospecific males in safer conditions (Willis et al., 2012), possibly bearing the reproductive cost of producing hybrids to increase chances of survival. These examples illustrate the fact that predation risk can not only weaken intersexual selection relative to male conspicuous sexual signals but can also change the direction of selection towards more cryptic traits.

Sexual selection through female choice may enhance conspicuousness of a signal (Darwin, 1871), but natural selection may drive signal evolution in the opposite direction if females associate with less conspicuous males under high predation risk (Godin & Briggs, 1996; Johnson & Basolo, 2003). Given the natural diversity of forms and behaviors used by males for mate attraction across different taxa, it is important to ask how females from different species modify their mate choice under different levels of predation risk. To develop this concept, we used a Neotropical bird, the blue-black grassquit (*Volatinia jacarina*), which exhibits a collection of traits that make it an excellent subject to explore questions about predation risk and mate choice. This socially monogamous passerine presents elevated rates of extrapair fertilization associated with a complex repertoire of courtship behaviors and ornaments (Macedo et al., 2012; Manica et al., 2016), and breeds in regions of high levels of predation risk (Ricklefs, 1969; Skutch, 1985). Grassquit males molt from a brownish cryptic to a blue-black iridescent nuptial plumage during the breeding season (Maia & Macedo, 2011), which color is highly contrasting with the savannah vegetation of their breeding grounds. Additionally, males perform long bouts of a multimodal flight display throughout the day, which they intensify especially when sunlight shines directly upon them (Sicsú et al., 2013). Males maintain their sexual displays even after establishing a socially monogamous breeding pair (Macedo et al., 2012), probably to attract neighboring females to obtain extrapair copulations (Carvalho et al., 2006; Manica et al., 2016). Males perform these conspicuous displays despite having an active nest in their territories, and this has been shown to increase predation risk for nests (Dias et al., 2010). Because females associate with males for most of the breeding season (Carvalho et al., 2007), it is possible that the predator attention that males draw to both themselves and their nests also affects females. This raises the question of whether grassquit females benefit by associating with vigorous and conspicuous males (for example, by ensuring that their offspring will inherit “good genes”), or whether this association may be costly in situations of high predation risk, wherein the female and her offspring are at a greater risk due to intense male sexual displays.

To address these issues, we asked whether blue-black grassquit females modify their partner choice preferences under different predation risk regimes using predator simulation experiments. Specifically, we tested two hypotheses, each one predicting a different outcome (table 1), using a simple mate choice paradigm experiment. The first hypothesis, based on the handicap principle (Zahavi, 1975), is that females benefit from choosing conspicuous males as their mates. Thus, males that perform intense sexual displays in situations of high predation risk are probably of higher quality and should be chosen by females, despite the possible costs associated with the risk of predation. Based on this hypothesis, we expected an interaction between male display conspicuousness and predation risk affecting female choice. In our experiment, this hypothesis would be supported if females favored conspicuous males in situations of greater risk (simulation of a predator of adults) than in situations of no risk (control treatment). The second hypothesis states that females increase predation risk to themselves (Marzal et al., 2016) and to their offspring (Dias et al., 2010) by associating with a conspicuous male. By including a predation risk cost in female partner choice, we expected a different interaction between male display conspicuousness and predation risk affecting female response. The second hypothesis would be supported if females favored conspicuous males in situations of no risk (control treatment); in contrast, females should be nonresponsive to male display conspicuousness when predation risk is high (simulation of adult and/or nest predators), or even show a greater response to less conspicuous males.

[Place table 1 here]

## METHODS

### *Study subjects and field methods*

Blue-black grassquits are small, granivorous passerines that breed in central Brazil during the local rainy season (November to April; Carvalho et al., 2007). After establishing their territories, males perform conspicuous multimodal displays consisting of bouts of repeated leaps that include high-speed wingbeats, with emission of a high-pitched song at the peak of the leaps (Macedo et al., 2012). Males exhibit this sexual display for most of the day; natural variation in display rate ranges from 5.0 to 20.8 displays/min (mean ± SD; 14.2 ± 3.2; Manica et al., 2014). After establishment of the breeding pair, both parents build an open cup nest using herbaceous vegetation as substrate (10-50 cm off the ground) and care for broods of 2-3 nestlings (Almeida & Macedo, 2001). Extrapair fertilization rates in this species are exceptionally high, ranging from 11 to 47% of all broods (Manica et al., 2016). Nest predation is very high, with up to 80% of nests being depredated in some breeding seasons (Diniz et al., 2015). An aggravating factor related to nest predation is that males continue to perform sexual displays during the nesting period within their typically extremely small territories (as small as 13.0 $m^2$ and average of 72 m$^2$; Almeida & Macedo, 2001; Carvalho et al., 2006). These displays apparently are excellent indicators of the location of male territories and nests, increasing nest predation risk (Dias et al., 2010).

We used mist nets (2.5 m x 12 m, 10 mm mesh) to capture 26 female blue-black grassquits in savanna habitat patches within the Universidade de Brasília campus (15°46'S, 47°52' W) during the breeding season of 2014/2015. The females were banded with a unique combination of two plastic color bands and then housed in an outdoors aviary (2,56 m x 1,38 m x 2,06 m), and supplied with *ad libitum* water and a diet of specific seeds for granivorous birds (Nutrópica®). We kept the females in the aviary for 10 days before using them in any experimental trial to minimally standardize their reproductive status, since it was impossible to know their reproductive condition prior to capture. Before each experimental trial, we measured female body mass to the nearest 0.2 g with a scale, and the length of the left tarsus with calipers (accuracy: ± 0.01 mm). We then created a body condition index (body mass divided by tarsus length) and used it as a proxy of female condition, in terms of body fat deposition (Aguilar et al., 2008; Costa & Macedo, 2005; Magalhães et al., 2014; Santos et al., 2009). We also visually counted the number of feather lice on both wings (data pooled) and used it to estimate female ectoparasite load. After the experiment, females were kept in the aviary to be used as experimental subjects in other studies in our lab. All experimental procedures were in agreement with the Universidade de Brasília ethical standards for animal welfare (UnBDoC #92808/2014) and we captured animals with the endorsement of the Brazilian Ministry of the Environment (permit #42365-3).

### *Experimental stimuli and design*

The experiment took place in a second outdoors aviary containing the experimental arena (100 cm x 35 cm x 65 cm). We placed one female at a time in the arena, which contained two wooden perches at a height of 10 cm, each one at a 10 cm distance from the lateral sides of the arena. Bird food and water *ad libitum* were placed in a central position in the arena. We left the female in the arena for 12 hours for habituation in the new environment before starting the experimental trial. We used one female per trial, and the experimental protocol (detailed below) consisted of exposing females to songs of two contrasting types of males, while simulating three different levels of predation risk by broadcasting predator vocalizations.

Two sound-speakers (Kaiyue KY-907, frequency response: 0.15 – 18 kHz) were placed outside of the arena, each one adjacent to one of the perches and close to the sides of the arena. These two speakers were used to broadcast male blue-black grassquit songs. A third similar sound-speaker was positioned 2 m at the front of and aligned with the center of the arena. This centrally-located speaker was used to broadcast the predator treatment types.

To create the contrasting grassquit songs, we recorded songs from 26 males in the field with a Marantz PMD660 recorder (WAV, sampling rate of 48 kHz, 24 bits of resolution, mono), and a unidirectional microphone (Sennheiser ME66). We edited the audio files from each male with Audacity® to create two categories of male song rates: (1) high song rate (20 songs per minute), simulating a more acoustically conspicuous male; and (2) low song rate (10 songs per minute), simulating a more acoustically discrete male. These high and low song repetition values are naturally observed in the wild (Manica et al., 2014). We filtered acoustic frequencies below 2 kHz to minimize noise interference in stimuli creation, and standardized stimuli amplitude. The final one-hour audio files were comprised of one-minute bouts of male songs (at either high or low song rates) intercalated by 30 s of silence.

The potential predator vocalizations emitted by the centrally located and farthest speaker from the experimental arena were taken from an online library of avian songs (Xeno-Canto Foundation©). We used these audios to create three treatments with different levels of acoustically simulated predation risk: (1) an adult grassquit predator (aplomado falcon, *Falco femoralis*; Bó, 1999; Hector, 1985), which represents a great risk to females; (2) a grassquit nest predator (guira cuckoo, *Guira guira*; Menezes & Marini, 2017), representing a greater risk to the offspring, but still somewhat dangerous to the female; and (3) a no-risk control (sayaca tanager, *Tangara sayaca*), that offers no predation risk to either female or offspring.

Experimental treatments lasted two hours and were conducted separately at fixed times (0900 h and 1500 h), totaling six hours of observation per female (three treatments, two hours each). During experimental trials we recorded female activity within the arena, while the vocalizations of potential predators or nonpredator (control) were broadcast by the central speaker, and male songs with different attributes (low versus high rates) were broadcast from the lateral speakers. Both audios were created from the songs of the same male (high and low song rate) and were presented simultaneously to the female to control for male identity and other song attributes. We used the audios of the same male for all treatments within an experimental trial, but there was no repetition of male stimuli between trials. We changed the positions of the lateral speakers after one hour of each treatment, thus inverting the emission site of each category of male song to avoid any possible biases in female preference for sides of the arena. The day-shift (morning or afternoon) for execution of the experimental treatments, order of control and predator stimuli presentation (adult predator, nest predator), and the initial position of male song rate categories (left or right side of the arena) varied randomly across trials.

