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INSTITUTO DE BIOLOGIA

GUILHERME AUGUSTO ALVES

ANURANS ACOUSTIC AND VISUAL COMMUNICATION,
THEIR MULTICOMPONENTS, AND IMPLICATIONS IN THE
MULTIMODAL CONTEXT

COMUNICAÇÃO ACÚSTICA E VISUAL EM ANUROS, SEUS
MULTICOMPONENTES E IMPLICAÇÕES NO CONTEXTO
MULTIMODAL

CAMPINAS

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MULTIMODAL**

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Orientador: PROF. DR. LUÍS FELIPE DE TOLEDO RAMOS PEREIRA

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RESUMO

Os sapos mediam suas interações sociais principalmente por meio de vocalizações. Através do canto, os machos atraem as fêmeas e repelem machos vizinhos. Entretanto, o repertório comportamental dos anuros não está restrito aos cantos, sinais químicos, táteis, sísmicos/vibracionais e visuais também são utilizados nas interações intraespecíficas. Além disso, esses sinais, provenientes de diferentes modos sensoriais, podem ser emitidos em conjunto, de forma simultânea ou alternada, o que é denominado comunicação multimodal. A presente tese lança o olhar para a comunicação acústica e visual em anuros por duas diferentes perspectivas, que são separadas em duas seções. A primeira seção, que dá origem a dois capítulos, traz como objeto de estudo a família reofílica endêmica da Mata Atlântica, Hylodidae. No capítulo I, busca-se entender as implicações da alometria acústica, discutindo as consequências do uso de sinais acústicos em ambientes naturalmente ruidosos, como ambientes lóticos. Para isso, realizamos uma revisão bibliográfica, obtendo informações sobre frequências do canto e tamanho do corpo para todas as espécies da família Hylodidae. A partir desses dados, demonstramos que a relação alométrica se mantém para as espécies dos gêneros cantantes, *Crossodactylus* e *Hylodes*, e discutimos como essa relação alométrica, juntamente com o alto ruído, pode ter implicado na evolução inesperada da falta de vocalização nos gêneros *Megaelosia* e *Phantasmarana*. No capítulo II, realizamos observações de campo, análises de áudios e vídeos, e apresentamos uma descrição detalhada, bem como a quantificação dos sinais acústicos e visuais da espécie *Hylodes phyllodes*, tanto em nível individual quanto populacional. Identificamos e caracterizamos cantos de anúncio, territorial e de encontro, além de registrar 16 exposições visuais. Por fim, demonstramos que a sinalização visual é facilmente quantificável e pode ser usada para comparar indivíduos, populações e espécies, de forma semelhante ao que é comumente feito com sinais acústicos em anuro. A segunda seção, também composta por dois capítulos e denominados em sequência aos anteriores como III e IV respectivamente, investiga-se características da coloração corpórea de duas pererecas, *Boana albomarginata* e *Dryophytes versicolor* (Hylidae), são utilizadas na transmissão de informação nas relações sociais, levando em consideração o contexto multimodal (canto + característica da coloração corpórea como pista visual). O capítulo III desta tese examinou como as características de coloração e canto podem prever as características físicas dos indivíduos de *B. albomarginata*, em diferentes populações. Para isso, utilizamos gravações dos cantos e fotografias para acessar características individuais dos cantos e cores, além de medidas de massa e tamanho. Nossos resultados demonstram que os machos em melhores condições corporais apresentam manchas maiores e

mais intensas, além de melhores características acústicas. Por fim, para o capítulo IV usamos uma abordagem experimental de testes de duas escolhas para testar se diversos traços de coloração corpórea e movimentação do saco vocal transmitem informações visuais secundárias em *D. versicolor*, aumentando a atratividade dos machos. Nenhum dos estímulos visuais testados impactou a escolha das fêmeas, e não houve diferença na taxa de resposta, tempo de resposta, ângulo de escolha ou distância percorrida na arena entre estímulos multimodais e unimodais.

Palavras-chave: alometria; barreiras acústicas; bioacústica; coloração corpórea; ecologia comportamental; história natural; sinalização visual.