Before each experimental treatment, we used a decibel meter (SEW® 2310 SL) to calibrate the sound-speakers to a standard amplitude of 69 dB. We calculated this value using the same equipment to measure the singing amplitude of grassquits in the wild and also in captivity. After sampling the grassquits´ singing amplitudes, we used a measuring tape to calculate the distance between the sampled grassquits and the decibel meter. We applied these two values in the formula $L1 = Lr + 20 \times \log_{10}(r)$, which calculates the amplitude that should be sampled by a decibel meter when it is one meter away from the sound-speaker (van den Heuvel et al. 2012). In this formula, $Lr$ represents the singing amplitude measured at distance $r$, resulting in the singing amplitude measured at one meter from the emitter L1 (regarding the grassquits, L1 = 69 dB).

We used a video camera (Kodak Zx1) to record experimental trials for all 26 females used as experimental subjects (156 h of observation). During the video analyses, we ignored the first 10 min of each experimental treatment and the first 10 min after the repositioning of the lateral speakers to avoid any effect of researcher presence on female behavior. We used a virtual line dividing the arena into two halves and then identified which side corresponded to which male song rate category. We used scan sampling (Altmann, 1974) to score the position of the female in the arena at 2-min intervals. We created two mate preference scores: "mate preference score 1" was the total number of scans where the female was observed in each side of the arena; "mate preference score 2" consisted of the difference in the number of scans between 'high male song rate' side and 'low male song rate' side. A high value in the mate preference score 2 means that the female was more frequently observed closer to the high male song rate stimulus, while a low value means that the female spent more time in close proximity to the low male song rate stimulus.

### *Data analysis*

We analyzed data with R version 3.4.1 (R Development Core Team, 2017). We used Generalized Linear Mixed Models (GLMM, package lme4) to analyze variation in the mate preference scores. To avoid model overfitting, we conducted the analyses in two steps. First, we investigated whether grassquit females expressed a preference for the playback of male song bouts that varied in song rate (high male song rate vs low male song rate, hereafter) and under predation risk, using GLMM with a Negative Binomial error distribution (to deal with overdispersion). We used the number of scans wherein a female was observed in each of the two sides of the arena (i.e. "mate preference score 1") as a response variable. We included the main effects of, and the interactions between, male song type (i.e. that corresponded to each side of the arena) and predation risk treatment. Finally, we included female identity as a random effect.

Since female condition can affect mate choice behavior (Hunt et al., 2005) we performed a second analysis to control for confounding effects on mate choice unrelated to predation risk. This time, we used a global model to test whether variation in female preference for the variable male song rate varied with female body condition index (scaled) and ectoparasite load (scaled). For this analysis, the "mate preference score 2" was used as response variable. Accordingly, we used a GLMM with a Gaussian error distribution. We included the main effects of predation risk treatment, female body condition and female ectoparasite count. We also added the interactions predation risk treatment * female body condition, and predation risk treatment * female ectoparasite count, as fixed effects in this global model. Finally, we included female identity as a random factor in this model.

We conducted likelihood ratio tests in a backward stepwise approach for model selection. For each variable, we present the significance of these tests for the last model that the variable occurred.

## RESULTS

Grassquit females did not show any preference for the playbacks of high song rate bouts over low song rate bouts (table 2). This pattern of no preference was not influenced by simulated predation risk offered by adult or nest predators (figure 1), female body condition or female ectoparasite load (table 2). In other words, subject females spent equal amounts of time in the high song rate and low song rate sides of the arena, irrespective of predation risk regimes. The scans for females in the three predator risk regimes exhibited very similar scores for the alternative male song rate categories (mean ± SD): (1) control high song rate side = 32.58 ± 7.44 vs. low song rate side = 30.23 ± 7.41; (2) adult predator high song rate side = 29.96 ± 7.70 vs. low song rate side = 32.85 ± 7.31; and (3) nest predator high song rate side = 31.88 ± 6.46 vs. low song rate side = 31.04 ± 6.47. These results are in sharp contrast to the predicted outcome that females should modify their mate choice preferences under different predation risk levels.

[Place table 2 and figure 1 here]

## DISCUSSION

Our first prediction, based on the handicap principle (Zahavi, 1975), was that females would choose more conspicuous male stimuli across every predation risk treatment. Specifically, we expected that female preference would interact with predation risk and be more intense under higher predation risk (i.e. adult predator treatment) than in the no-risk situation (i.e. control treatment). Our second prediction was that females would select more conspicuous male stimuli only in situations of low risk, and under high predation risk they would choose male stimuli at random or even prefer more muted stimuli. This latter outcome was based on the assumption that association with vigorously displaying males might impose a great survival cost on females and their potential offspring (Dias et al., 2010; Marzal et al., 2016). Unexpectedly, our results did not support either of the two hypotheses, given that females were nonresponsive to male song stimuli, whether low or high rate, across all experimental treatments.

Despite the surprising nonresponsive pattern for grassquit female mate choice, our data confirm previous findings for this species to a certain degree, suggesting alternative explanations that advance our understanding of how sexual selection operates. Initial studies of this species indicated that, regardless of male attributes, grassquit females do not appear to choose their mates based on male territory quality and resource availability (Almeida & Macedo, 2001). A previous mate choice experiment using live grassquits exposed females to two male groups: healthy males treated with coccidiostatic drugs and parasite-infested males (Aguilar et al., 2007). The study showed that the coccidian infection significantly affected several sexual display attributes, and that healthy males performed more intense and vigorous displays than parasitized males. However, grassquit females were nonresponsive to healthy or parasitized male displays, although male performance honestly reflected male health. A subsequent experiment investigated how socially paired females reacted to potential extrapair mates (Dias et al., 2014). Paired female subjects were exposed to extrapair males to assess how they would react to novel versus familiar male attributes. Expectations were that females would be more attentive to novel males with more vigorous sexual attributes when compared with familiar males, while ignoring males in worse condition than their mates. However, females were interested in novel males even when familiar male attributes were of higher quality.

Taken together these previous studies of grassquit female mate choice and our results, we propose that female grassquits exhibit passive or indirect mate choice, that is, dependent on male competition (Wiley & Poston, 1996). During the breeding season, males compete to defend small territories with available substrate for nest building (Almeida & Macedo, 2001). Females then pair with bachelor territorial males, apparently ignoring most male attributes (Dias et al., 2014). They also appear to choose their mates disregarding male health (Aguilar et al., 2007), territory quality (Almeida & Macedo 2001; Carvalho et al., 2006) and predation risk (this study). Past studies have indicated that successfully paired males in the wild exhibit some particularities, such as spending more time in their territories, conducting displays for longer periods and at greater rates, and leaping higher (Carvalho et al., 2006; Manica et al., 2016). Possibly, female grassquits are attracted to males that are simply able to establish and defend a territory with appropriate substrate vegetation. In such a context, intrasexual competition may be the key factor, where male attributes such as persistence in displaying and conducting higher leaps may determine male status. Experimental evidence supports the proposal that male intrasexual competition may be strongly associated with male ornamentation. Males living in all-male environments, in contrast with males in mixed groups or in pairs, are more aggressive and present a higher plasmatic concentration of testosterone (Lacava et al., 2011). Such males also develop nuptial plumage faster, earlier and with more ultraviolet reflectance (Maia et al., 2012), which may signal male quality (Keyser & Hill, 1999). After pair formation, females could mate with neighboring males not necessarily for their inherent quality (Dias et al., 2014), but to increase offspring genetic diversity (Yasui, 2001) or to obtain access to resources (e.g.

Tryjanowski & Hromada, 2005). Therefore, mating decision in blue-black grassquits may not result primarily from female choice for male attributes, but on male status and intrasexual competition (see Arak, 1988; Okamura & Goshima, 2010; Rebout et al., 2017).

An alternative reason for the lack of female grassquit preference in the context of elevated predator risk may be related to our use of only one male display attribute (i.e. song rate) as the experimental stimulus. Blue-black grassquit males perform multimodal displays that involve not only acoustic components, but also visual cues: males leap at heights of 25 ± 9 cm (Costa & Macedo, 2005) while clapping their wings behind their heads and presenting white underwing plumage patches (Maia & Macedo, 2011). At the peak of the leap, males emit a high-pitched vocalization and return to the perched position to likely start a new display a few seconds later (Manica et al., 2017). Previous studies showed that the height of the leap is important for male pairing success (Carvalho et al., 2006; Manica et al., 2016). Given that avian predators can capture males during their leap display (Macedo pers. observ.) and grassquit males often emit sexual display songs without performing the leap display (Manica et al., 2017), singing without leaping may be a low-risk male strategy for mate attraction. However, the singing without leaping strategy may have low appeal to females, which may explain the lack of female responsiveness to male song in our experiments.

A final possible explanation for female indifference towards predatory stimuli is that they do not perceive the experimental treatments as a threat. While grassquit males present flashy colors and perform conspicuous displays that attract predatory attention (Dias et al., 2010), grassquit females have a brownish plumage that blends with the savannah background and that may be hard for predators to detect. Therefore, grassquit females possibly do not experience the same predation risk levels as males, especially considering predation by avian predators. This interpretation probably changes when nest predation is taken into account, since incubation and brooding are performed exclusively by females in this species (Carvalho et al., 2007). Female plumage coloration in dichromatic bird species may be an adaptation to open-nest camouflage (Soler & Moreno, 2012), and they do not present striking colors similar to males as that would increase nest predation risk (Ekanayake et al., 2015). Therefore, we expect that grassquit females may be more responsive to nest than to adult predators, a proposition that is worth testing in field conditions where parental activity is taking place.

In conclusion, we found that blue-black grassquit females do not respond to nest and adult predation risk under specific experimental conditions of mate choice. We discussed several alternative explanations, although we cannot substantiate any of them without further studies. To avoid any confusion over the relevance of male stimuli to female choice, we suggest that further mate choice studies focusing on multimodal displaying species should use the complete display as choice stimuli, instead of using segregated display components that may present incomplete information for females. Therefore, video playbacks with sufficient visual resolution are recommended in addition to vocal playbacks.

## ACKNOWLEDGEMENTS

We thank Universidade de Brasilia for logistic support, and all students of the Laboratório de Comportamento Animal (UnB) for field assistance. This study was funded by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Apoio à Pesquisa do Distrito Federal (FAP-DF), Developing Nations Research Grants of the Animal Behavior Society, and Programa de Pós-Graduação em Ecologia da Universidade de Brasília.

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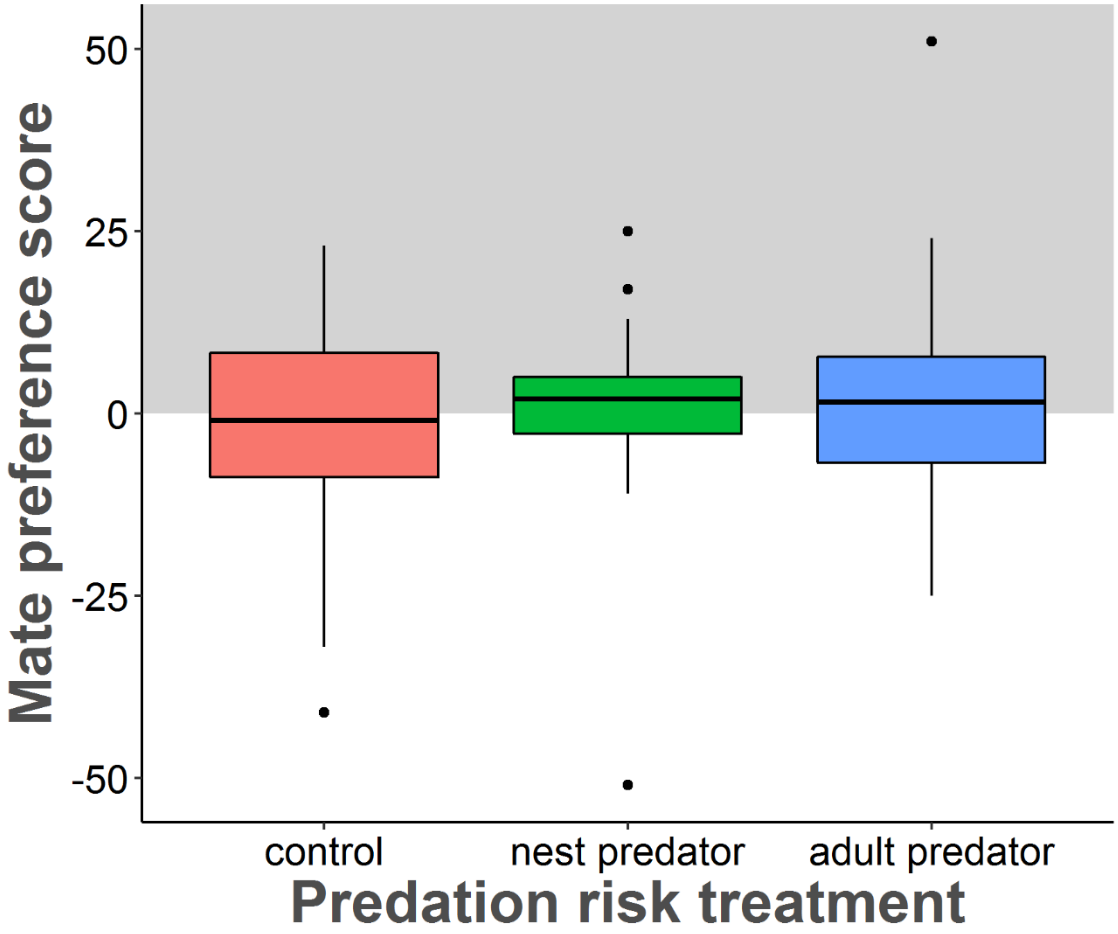
## FIGURE LEGENDS

Figure 1. Boxplot showing variation in female mate preference scores for the playback of variable male song rates (high vs low) under different scenarios of predation risk (N = 26 females, three treatments per female). Grey area corresponds to preference for high male song rate, whereas white area corresponds to preference for low male song rate. Scenarios of manipulated predation risk by playback: control = sayaca tanager, *Tangara sayaca*; nest predator = guira cuckoo, *Guira guira*; adult predator = aplomado falcon, *Falco femoralis*.

Figure 1.


50
25
0
-25
-50
Mate preference score
control
nest predator
adult predator
Predation risk treatment

TABLES

Table 1: Summary of hypotheses tested in this study and their predicted female mate preference outcomes. Blue-black grassquit females were used as experimental subjects. Symbols indicate that females favor males strongly (++), moderately (+) or not at all (-), as assessed by time spent in close proximity with the specific male stimulus (high or low song rate).

| **Hypotheses** | **Description** | **Experimental Treatment** | **Male conspicuousness** | |
|---|---|---|---|---|
| | | | High | Low |
| **Handicap principle**[1] | Associating with conspicuous males: greater reproductive benefits to females | Predation risk | ++ | - |
| | | Control | + | - |
| **Female/offspring predation risk**[2] | Associating with conspicuous males: greater survival costs to females and offspring | Predation risk | - | - or + |
| | | Control | + | - |

[1] based on Zahavi (1975); [2] based on Dias et al. (2010) and Marzal et al. (2016).

Table 2: Model selection through backward stepwise procedure to assess variation in mate preference scores by blue-black grassquit females for variable male song rates. Female identity is included in all models. Mate preference score 1 is the number of scans on each side of the cage. Mate preference score 2 is the difference in the number of scans recorded for the female between cage sides (‘high male song rate’ minus ‘low male song rate’). Variables considered include: condition (female body condition index = mass/tarsus), ectoparasite (ectoparasite count for female), predation (predation risk treatment = control, adult predator, nest predator); and song (high and low male song rate).

| Global model | Family | Response variable | Variable | Model selection step | Likelihood ratio test | | |
|---|---|---|---|---|---|---|---|
| | | | | | $\chi^2$ | df | P |
| 1 | Negative binomial | Mate preference score 1 | song * predation | 1 | 3.69 | 2 | 0.16 |
| | | | predation | 2 | 0.002 | 2 | 1.00 |
| | | | song | 3 | 0.008 | 1 | 0.93 |
| 2 | Gaussian | Mate preference score 2 | predation * ectoparasite | 1 | 0.66 | 2 | 0.72 |
| | | | ectoparasite | 2 | 0.79 | 1 | 0.37 |
| | | | predation * condition | 3 | 2.41 | 2 | 0.30 |
| | | | predation | 4 | 2.37 | 2 | 0.31 |
| | | | condition | 5 | 2.47 | 1 | 0.12 |

# CAPÍTULO 3

# Predation risk affects biparental care in a sexually dichromatic Neotropical songbird

# ABSTRACT

Predation risk has been shown to modify a broad range of behaviors in birds, mainly involving parental care. However, most of the studies to date focus on temperate species as animal models, which suffer a small risk of predation in comparison to tropical species. Here, we investigated how predation risk affects the parental behavior of a Neotropical passerine, the blue-black grassquit (*Volatinia jacarina*). We also observed whether grassquits present different responses to predation risk in relation to sex, since this is a sexually dichromatic species and detection by predators may impose different costs on parental care performed by each sex. Playbacks of known predators and non-predatorial control sympatric species were used near nesting pairs in the field. Results show that grassquits modify their behavior according to predation risk, and this behavioral response is linked to parental sex. Males decrease their nest visit times, become more discreet in their movements approaching the nest, and stop performing sexual displays after leaving the nest. Alternatively, females decrease latency to visit the nest and increase duration of brooding bouts. These different sex responses achieve the same goal: they reduce nest visual and acoustic detectability, since conspicuous males are readily spotted on nests and cryptic females camouflage nestlings and suppress begging calls. Although these behavioral changes reduce predation risk, there is a cost of reduced food load to nestlings and increased brood starvation risk. These changes in nesting activities illustrate the behavioral adaptability of grassquits to ensure offspring survival in tropical high predation risk environments.

Keywords: natural selection; nest predation; parental behavior; blue-black grassquit; antipredator behavior; sex roles.

## INTRODUCTION

The central paradigm of parental care focuses on the trade-off between current and future reproduction, that is, parental behavior that increases current offspring fitness may decrease parental capacity to invest in future reproduction (Clutton-Brock, 1991; Trivers, 1972). Parental investment may occur even before fertilization, with females allocating resources to eggs (Coslovsky & Richner, 2011), and may last long after offspring sexual maturation, when different overlapping generations stay in close proximity in species with long lifespans (Hawkes et al., 1997). The level of care allocated to a specific offspring may vary with environmental factors, such as food availability (Dewey & Kennedy, 2001) and weather (Öberg et al., 2014). Because of energetic and time constraints, parents frequently face trade-offs between continuing to care for an offspring that has already received considerable investment but currently presents a low survival probability or abandoning it to save resources to produce a new offspring when environmental conditions are favorable again (Clutton-Brock, 1991).