ABSTRACT

Frogs mainly mediate their social interactions through vocalizations. Males use their calls to attract females and repel neighboring males. However, the behavioral repertoire of anurans is not limited to calls; chemical, tactile, seismic/vibrational, and visual signals are also used in intraspecific interactions. Moreover, these signals, originating from different sensory modes, can be emitted simultaneously or alternately, which is termed multimodal communication. This thesis focuses on the acoustic and visual communication in anurans from two different perspectives, which are divided into two sections. The first section gives rise to two chapters and focuses on the endemic reophilic family of the Atlantic Forest, Hylodidae. Chapter I seeks to understand the implications of acoustic allometry, discussing the consequences of using acoustic signals in naturally noisy environments such as lotic water bodies. To do this, we conducted a literature review, gathering information on the frequencies of calls and body size for all species in the Hylodidae family. Based on this data, we demonstrated that the allometric relationship holds true for species in the calling genera *Crossodactylus* and *Hylodes*. We also discussed how this allometric relationship, along with high noise levels, may have contributed to the unexpected evolution of the lack of vocalization in the genera *Megaelosia* and *Phantasmarana*. In Chapter II, we conducted field observations, audio and video analyses, and provided a detailed description, as well as quantification of acoustic and visual signals in the species *Hylodes phyllodes*, at both the individual and population levels. We identified and characterized advertisement, territorial, and encounter calls, as well as recorded 16 visual displays. Finally, we demonstrated that visual signaling is quantifiable and can be used to compare individuals, populations, and species, similar to what is commonly done with acoustic signals in anurans. The second section, also composed of two chapters and named in sequence to the previous ones as III and IV respectively, investigates whether characteristics of body coloration in two treefrogs, *Boana albomarginata* and *Dryophytes versicolor* (Hylidae), are used in the transmission of information in social relationships, taking into account the multimodal context (call + body coloration as visual cues). Chapter III of this thesis examines how coloration and calls can predict the physical characteristics of individuals in different populations of *B. albomarginata*. To do this, we used recordings of calls and photographs to assess individual characteristics of calls and colors, as well as measurements of mass and size. Our results demonstrate that males in better physical condition display larger and more intense orange patches, as well as better acoustic characteristics. Finally, for Chapter IV, we employed an experimental two-choice test approach

to examine whether various body coloration traits and vocal sac movements convey secondary visual information in *D. versicolor*, enhancing male attractiveness. None of the visual stimuli tested impacted female choice, and there were no differences in response rate, latency, choice angle, or path length in the arena between multimodal and unimodal stimuli.

Keywords: allometry; acoustic barriers; bioacoustics; behavioral ecology; body coloration; natural history; visual signaling.

SUMÁRIO

INTRODUÇÃO GERAL	14
CHAPTER I - Acoustic allometry, background stream noise and its relationship with large-bodied and voiceless rheophilic frogs	19
Abstract.....	21
Introduction.....	22
Methods	23
Results.....	28
Discussion.....	30
Acknowledgements.....	32
Supplementary material	34
CHAPTER II - Communication across multiple sensory modes: quantifying the rich behavioural repertoire of a Neotropical torrent frog.....	36
Abstract.....	38
Introduction.....	39
Methods	40
Results.....	42
Discussion.....	51
Acknowledgments	55
Supplementary material	56
CHAPTER III - Geographic variation in acoustic and visual cues and their potential to signal body condition in the Brazilian treefrog, <i>Boana albomarginata</i>	68
Abstract.....	70
Introduction.....	71
Methods	73
Results.....	78
Discussion.....	84
Acknowledgments	86
Supplementary material	87
CHAPTER IV - Visual cues do not function in a multimodal signaling context for mate attraction in Eastern Gray Treefrogs	103
Abstract.....	105
Introduction.....	106

Methods	107
Results.....	114
Discussion.....	121
Acknowledgments	125
Supplementary material	126
CONSIDERAÇÕES FINAIS	129
REFERÊNCIAS BIBLIOGRÁFICAS	131
ANEXOS	155
Anexo I – Licença de coleta: Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio #63697-2)	155
Anexo II – Licença de coleta: Instituto Florestal (Carta COTEC #483/2018 D98/2018 PH)	158
Anexo III – Parecer da Comissão de Ética no Uso de Animais da Universidade Estadual de Campinas (CEUA/Unicamp #4983-1/2018).....	163
Anexo IV – Registro no Sistema Nacional de Gestão do Patrimônio Genético (SISGen #AE9A0E0)	164
Anexo V – Declaração de Bioética e Biossegurança.....	165
Anexo VI – Declaração de Direitos Autorais	166
Anexo VII – comprovante submissão do capítulo III.....	167
Anexo VIII – Comprovante submissão do capítulo IV	168
Anexo IX– Produção científica durante a vigência do curso de doutorado.....	169