Predation risk is an environmental factor that weighs heavily on parental decision to care for or abandon an offspring and is also one of the main causes of nest failure in birds (Martin, 1995). Since predators can use parental behavior as a cue to locate nests, parents have to modulate their parental care strategies to ensure offspring survival (Martin et al., 2000b). Birds are known for their flexible parental care in situations of high predation risk, and under such conditions parents have been found to change brood size (Martin et al., 2000a), food provisioning rate (Ghalambor & Martin, 2000), and incubation bout duration (Massaro et al., 2008). This behavioral plasticity depends not only on the fleeting presence of a predator around the nest, but is also related to the historical predation risk level that a species has faced in its environment (Ghalambor et al., 2013). Results of a comparative study between subtropical and temperate species, which suffer different historical levels of predation (Ricklefs, 1969; Skutch, 1985), indicated that all studied species had a significant variation in parental care due to increased perception of predation risk (Ghalambor et al., 2013). However, subtropical species living in environments with greater historical predation rates presented more intense responses to predatory cues, reducing nest predation risk by decreasing offspring provisioning.

An evolutionary response to nest predation risk is also expected in sexually dichromatic species, likely resulting in a gender-bias in parental care since conspicuous individuals (usually males) are readily detected by visually oriented predators (Endler, 1992). Some studies with dichromatic birds confirmed this expected gender difference in biparental care under predation risk (Colombelli-Négrel & Kleindorfer, 2010; Ekanayake et al., 2015; Krištofková et al., 2011). For instance, male red-capped plovers (*Charadrius ruficapillus*), an open-nesting shorebird, present redder and brighter head coloration than females. In an experimental manipulation, male dummies were attacked by predators significantly more than female dummies during the day, but not at night (Ekanayake et al., 2015). Probably due to this gender difference in predation risk, red-capped plover females incubate during the day while males perform the same task at night when visually oriented predators are inactive (Ekanayake et al., 2015).

In this study, we examine plasticity in biparental care presented by a Neotropical species, the blue-black grassquit (*Volatinia jacarina*), which has very high nest predation rates, typical of tropical environments (Ricklefs, 1969; Skutch, 1985). The blue-black grassquit is a small passerine that migrates to savannahs of central Brazil to breed during the local rainy season (November to April; Almeida & Macedo, 2001; Sick, 1997), with nearly 80% of nests being depredated in some seasons (Diniz et al., 2015; Macedo et al., 2012). Nest predators are mostly birds, like the Guira cuckoo (*Guira guira*; Menezes & Marini, 2017). Blue-black grassquits are sexually dichromatic, with males molting into a blue-black iridescent nuptial plumage prior to the breeding season while females have a brownish cryptic coloration (Maia & Macedo, 2010). During the breeding season, males and females form socially monogamous breeding pairs and care for the offspring together (Almeida & Macedo, 2001). The species has very high rates of extrapair fertilization (Carvalho et al., 2006; Manica et al., 2016), which is likely the cause of males continuing to perform sexual displays even with active nests in their territories. Grassquit male sexual displays consist of a vertical leap from a perch, followed by several high-speed wing beats behind the head and a stereotypical high-pitched song at the peak of the leap (Manica et al., 2016). Although performing this conspicuous display may increase male fitness through extra-pair fertilization, it draws predators to the displaying male territory and, therefore, puts offspring at risk (Dias et al., 2010).

The blue-black grassquit system provides a unique opportunity to examine the influence of predation upon biparental care patterns, given the conflicts between social monogamy with bi-parental care and extrapair copulation, all evolved within a historical context of high nest predation rates. In this study, we experimentally tested whether sex-related behaviors are influenced by nest predation risk by avian predators. We predicted that parenting activities at the nest would change according to sex and predation risk, where males would decrease the number of nest visits and the time spent at the nest under high predation risk, while females would present the opposite pattern. This difference was expected because of the species´ sexual dichromatism and predator detection capacity: nest predation risk may increase with the presence of conspicuous males and may decrease with cryptic females. In a context of increased predation risk, possibly resulting in reduced nest visits by males and increased brooding bouts by females, we expected a decrease in the time invested in feeding the nestlings. Therefore, we also predicted that parents would increase the amount of food provisioned to nestlings per feeding visit to avoid nestling starvation risk. Finally, we examined whether predation risk affected other adult behaviors, such as the way parents enter the nest and the execution of sexual displays nearby.

## METHODS

### *Study subjects*

We conducted fieldwork during the blue-black grassquit breeding season of 2013/2014 (December to March) in a savannah area located within the Universidade de Brasília campus (15°44'S, 47°52'W). We used mist nets (2.5 m x 12 m, 10 mm mesh) to capture adults of both sexes and banded them with unique combinations of three plastic colored bands and one aluminum band (CEMAVE). We searched for active nests that, once found, were monitored every two days to estimate hatching date. Blue-black grassquit nests are small open cups (external diameter of 6.9 ± 0.7 cm, internal depth of 4.7 ± 0.9 cm) made of grass and roots (Carvalho et al., 2007), attached to grasses and shrubs 10 to 50 cm off the ground (Almeida & Macedo, 2001; Carvalho et al., 2007). Clutch size varies from two to three eggs that hatch after 10 days of incubation, and nestlings take 10 additional days to fledge (Carvalho et al., 2007). We performed the predation risk experiment (described below) on the fifth day (± 1 day) after hatching to control for possible ontogenetic variation in parental behavior. To avoid pseudo replication in our experiment, we used only nests in which at least one of the social parents was banded. All animal procedures were in accordance with the Universidade de Brasília ethical standards for animal welfare (UnBDoC #92808/2014) and were approved by the Brazilian Ministry of the Environment (permit #42365-3).

### *Experimental design*

The purpose of our experiment was to test whether predation risk affected blue-black grassquit parental behaviors both at the nest and in its immediate surroundings. The experiment consisted of exposing parents of active nests to two experimental treatments at the 5th ± 1 day after the eggs ecloded (to control possible ontogenetic demands present by nestling at different ages). We used vocalizations of two bird species as treatments: the guira cuckoo (*Guira guira*), a local bird that depredates nests (Menezes & Marini, 2017); and the sayaca tanager (*Tangara sayaca*) as a control that offers no risk to the nest. All vocalizations were taken from an online library of avian songs (Xeno-Canto Foundation©; https://www.xeno-canto.org/). An experimental trial consisted of the presentation of both treatments at a single nest. We presented treatments separately at fixed times (0700 h and 1600 h), when grassquits were most active (pers. obsv.). Each treatment lasted two hours (totaling four hours per nest) and vocalizations were broadcast during the whole treatment by a sound-speaker (Kayue KY-907) positioned two meters from the nest. The order and time of treatment presentation varied randomly, and we exposed each nest to each treatment (predator and control) only once.

We used a video camera (Kodak Zx1) fixed to a tripod at 50 cm from the nest to record nest activity in all experimental trials. Pilot studies showed that parents usually avoided entering the nest with the camera positioned at this distance, so we conducted a habituation period of 24 h before each experimental trial. During this habituation, we placed a dummy camera made of Styrofoam and dark fabric, which looked similar to the real camera, 50 cm from the nest. One hour before each treatment started (0600 h and 1500 h), we replaced the dummy camera with the real camera, and then waited for one hour before starting video recording. Habituation proved to be a successful measure since parents carried out nesting activities for the duration of the trials.

We used 20 nests, each exposed to two experimental treatments (2 hours/treatment), totaling 80 hours of nest observations. During video analysis, we quantified seven nest-related behavioral categories as response variables to our experimental treatments: (1) latency to visit the nest for the first time after the playback treatment started; (2) number of visits and total time spent at the nest by both parents; (3) food load transfers to nestlings (i.e. number of beak-to-beak interactions between parents and nestling) and food delivery rate (i.e. number of beak-to-beak interactions / number of nest visits) by both parents; (4) number and duration of brooding bouts performed by females; (5) nest-approaching strategy, which was the mean difference between the number of arrivals under and above the nest (grassquits would land directly over the nest, or land farther away. approach through the vegetation, and climb up to the nest from below); (6) nest-departing strategy, which was the mean difference between the number of nest departures under and above the nest (grassquits would either fly straight up from the nest or leave through the vegetation below the nest); (7) sexual displays performed by males while departing from the nest. Relative to these behaviors, it is important to clarify that we used beak-to-beak interactions to quantify food transfers because parents give a large number of seeds in single loads to nestlings, making it impossible to visually count the seeds using the videos. Likewise, males typically perform sexual displays during long bouts in visible perches across their territories (Manica et al., 2013). However, early in the study we found that they also perform the display while leaving the nest (using the nest as a display perch) and wondered if nest predation risk affects this conspicuous behavior.

### *Statistical analyses*

We tested whether predation risk affected blue-black grassquit adult behaviors and parental care in the nestling stage using linear mixed models and generalized linear mixed models in R (Zuur et al., 2009, 2013) (package lme4; Bates et al., 2015). We added predation treatment and sex as fixed effects in all models. We added sex and its interaction term with predation treatment to all models, except the models built to analyze variation in time spent brooding (only females), time spent in the nest (only males), and displays while leaving the nest (only males). The time spent brooding was highly correlated with the time spent in the nest for females (rs = 0.81), and so we only used the former.

Daytime (morning or afternoon) affects nest-feeding rates in blue-black grassquits (Diniz et al., 2015), so we added this variable as a covariable (fixed effect) in all models. Finally, we controlled between-individual and between-pair variation in parental behaviors by adding individual identity in all the models that included one of the parents, and individual identity nested within pair identity in all the models that included both parents.

We evaluated the significance of predictors in the full models using Type II Wald chi-square tests (package car; Fox & Weisber, 2011). We carried out multiple comparisons using multcomp package (Hothorn et al., 2008) to obtain estimates and respective standard errors for paired-differences between levels of significant interaction terms (i.e. Predation Treatment * Sex). To analyze variation in the nest approaching strategy, nest departure strategy and leap display during nest departure (only males), we excluded cases where the focal parent did not visit the nest.

## RESULTS

Predation risk did not affect the number of visits for both parents (figure 1a). However, it affected provisioning behavior (table 1). Both parents reduced food load (total number of beak-to-beak interactions across nesting visits) to nestlings during the predation treatment in comparison with the control treatment (figure 1b). This was even more pronounced when the model was run without the non-significant interaction between sex and treatment (Wald $\chi2$ test; Treatment: $\chi2 = 7.67$, $p = 0.006$; Estimate [95% CI]: -0.34 [-0.58, -0.10]).

[Place figure 1 here]

Predation risk also affected nest attendance by both parents, but in opposite directions (table 1). Males spent approximately 30% less time in the nest when under elevated predation risk in comparison with the control treatment (figure 1c). Females, on the other hand, spent on average about 50% more time brooding when under elevated predation risk in comparison with the control treatment (figure 1d). This latter effect was even stronger if we removed an outlier female that behaved very differently from other females (Wald $\chi2$ test; Treatment: $\chi2 = 15.72$, $p < 0.0001$; Estimate [95% CI]: 13.27 [6.51, 20.03]).

[Place table 1 here]

Predation risk influenced the latency and the way parents entered the nest (table 1). Females took less time to visit the nest than their partners when under increased predation risk (figure 2a; post hoc contrasts: Estimate [95% CI]: 0.65 (0.04, 1.27); appendices, table A1). The nest approaching strategy (expressed by the mean difference between the number of nest arrivals beneath the nest and the number of nest arrivals above the nest) was not affected by treatment. However, when we analyzed the sexes separately, males (Wald $\chi2$ test; Treatment: $\chi2 = 4.51$, $p <$

0.03; Estimate [95% CI]: 1.31 [0.09, 2.52]), but not females (Wald $\chi2$ test; Treatment: $\chi2 = 0.05$, $p = 0.82$), were more likely to arrive from below the nest during the predation risk treatment in comparison with the control treatment (figure 2b).

[Place figure 2 here]

Finally, nest departure location (expressed by the mean difference between the number of nest departures from under the nest and the number of nest departures from above the nest) was not affected by treatment (figure 2c). However, predation risk influenced the leap display executed by males while departing from nests. Males were around 200% less likely to execute leap displays during nest departure under predation risk compared with the control treatment (table 1; figure 3).

[Place figure 3 here]

## DISCUSSION

We found that predation risk affected blue-black grassquit biparental care and other nest related behaviors in several ways. First, contrary to our expectations, the number of nest visits by either males or females was not affected by predation risk treatment. However, predation risk affected the time both parents spent at the nest as we expected: males performed shorter visits while females spent more time brooding during higher predation risk periods, in comparison to low-risk situations. Despite the fact that the number of parental visits did not change based on predation risk, they provided less food to offspring during the predation versus control treatment. Females returned faster to nests under high predation risk, while males showed no difference in latency to return to nests in this context. Females also preferred to enter nests from below independent of predation risk, while males showed the same preference only during the predation treatment. Lastly, males significantly decreased sexual display performances while departing from nests when perceived predation risk was higher.

Our results show a general pattern of changes in sex-specific behaviors suggesting that in high predation risk levels parents can decrease nest visual and acoustic detectability. These patterns may be associated with the dichromatism of blue-black grassquits during the breeding season. Grassquit males spent less time attending the nest during the predator treatments. Because of their conspicuous colors in contrast to the savanna vegetation, males may facilitate nest detection by predators during feeding visits. For example, results from a study of the highly sexually dichromatic superb fairy-wren (*Malurus cyaneus*) showed that nest predation was influenced by the time that males, but not females, spent at the nest (Colombelli-Négrel & Kleindorfer, 2010). For the grassquits, not only are nests more easily detected visually when males are present, but adult presence also entails nestling begging calls and this elevated noise may increase nest acoustic detection (Briskie et al., 1999). Grassquit males solved this problem during the predator treatment by conducting shorter feeding visits, which we can assume would reduce nest exposure time to predator visual and acoustic detection.

A problem with shorter visits is the reduction of food load to offspring. This is possibly a solution to the trade-off between offspring predation risk versus starvation risk: by reducing food load to nestlings (i.e. increasing starvation risk) in shorter visits, males can reduce nest predation risk. Other studies have found the same pattern across several bird species, where parents decrease food provisioning when faced with an immediate increase in nest predation risk (reviewed by Martin & Briskie, 2009). Our data also suggest another way that males adjust their behavior to decrease nest visual detectability. In the predation treatment males stopped landing directly over the nests and preferred to land farther away, entering the nest from the vegetation below. This strategy may confuse predators that visually stalk grassquit males, since it is harder to follow prey through a dense vegetation structure (Denno et al., 2005). Finally, males drastically reduced their frequency of sexual display performance while leaving the nest when predation risk was high. Predators can find nests using male sexual displays as a visual cue (Dias et al., 2010) and waiting for males to emerge from vegetation and monitoring for sexual displays is an efficient method for detecting grassquit nests (pers. observ.). Therefore, males presumably are decreasing nest predation risk by reducing sexual display performance near the nest.

Meanwhile, grassquit females exhibit a different behavioral repertoire with the similar task of decreasing nest detectability. Grassquit females have a barred plumage that blends with the vegetation background (Gluckman & Cardoso, 2010). When predation risk is high, females return sooner to the nest and perform longer brooding bouts. This may be a strategy to disrupt the predator´s search image (Langley, 1996). There are two typical search images that are helpful when visually searching for grassquit nests: the white grassquit eggs against the nest material and the nestlings´ reddish gaping beaks when begging for food (pers. obs.). By sitting on the nest, females hide eggs and suppress nestling begging. Nestlings start to beg with sudden movements on the vegetation supporting the nest and this may happen when a predator lands or moves along the same substrate where the nest is located (Leonard et al., 2005), which may not only increase nest visual detectability (red gaping beaks) but also increases noise from nestling begging calls. Therefore, females can decrease nest visual and acoustic detectability by increasing duration of brooding bouts. Although this strategy may decrease nest predation risk, it increases offspring starvation risk since females reduce nestling food provisioning during brooding. Thus, grassquit females exhibit a behavior similar to that of males in face of the predation versus starvation risk trade-off by choosing to decrease nestling food load when predators are nearby (Martin & Briskie, 2009).

Unlike males, however, grassquit females tend to enter the nest from below independent of predation risk. Such female cautious behavior independent of predation risk may be associated with another facet of the grassquit breeding system, that is, the high rates of extra-pair copulation (Carvalho et al., 2006; Manica et al., 2016). Grassquit males engage in extra-pair copulations with neighboring females and may thus distribute their offspring across several nests during the same breeding season. On the other hand, intraspecific parasitism is rare for this species (Carvalho et al., 2006), so grassquit females typically concentrate their offspring in one nest at a time. Therefore, the cost associated with nest predation is higher for females than for males. Accordingly, we should expect the evolution of more cautious behaviors around the nest (i.e. entering the nest more discreetly) for females, while males can be expected to invest in strategies that increase extra-pair copulation opportunities, such as performing showy sexual displays while departing from the nest, when predation risk is low.

A tricky problem faced by grassquits when adopting antipredator strategies is the increased starvation risk of the offspring. Our experiment used a brief predation stimulus that lasted for only two hours and increased the perceived risk during a specific offspring ontogenetic stage (5 ± 1 days old). Different predation risk regimes (e.g. chronic predation risk or taxidermized predator models as stimulus) may interact with different ontogenetic pressures and yield different solutions to the predation versus starvation risk trade-off. Therefore, further studies using other types of predation risk treatment and at different offspring ontogenetic stages may uncover other adaptive parental behaviors (e.g. Pavel & Bureš, 2001).

There is a marked geographic bias in nest predation research (Ibáñez-Álamo et al., 2015). This scarcity of data from underrepresented tropical regions does not allow generalizations about the evolution of anti-predator behaviors in predator-rich environments. In this context, our findings are important because they suggest that predation risk affects biparental care in blue-black grassquits, and that the sexes respond with different strategies that together presumably reduce nest visual and acoustic detectability. The different sex roles exhibited by grassquit parents are probably linked to the sexual dichromatism in this species. Further studies with tropical species should take sexual dichromatism into account when analyzing the effects of nest predation risk on biparental care. Likewise, the response of monochromatic species to nest predation should also be investigated as a control to sexual dichromatism.

## ACKNOWLEDGEMENTS

We thank Universidade de Brasilia for logistic support, and all students of the Laboratório de Comportamento Animal (UnB) for field assistance. This study was funded by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Apoio à Pesquisa do Distrito Federal (FAP-DF), Developing Nations Research Grants of the Animal Behavior Society, and Programa de Pós-Graduação em Ecologia da Universidade de Brasília.