INTRODUÇÃO GERAL

Anuros contam com um diverso repertório comportamental baseado em sinalizações realizadas por diversas vias sensoriais, i.e., estímulos acústicos, químicos, sísmicos/vibracionais, táteis e visuais (e.g. Narins 1990; Bourne et al. 2001; Lewis et al. 2001; Byrne & Keogh 2007; Caldwell et al. 2010; Poth et al. 2012; Starnberger et al. 2014; Brunetti et al. 2014; Zornosa-Torres & Toledo 2019). Dentre estas vias, a sinalização acústica é a via mais utilizada para mediar as interações sociais entre os indivíduos (Wells 2007; Arch & Narins 2009; Starnberger et al. 2014; Folly & Hepp 2019; Chen & Wiens 2020), quando comparada às outras vias de comunicação.

Através do canto, machos atraem as fêmeas, as quais acessam informações do indivíduo emissor, tais como maturidade reprodutiva, tamanho, condição corpórea, e localização precisa (Ryan 1980; Forester & Czarnowsky 1985; Höbel & Gerhardt 2003; Toledo et al. 2015; Köhler et al. 2017). Menos comum e restrito a poucas espécies, as fêmeas podem emitir cantos em resposta aos machos, ou até mesmo para atraí-los durante os períodos reprodutivos (Emerson 1992; Emerson & Boyd 1999). Ainda, os cantos emitidos regulam as relações intrasexuais (macho-macho). Por meio do sinal acústico os machos mediam interações agressivas, protegem seus territórios e se organizam espacialmente no ambiente, mantendo o distanciamento (Wilczynski & Brenowitz 1988; Rose & Brenowitz 1991; Bee 2003; Toledo & Haddad 2005; Wells & Schwartz 2006; Chuang et al. 2017). A função de atração de fêmeas e mediação das relações entre machos nos coros é atribuída a um tipo específico de canto, chamado de canto de anúncio, mas o repertório acústico dos anuros não é limitado a um tipo de vocalização, revisões apontam um repertório extenso, com 14 tipos diferentes de cantos, classificados em quatro categorias: reprodutiva, agressiva, defensiva e alimentação (Toledo et al. 2015; Köhler et al. 2017).

Outra via sensorial amplamente observada no repertório comportamental dos anuros é a sinalização visual, ela envolve a transmissão de informação por meio da realização de gestos, movimentos e posturas estereotipadas ou pela exibição de características físicas como, por exemplo, traços da coloração corpórea (Hartmann et al. 2005; Giasson & Haddad 2006; Hirschmann & Hödl 2006; Vásquez & Pfennig; 2007; Furtado et al. 2019).

A sinalização visual está presente em grande parte das espécies com atividade diurna e/ou com atividade reprodutiva ligada a ambientes com alto ruído abiótico, como cachoeiras e riachos (Pombal et al. 1994; Haddad & Giaretta 1999; Hödl & Amézquita 2001; Narvaes & Rodrigues 2005; Hirschmann & Hödl 2006; Preininger et al. 2009). Desta maneira, a presença

de luminosidade e o alto ruído de fundo podem ter operado como pressões seletivas no desenvolvimento dessa forma de comunicação (Hödl & Amézquita 2001). Neste sentido, a sinalização visual representa um canal complementar de comunicação para as espécies que se reproduzem em ambientes lóticos ruidosos, uma vez que ruído representa uma barreira na dissipação da informação acústica (Boeckle et al. 2009; Schwartz and Bee 2013; Caldart et al. 2016), comprometendo a recepção do sinal pelo indivíduo receptor (Hödl & Amézquita 2001). Este é o caso da família Hylodidae, um dos taxa focais do presente estudo. A família contém quatro gêneros (Vittorazzi et al. 2021): *Megaelosia* e *Phantasmarana* que são mudos, i.e., não emitem canto de anúncio, e se comunicam por sinais visuais (há o registro do uso de sinalização visual na interação agressiva entre machos de *P. apuana*: Augusto-Alves et al. 2018); e *Crossodactylus* e *Hylodes* que possuem um amplo repertório comportamental conhecido, envolvendo sinais acústicos, visuais e táteis (Weygoldt & Carvalho e Silva 1992; Haddad & Giaretta 1999; Narvaes & Rodrigues 2005; Caldart et al. 2014; de Sá et al. 2016; Furtado et al. 2019).