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Table 1. Effect of predator risk on parental behaviors in blue-black grassquits. We show results of Type II Wald chi-square tests on (generalized) linear mixed models for eight response variables. We also show estimates for predictor effects. Degrees of freedom = 1. Significant effects including estimates with 95% confidence intervals not crossing zero are highlighted in bold.

| | Provisioning behavior | | Nest attendance | | Nest arrival and departure | | | |
|---|---|---|---|---|---|---|---|---|
| | Feeding visits[1] | Food load[2] | Time males spent at nest (min) | Time females spent brooding (min) | Latency to visit (s, log) | Nest arrival[3†] | Nest departure[4†] | Leap display[5†] |
| Fixed effects | $\chi^2$ (*P*) | | | | | | | |
| Sex | 0.40 (0.53) | 0.02 (0.90) | - | - | 0.59 (0.44) | 3.03 (0.08) | 1.62 (0.20) | |
| Treatment | 1.50 (0.22) | **7.67 (0.006)** | **6.01 (0.01)** | **3.85 (0.0497)** | 0.01 (0.91) | 1.90 (0.17) | 0.69 (0.41) | **12.29 (0.0005)** |
| Sex * Treatment | 0.41 (0.52) | 0.0054 (0.94) | - | - | **5.48 (0.02)** | 1.35 (0.25) | 0.86 (0.35) | |
| Daytime | **7.02 (0.008)** | **10.85 (0.001)** | 0.82 (0.37) | 0.88 (0.35) | 0.88 (0.35) | 0.004 (0.95) | 0.30 (0.59) | 1.42 (0.23) |
| | | | | | | | | |
| | Estimate (95% CI) | | | | | | | |
| Intercept | 1.21 (1.48, 0.94) | 3.07 (3.46, 2.68) | 2.80 (1.35, 4.25) | 20.70 (8.32, 33.10) | 7.02 (6.57, 7.47) | 2.08 (0.41, 3.75) | -1.95 (-3.30, -0.60) | 0.80 (-0.36, 1.95) |
| Sex (male) | -0.02 (-0.37, 0.33) | -0.02 (-0.55, 0.51) | | | -0.23 (-0.82, 0.36) | **-2.31 (-0.11, -4.51)** | -1.40 (-3.14, 0.34) | |
| Treatment (Predator) | -0.07 (-0.38, 0.24) | **-0.33 (-0.66, 0)** | **-0.91 (-1.63, -0.18)** | **9.70 (0.02, 19.38)** | -0.44 (-0.95, 0.07) | 0.16 (-1.25, 1.57) | -0.02 (-1.47, 1.43) | **-1.89 (-2.95, -0.83)** |
| Sex * Treatment | -0.15 (-0.60, 0.30) | -0.02 (-0.49, 0.45) | - | - | **0.88 (0.51, 1.25)** | 1.21 (-0.83, 3.25) | 1.00 (-1.12, 3.12) | |
| Daytime | **0.31 (0.07, 0.55)** | **0.41 (0.17, 0.65)** | 0.34 (-0.40, 1.08) | -4.72 (-14.60, 5.16) | -0.18 (-0.92, 0.56) | 0.03 (-1.03, 1.09) | -0.30 (-1.38, 0.78) | -0.60 (-1.58, 0.38) |

[1] In two-h focal session; [2] number of times parent feeds nestlings during visit; [3] mean difference between number of nest arrivals under and above the nest; [4] mean difference between number of nest departures under and above the nest; [5] proportion of nesting visits that male conducted display while leaving the nest; [†] cases where parent did not visit the nest were excluded.

FIGURE LEGENDS

Figure 1. Effect of simulated increase in predation risk upon provisioning behavior and nest attendance in blue-black grassquits (*Volatinia jacarina*). (a) Number of nest visits performed by females (green) and males (blue) in two predation risk treatments (control and predator); (b) Food load given to nestlings by females and males; (c) Time spent in the nest by males an time spent brooding by females.

Figure 2. Effect of simulated increase in predation risk upon parental behavior during nest arrival and nest departure in blue-black grassquits (*Volatinia jacarina*). (a) Latency to visit the nest by females (green) and males (blue) in two predation risk treatments (control and predator); (b) Frequency of nest arrivals under and above the nest by females (left) and males (right) in two predation risk treatments: control (dark bars) and predator (light bars); (c) Frequency of nest departures from under and above the nest by females (left) and males (right) in two predation risk treatments. Means and 95% CI in figures b and c. Numbers on the bottom of the bars indicate the number of individuals with ≥ 1 nesting visits.

Figure 3. Effect of simulated increase in predation risk upon male leaping display during nest departure in blue-black grassquits (*Volatinia jacarina*).

Figure 1

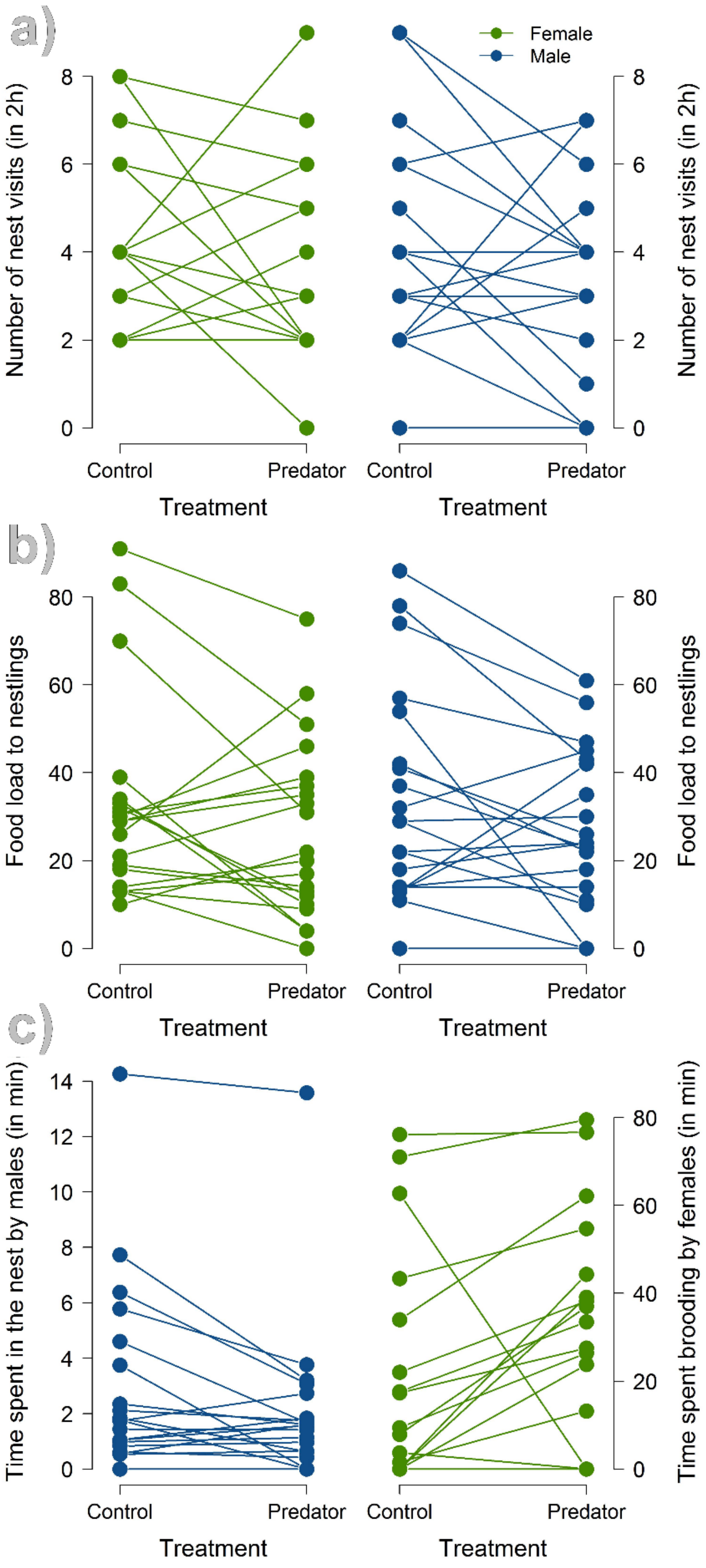
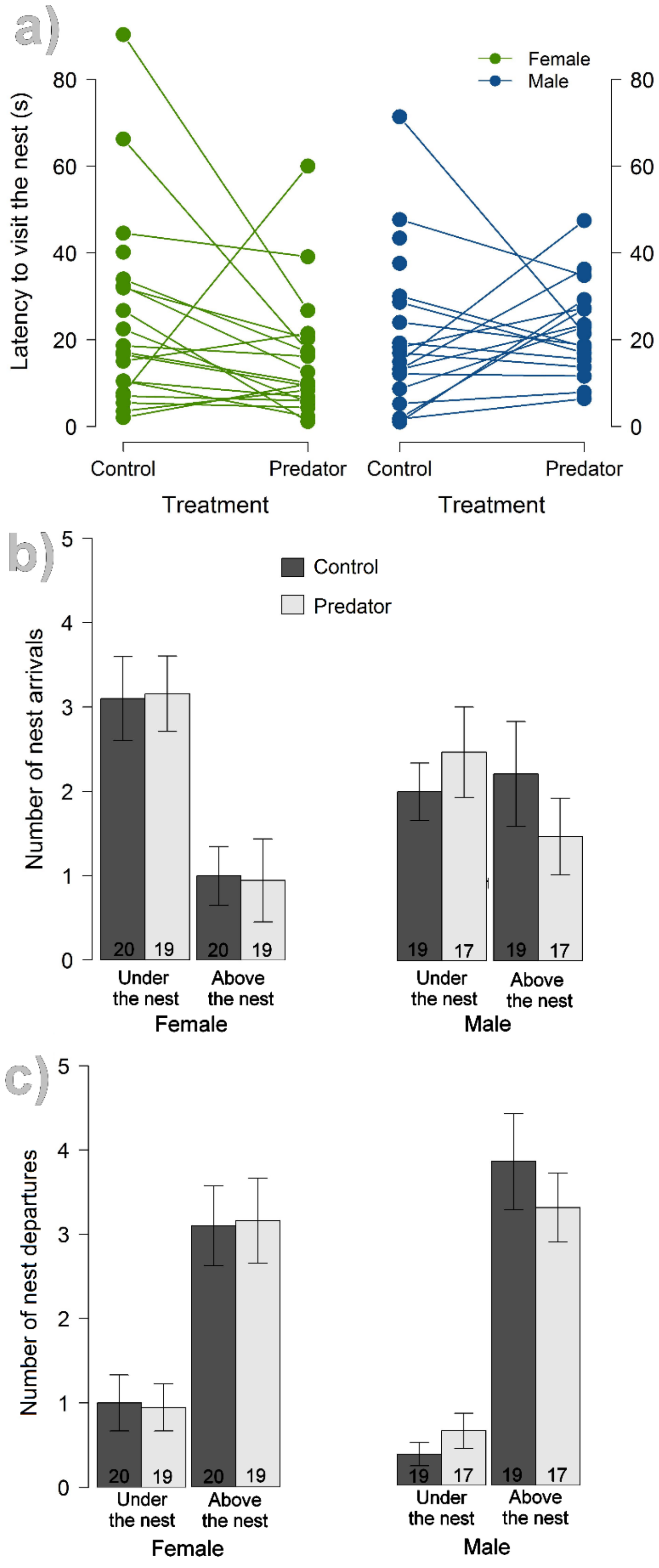
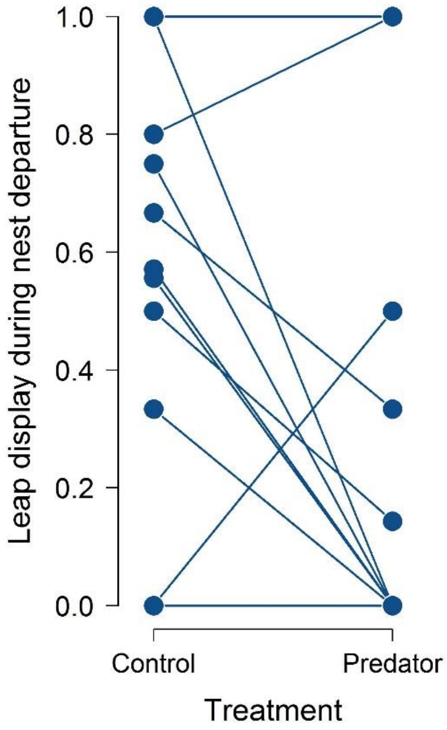

a)
Female
Male
Number of nest visits (in 2h)
0
2
4
6
8
Control
Predator
Treatment
b)
Food load to nestlings
0
20
40
60
80
Control
Predator
Treatment
c)
Time spent in the nest by males (in min)
0
2
4
6
8
10
12
14
Time spent brooding by females (in min)
0
20
40
60
80
Control
Predator
Treatment

Figure 2



a)
Latency to visit the nest (s)
0
20
40
60
80
Female
Male
Control
Predator
Treatment
Control
Predator
Treatment
b)
Number of nest arrivals
0
1
2
3
4
5
Control
Predator
20
19
20
19
Under the nest
Above the nest
Female
19
17
19
17
Under the nest
Above the nest
Male
c)
Number of nest departures
0
1
2
3
4
5
20
19
20
19
Under the nest
Above the nest
Female
19
17
19
17
Under the nest
Above the nest
Male

Figure 3


1.0
0.8
0.6
0.4
0.2
0.0
Leap display during nest departure
Control
Predator
Treatment

APPENDICES

Table A1. Full models built to analyze variation in parental behaviors in response to manipulated predation risk in blue-black grassquits.

| Response variable | Family | Predictor variables | Random effects | Sample size | |
|---|---|---|---|---|---|
| | | | | Female (Control, Treatment) | Male (Control, Treatment) |
| **Provisioning behavior** | | | | | |
| Feeding visits[1] | Poisson | Sex * Treatment + Daytime | Bird ID nested in Pair ID | 20, 20 | 20, 20 |
| Food load[2] | Poisson | Sex * Treatment + Daytime | Bird ID nested in Pair ID + Observation level[7] | 20, 20 | 20, 20 |
| **Nest attendance** | | | | | |
| Time spent at nest[†] | Gaussian | Treatment + Daytime | Male ID | | 20, 20 |
| Time spent brooding[‡] | Gaussian | Treatment + Daytime | Female ID | 20, 20 | |
| **Nest arrival and departure** | | | | | |
| Latency to visit[3*] | Gaussian | Sex * Treatment + Daytime | Bird ID nested in Pair ID | 20, 19 | 19, 17 |
| Nest arrival position[4]* | Gaussian | Sex * Treatment + Daytime | Bird ID nested in Pair ID | 20, 19 | 19, 17 |
| Nest departure position[5]* | Gaussian | Sex * Treatment + Daytime | Bird ID nested in Pair ID | 20, 19 | 19, 17 |

|  |  |  |  |  |
|---|---|---|---|---|
| Display performance$^{6*\dagger}$ | Binomial | Treatment + Daytime | Male ID | 19, 17 |

[1]in 2-h focal session; [2]number of times a parental gives food to nestlings during a nesting visit; [3]in seconds, log-transformed; [4]mean difference between the number of nest arrivals under the nest and the number of nest arrivals above the nest; [5]mean difference between the number of nest departures under the nest and the number of nest departures above the nest; [6]proportion of nesting visits that a male conducted a leaping display when flew to leave the nest; [7]factor containing observation level effect to control for overdispersion in Poisson model; *cases where parent did not visit the nest were excluded; † only males; ‡only females.

# DISCUSSÃO GERAL

Nesta tese, eu investiguei como o risco de predação afeta diversos comportamentos do repertório reprodutivo do tiziu. O risco de predação é uma das pressões seletivas mais importantes a moldar traços morfológicos e comportamentais em animais (Lima & Dill, 1990). Logo, espera-se que animais que convivem com alto risco de predação apresentem adaptações que promovam o seu sucesso reprodutivo nestas condições. Como a região Neotropical apresenta altas taxas de predação (Ricklefs, 1969; Skutch,1985), espécies neotropicais podem servir de modelos de estudos que investiguem como animais se adaptam, sobrevivem e reproduzem em ambientes de alto risco. Assim, eu utilizei o tiziu (*Volatinia jacarina*) como espécie de estudo e executei experimentos durante a estação reprodutiva desta ave no planalto central brasileiro.

No primeiro capítulo, eu investiguei como tizius machos modificam seus *displays* aéreos de acordo com o risco de predação. Três hipóteses foram testadas, cada qual prevendo um cenário diferente de resposta dos machos: (1) a hipótese focada na sobrevivência , onde machos devem priorizar sobrevivência e reduzir a intensidade de seus *displays* com o maior risco de predação (Lima & Dill, 1990; Magnhagen, 1991); (2) a hipótese do princípio da desvantagem (Zahavi, 1975), prevendo que machos devem aumentar a intensidade de seus *displays* com o maior risco; (3) a hipótese da proteção de recursos (Clark, 1994) combinada com a hipótese do investimento terminal (Pianka & Parker, 1975; Trivers, 1972; Williams, 1966), onde a resposta do macho ao risco de predação seria dependente de seu valor reprodutivo residual.

Como resultado, foi observado que o risco de predação afeta o *display* sexual de machos de tiziu, mais especificamente a duração dos saltos executados durante o *display*. Saltos mais longos também são mais altos e apresentam um maior número de batidas de asas (Manica et al., 2017), sendo, portanto, mais conspícuos. Este efeito do risco de predação sobre a duração do salto variou de acordo com duas características do macho: a carga de ectoparasitas nas asas e a cobertura de plumagem nupcial. Machos mais parasitados aumentaram a duração do salto em situações de maior risco, em comparação com o tratamento controle. Já machos menos parasitados não apresentaram uma variação na duração do salto em relação ao risco de predação. Machos menos ornamentados (i.e. menor cobertura de plumagem nupcial) apresentaram resultados semelhantes aos mais parasitados, aumentando a duração dos saltos com um maior risco de predação. Machos mais ornamentados, por sua vez, não modificaram a duração do salto de acordo com o risco de predação. Também foi encontrada uma relação negativa entre o índice de condição corporal (peso/tarso) e a duração dos saltos, onde machos em piores condições apresentaram saltos mais longos e machos em melhores condições, por sua vez, apresentaram saltos mais curtos. Contudo, esta relação não interagiu com o tratamento de risco de predação.

Os resultados do capítulo 1 deram suporte à hipótese do investimento terminal (Pianka & Parker, 1975; Trivers, 1972; Williams, 1966). Primeiro, porque indivíduos mais parasitados aumentaram o investimento reprodutivo (i.e. maior intensidade de *display*) com o maior risco de mortalidade extrínseca. A depender do custo do parasitismo, alguns indivíduos podem não sobreviver até a próxima estação reprodutiva (Brown et al., 1995; Richner & Tripet, 1999), de modo que seu potencial reprodutivo residual será baixo. Ao aumentarem a duração dos saltos com o maior risco de predação, machos mais parasitados estão investindo em reprodução atual possivelmente para compensar a sua baixa probabilidade de reproduzir no futuro. De modo semelhante, machos menos ornamentados também passaram a investir mais em reprodução atual com o aumento do risco de mortalidade extrínseca. Na visão tradicional da seleção intersexual, machos sinalizam saúde e resistência a parasitas através da exibição de ornamentos sexuais plenamente desenvolvidos e realizando *displays* sexuais dinâmicos (Andersson, 1994; Hamilton & Zuk, 1982). A expressão honesta destes caracteres sexuais secundários é comumente associada a um maior sucesso reprodutivo (Sheldon et al., 1997) e, sendo assim, machos que apresentam ornamentos sexuais incompletos podem ter um menor sucesso reprodutivo residual. Em resumo, machos de tiziu com menor valor reprodutivo residual (i.e. mais parasitados e menos ornamentados) aumentaram a intensidade de seus *displays* com o maior risco de predação e, deste modo, passaram a apresentar uma estratégia de investimento terminal.