Por outro lado, sapos têm uma visão altamente adaptada às condições de pouca luz, sendo capazes até mesmo de distinguir cores em ambientes com baixa luminosidade (Gomez et al. 2010; Kelber et al. 2017; Yovanovich et al. 2017; Robertson et al. 2022). Desta maneira, a comunicação visual em espécies de hábitos noturnos pode ter apenas sido negligenciada por algum tempo, o que atualmente vem se refletindo no aumento de estudos (e.g., Hartmann et al. 2004; Toledo et al. 2007; Abrunhosa & Wogel 2008; Miranda et al. 2008; Gomez et al. 2009; Barros & Feio 2011; Furtado et al. 2017; Moroti et al. 2017). Crescimento que abrange nossas espécies focais *Boana albomarginata* (Hartmann et al. 2005; Giasson & Haddad 2006; Furtado & Nomura 2014) e *Dryophytes versicolor* (Reichert 2013).

Independente da via de transmissão da informação, o processo de comunicação é complexo e pode envolver mais de um componente dentro do mesmo modo sensorial (Candolin 2003; Hebets & Papaj 2005; Partan & Marler 2005). Exemplificando, dentro da comunicação acústica, diferentes parâmetros temporais e espectrais podem ser utilizados pelos receptores para acessar as características dos indivíduos emissores (Vignal & Kelley 2007). Ainda, diferentes partes do corpo, com traços de coloração distintos, podem enviar mensagens aos receptores como sinais visuais (Jones et al. 2017; Ferrer et al. 2021). Por outro lado, para além dessas vias sensoriais, as interações sociais frequentemente são dependentes de suas intersecções multimodais. A comunicação multimodal (assim como as variações terminológicas comunicação/sinalização multimodal/multissensorial) vem recebendo atenção especial dos ecólogos comportamentais nas últimas décadas (Møller & Pomiankowski 1993;

Johnstone 1996; Candolin 2003; Hebets & Papaj 2005; Partan & Marler 2005; Bro-Jørgensen 2010; de Luna et al. 2010; Starnberger et al. 2014a; 2014b; Caldart et al. 2022), e é caracterizada pela transmissão de informação por duas ou mais vias sensoriais de forma simultânea ou alternada. A sinalização multimodal pode ter evoluído para diferentes funcionalidades na comunicação animal, e diferentes hipóteses são discutidas - a hipótese do sinal redundante propõe que os diferentes componentes servem como *back-up*, permitindo que o receptor, de uma só vez, acesse a mesma informação codificada em diferentes modalidades (Møller & Pomiankowski 1993; Johnstone 1996; Hebets & Papaj 2005). Por outro lado, a hipótese das mensagens múltiplas propõe que os diferentes componentes codificam diferentes características (Møller & Pomiankowski 1993; Johnstone 1996; Hebets & Papaj 2005), ou que são usados em diferentes contextos, por exemplo, um componente para atrair fêmeas e o outro para a defesa territorial (Hebets & Papaj 2005).

Existem diferenças na forma de propagação e percepção dos sinais de diferentes vias, assim a combinação do uso de vários modos sensoriais é benéfica ao emissor porque aumenta a chance de transferência precisa das informações. Por exemplo, vocalizações possuem longo alcance, sendo transmitidas em distâncias maiores (Forrest 1994; Velásquez et al. 2018), assim mediam interações de curtas a longas distâncias nos mais diferentes contextos sociais (Toledo et al. 2015; Köhler et al. 2017). Em contraste, a sinalização visual é mais eficaz em curtas distâncias, funcionando principalmente para avaliação de curto alcance da localização e condição do emissor (Giasson & Haddad 2007; Robertson & Greene 2017). Adicionalmente, cada modo sensorial precisa lidar com diferentes barreiras. Sinais acústicos podem ser mascarados por ruído abiótico (e.g., chuva: Lengagne & Slater 2002; Augusto-Alves et al. 2020; fluxo de água: Boeckle et al. 2009; Schwartz & Bee 2013; vento: Luther & Gentry 2013; e ruído abiótico não natural: Parris et al. 2009). Por sua vez, os sinais visuais também enfrentam desafios ambientais, como a presença de vegetação movida pelo vento ao fundo ou condições de iluminação insuficiente (Fleishman 1992; Ord et al. 2007; Ord & Stamps 2008). Por fim, o uso de sinais multissensoriais reduz erros de avaliação e dificulta trapaças, favorecendo uma decisão mais precisa para os receptores (Candolin 2003; Hebets & Papaj 2005).

Deste modo, dada a variedade de vias de comunicação utilizadas pelos anuros, assim como as suas complexidades e possíveis interações em um contexto multimodal, apresento esta tese, que foi organizada em duas principais seções. A primeira seção, denominada “Ruído de fundo natural como pressão seletiva na comunicação acústica e visual”, traz como objeto de estudo as espécies pertencentes a família Hylodidae. O capítulo I traz uma revisão de características acústicas e morfológicas da família, evidenciando a complexidade da alometria

acústica em espécies reofílicas, e discutindo as estratégias de eficácia da comunicação intraespecífica em ambientes com amplo ruído do fluxo de água (capítulo I: *Zoologischer Anzeiger*, 295: 156–162, *special issue: 'Fascinating adaptations in amphibians'*). O capítulo II é focado em observações da história natural de *Hylodes phyllodes*, e traz a descrição do repertório vocal e visual da espécie, detalhando as variações encontradas nos níveis intra- e interpopulacionais, discutindo como sinais visuais podem ser quantificados e, portanto, podem ser utilizados para comparar indivíduos, populações e espécies, assim como frequentemente realizado com o sinal acústico (capítulo II: *Behaviour*, 159: 351–375). Já na segunda seção, denominada “Características de coloração corpórea como pistas visuais em um contexto multimodal” foram investigadas duas espécies de pererecas, *Boana albomarginata* e *Dryophytes versicolor*, com o objetivo de desvendar se machos dessas espécies utilizam características de coloração corpórea para transmitir informações. No capítulo III examinou-se como as características de coloração e canto podem predizer as características físicas dos indivíduos de *B. albomarginata*. Por fim, por meio de uma abordagem experimental, o capítulo IV investiga se as características de coloração de diferentes partes corpóreas são utilizadas como pistas visuais durante o processo de escolha dos parceiros pelas fêmeas de *D. versicolor*.

FISRT SECTION.**Natural background noise as selective pressure in acoustic and visual communication****Content:**

Chapter I. Augusto-Alves, G., Dena, D. & Toledo, L. F. Acoustic allometry, background stream noise and its relationship with large-bodied and voiceless rheophilic frogs.

Chapter II. Augusto-Alves, G. & Toledo, L. F. Communication across multiple sensory modes: quantifying the rich behavioural repertoire of a Neotropical torrent frog.

CHAPTER I

ACOUSTIC ALLOMETRY, BACKGROUND STREAM NOISE AND ITS RELATIONSHIP WITH LARGE-BODIED AND VOICELESS RHEOPHILIC FROGS

Alometria acústica, ruído de fluxo de fundo e sua relação com rãs reofílicas de corpo grande e mudas

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Acoustic allometry, background stream noise and its relationship with large-bodied and voiceless rheophilic frogs

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Abstract

Anurans that breed in rivers and cascade streams need to deal with the influence of the low-frequency water flow noise that could interfere with their acoustic communication. To overcome that, some species display visual signals or emit high-pitched calls. However, there is a constraint related to body size [snout-vent length (SVL)] that limits the anurans' calling frequency range. Thus, to evaluate if water noise influences this allometric relationship, we analysed hylodid frogs' SVL, dominant frequencies of advertisement call, and background noise to which individuals are submitted. Although *Hylodes* and *Crossodactylus* have differences in morphology and natural history, we found negative acoustic allometry in both genera. This allometry may be part of the explanation of the absence of advertisement calls in *Megaelosia* and *Phantasmarana* genera. Based on the large body lengths of *Megaelosia* and *Phantasmarana* species, if they could call, it would be low-pitched and acoustically masked by the stream noise. Besides, we found a positive relationship between *Hylodes phyllodes* advertisement call and background sound pressure level (SPL), thus, the analysed individuals, emitted louder calls when submitted to louder background SPLs. This study exemplifies the complexity of acoustic allometry in rheophilic species: we evidenced a strategy for intraspecific communication effectiveness in rivers and streams with broad water flow background noise; and how acoustic allometry coped with background noise could be implying in unexpected trait evolution, as lack of vocalization in anurans.