No segundo capítulo, eu investiguei como fêmeas de tiziu modificam seus padrões de escolha de parceiro em diferentes níveis de risco de predação. Dois cenários hipotéticos foram testados, com o primeiro baseado na premissa de que comportamentos arriscados são sexualmente selecionados (Zahavi, 1975). Este cenário prevê que fêmeas devem preferir machos que apresentam *displays* mais intensos e que esta preferência deve aumentar com o maior risco de predação. A segunda hipótese testada tem como premissa o maior risco de predação sofrido por fêmeas (e sua prole) ao se associarem a machos que executam *displays* mais conspícuos (Dias et al., 2010; Marzal et al., 2016). Esta hipótese prevê, portanto, que fêmeas devem escolher seus parceiros ao acaso em situações de maior risco, ou mesmo evitar machos de *displays* mais chamativos, preferindo machos que sejam mais discretos.

Como resultado, não foi encontrada uma relação entre risco de predação e escolha de parceiro pelas fêmeas, as quais não apresentaram preferência por nenhum estímulo de macho nos diferentes níveis de risco de predação simulado aos quais foram submetidas. Estes resultados estão de acordo com outros estudos de escolha de parceiros já realizados com tizius. Até então, fêmeas de tiziu têm se mostrado indiferentes à qualidade do território de machos e à disponibilidade de recursos nos mesmos (Almeida & Macedo, 2001), ao nível de parasitismo (Aguilar et al., 2007) e a alguns atributos morfológicos dos machos (Dias et al., 2014). Possivelmente, fêmeas de tiziu escolhem seus parceiros de maneira passiva ou indireta, ou seja, dependente da competição intrassexual (Wiley & Poston, 1996). Neste cenário, fêmeas devem ser atraídas a machos que conseguem estabelecer e defender territórios com substratos apropriados para a construção de ninhos. Estudos com machos em ambiente natural mostraram que certos atributos dos mesmos podem resultar em um maior sucesso de cópula, como permanecer por mais tempo em seus territórios, executando *displays* por longos períodos, em maiores frequências e saltando mais alto (Carvalho et al., 2006; Manica et al., 2016). No contexto de seleção intrassexual, estes atributos podem determinar o status de um macho e, portanto, influenciar a sua capacidade de manter um território. Outra evidência para a proposta da escolha de parceiros por competição entre machos vem de experimentos com grupos com razões sexuais distintas, onde indivíduos em cativeiro eram divididos em grupos contendo apenas machos, grupos mistos ou apenas pares (Lacava et al., 2011; Maia et al., 2012). Estes experimentos demonstraram que machos em um ambiente mais competitivo apresentam maiores concentrações plasmáticas de testosterona, são mais agressivos, desenvolvem suas plumagens nupciais mais rápido, mais cedo e com maior reflexão de ultravioleta. Depois da formação do par reprodutivo, fêmeas de tiziu podem realizar cópulas extra-par não necessariamente pela qualidade dos machos disponíveis (Dias et al., 2014), mas sim para aumentar a viabilidade da prole (e.g. Simmons, 2005) ou para obter recursos (e.g. Tryjanowski & Hromada, 2005). Portanto, a escolha de parceiros no tiziu pode se dar não pela escolha direta da fêmea por atributos dos machos, mas através da competição sexual e do status do macho.

No terceiro capítulo, eu avaliei se o risco de predação influencia o cuidado biparental do tiziu e se este efeito depende do sexo. Como o comportamento dos pais pode indicar a predadores a localização do ninho (Martin et al., 2000), o cuidado parental deve ser reduzido a fim de se diminuir o risco de predação sobre o ninho. No entanto, filhotes em desenvolvimento demandam atenção constante e reduzir as visitas de alimentação ao ninho pode ser prejudicial ao desenvolvimento dos ninhegos. Portanto, o cuidado parental sob risco de predação deve ser otimizado de modo a se evitar a morte da ninhada por inanição e por predação (Ghalambor et al., 2013). Um possível fator a influenciar o cuidado parental dos tizius é o dicromatismo sexual desta espécie (Maia & Macedo, 2011). Espera-se que machos vistosos como o tiziu aumentem a detectabilidade do ninho quando visitam o mesmo, resultando no aumento do risco de predação sofrido pela prole (Colombelli-Négrel & Kleindorfer, 2010). Fêmeas de tiziu, por sua vez, apresentam uma plumagem amarronzada críptica que as camufla na vegetação de fundo (*barred plumage*; Gluckman & Cardoso, 2010). Portanto, fêmeas diminuem a detectabilidade do ninho ao incubar a prole, diminuindo o risco de predação sofrido pela mesma. Para avaliar todos estes pressupostos, eu expus ninhos de tiziu em campo a diferentes tratamentos de risco de predação e monitorei a resposta dos pais.

Os resultados mostraram que o risco de predação afetou o comportamento biparental no tiziu e que a resposta ao risco dependeu do sexo. Primeiro, o número de visitas ao ninho não foi afetado pelos tratamentos de risco de predação. No entanto, o tempo passado no ninho foi afetado e houve uma variação ligada ao sexo nesta resposta. Machos executaram visitas mais curtas quanto maior o risco de predação, enquanto fêmeas passaram mais tempo sobre os ninhegos com o aumento do risco. As fêmeas também voltaram mais rápido ao ninho na primeira visita após o início do tratamento predatório, enquanto machos não apresentaram este comportamento. Fêmeas também foram mais discretas em sua entrada no ninho independente do risco de predação, preferindo entrar no ninho pela vegetação embaixo do mesmo. Machos apenas apresentaram esta preferência quando o risco de predação era maior. Machos também reduziram drasticamente a frequência de *displays* executados ao saírem do ninho durante o tratamento predatório. Por fim, tizius forneceram menos alimento aos filhotes com o maior risco de predação.

Os resultados do capítulo 3 apontam uma estratégia parental de redução da detectabilidade visual e acústica do ninho com o maior risco de predação. Os machos, de coloração mais conspícua, diminuem o tempo em que são vistos no ninho e passam a ser mais discretos tanto na entrada (evitam pousar direto no ninho e preferem entrar por meio da vegetação de baixo) como na saída (param de executar *displays* ao saírem do ninho). Outro problema enfrentado pelos pais são as vocalizações de súplica emitidas pelos filhotes quando há movimentação no ninho ou na vegetação ao redor. O ruído gerado pelos filhotes pode guiar predadores até o ninho (Briskie et al. 1999). Deste modo, ao reduzir o tempo no ninho, machos também diminuem o tempo em que os filhotes vocalizam e, assim, reduzem a detecção acústica do ninho. Já as fêmeas, de coloração mais críptica, sentam-se sobre os filhotes e anulam a imagem de busca dos predadores (i.e. a coloração e formato dos ovos ou a boca avermelhada dos ninhegos durante o comportamento de súplica). Além disso, ao sentarem-se sobre os filhotes, as fêmeas suprimem a súplica que pode porventura ser iniciada caso o predador pouse ou se desloque pela vegetação que serve de substrato ao ninho. Assim, ao aumentarem o tempo no ninho, fêmeas reduzem a detectabilidade visual e acústica do mesmo, resultando em um menor risco de predação para a prole. Um custo decorrente da resposta dos pais ao risco de predação é o aumento do risco de inanição sofrido pela prole. Esta resposta é comumente observada em aves de zonas temperadas e tropicais (Ghalambor et al. 2013) e, no caso do tiziu, é possível que seja decorrente da breve exposição (apenas duas horas) do ninho a *playbacks* acústicos de predadores. Diferentes simulações de risco de predação, como a apresentação de predadores taxidermizados ou a exposição crônica ao risco, podem gerar novos padrões de resposta ao *trade-off* entre risco de predação e inanição.

## CONCLUSÕES

1. O risco de predação influencia o comportamento reprodutivo do tiziu, especificamente a execução de *displays* aéreos pelos machos e o cuidado com a prole por ambos os progenitores.

2. A influência do risco de predação sobre a performance dos *displays* depende também da qualidade do macho.

3. Machos menos parasitados e que apresentam mais ornamentos não são afetados pelo risco de predação quando executam seus *displays*.

4. Machos com mais parasitas e menos ornamentados aumentam a intensidade de seus *displays* com o maior risco de predação, uma estratégia de investimento terminal.

5. Fêmeas não se baseiam no risco de predação para escolher seus parceiros.

6. É provável que fêmeas façam esta escolha indiretamente, baseando-se no status do macho estabelecido em seleção intrassexual.

7. Durante o cuidado com a prole, ambos os sexos modificam seus comportamentos de maneira distinta, mas com o resultado final de diminuir a detectabilidade visual e acústica do ninho.

8. Com o maior risco de predação, machos diminuem o tempo que passam no ninho, são mais discretos em suas entradas e evitam decolar dos mesmos executando *displays* sexuais.

9. Fêmeas passam mais tempo no ninho incubando os ninhegos quando o risco de predação é alto.

10. Estas diferenças provavelmente estão ligadas ao dicromatismo sexual dessa espécie: machos vistosos atraem atenção visual para o ninho e ativam chamados de súplica dos filhotes,

enquanto fêmeas crípticas camuflam os filhotes e diminuem o ruído no ninho durante a incubação.

11. Todas estas respostas ao risco de predação resultam na menor oferta de alimento à prole, logo tizius escolhem diminuir o risco de predação sofrido pela prole mesmo que isso resulte em um maior risco de inanição.

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