Keywords: Acoustic communication; advertisement call; diurnal species; background noise; voiceless frogs; call evolution.

Introduction

Animals that communicate with sounds should overcome interferences present in the acoustic space to improve the signal transmission (Gerhardt & Huber 2002; Römer 2013). This is particularly true for species in which acoustic communication is pivotal to reproductive activities, as anurans. Frogs that use acoustic communication in noisy environments tend to have high-pitched frequency calls (Narins et al. 2004; Grafe et al. 2012; Caldart et al. 2016). Some species can even increase the dominant frequency of their calls when exposed to environments with higher noise intensity, as in urban habitats (Parris et al. 2009; Cunningham & Fahrig, 2010), or in the presence of low-pitched calling invasive species (Both & Grant 2012; Medeiros et al. 2017). However, even if anurans can modulate the frequency of their vocalizations (e.g., Foratto et al. 2021), this ability is constrained by their anatomy, since there is a negative correlation between anuran body size and call frequencies – the acoustic allometry (Bee et al. 2000; Gingras et al. 2013; Nunes-de-Almeida et al. 2016; Galvis et al. 2016; Tonini et al. 2020). Body size determines the mass of the vocal apparatus, which relates to the size of vocal folds (Narins & Smith 1986; McClelland et al. 1996; Suthers et al. 2006). Only a few escapes of acoustic allometry were recently reported, either in studies of the relationship within families and large lineages clades (Tonini et al. 2020) or within populations of a single species (Rebouças et al. 2020).

Based on an extensive dataset, Tonini and collaborators (2020) demonstrated that acoustic allometry is conservative for anurans, once allometric escape was detected in only four lineages around the globe. Among the hypotheses raised, calling site and background noise could be important factors influencing these escapes (Tonini et al. 2020). Species that breed in lotic water bodies, such as rivers and streams, need to deal with background noise produced by the water flow, including that of waterfalls. Solutions to this potential interference are the emission of high-pitched calls (Narins et al. 2007; Bilate et al. 2012; Grafe et al. 2012), including ultrasonic frequencies (Narins et al. 2004; Feng et al. 2006; Arch et al. 2008; Sheng et al. 2008; Feng & Narins 2008), presence of visual signalling (Haddad & Giaretta 1999; Hödl & Amézquita 2001; Caldart et al. 2014; de Sá et al. 2016), and diurnal activity (Haddad & Giaretta 1999; Grafe et al. 2012; Caldart et al. 2016). Besides, the low frequency and often high sound pressure level (SPL) of lotic water bodies are also pointed out as selective pressures in the evolution of voiceless species (Wells 1977; Emerson & Inger 1992). The terminologies “voiceless” or “mute” are applied to frog species that do not produce advertisement calls, even if these species can emit other calls, such as distress (Giaretta & Aguiar Jr., 1998; Bell 2010), release (Jacobson & Vanderberg 1991; Giaretta & Aguiar Jr. 1998) or calls with still

unidentified functions (Jacobson & Vanderberg 1991; Muscat et al. 2020). If losing the calling ability (or simply not producing it) could be considered a handicap, it also may imply in benefits. For example, frogs that do not vocalize are acoustically camouflaged, avoiding acoustically oriented predators (e.g., Ryan et al. 1981; 1982; Page & Ryan 2008), or they can establish breeding and viable populations in noisy environments, once the acoustic interference is not a barrier for their communication. For instance, in the clade [*Phantasmarana* + (*Hylodes* + *Megaelosia*)], large-bodied species, i.e., *Megaelosia goeldii* and *Phantasmarana* spp., can occupy large and noisy streams where species of *Hylodes* usually cannot be found (G. Augusto-Alves & L. F. Toledo, personal observations).

To evaluate whether the background noise from rivers and streams influences the acoustic-size allometry, we studied the relationship between snout-vent length (SVL) and advertisement call dominant frequency of Atlantic Forest rheophilic species of the Hylodidae family. We chose this family as a model for our study because it includes diurnal species (Nogueira et al. 2006; Almeida-Gomes et al. 2007; de Sá et al. 2016), which communicates and breeds in noisy rivers and streams (Haddad & Giaretta, 1999; Pombal et al. 2002; Augusto-Alves et al. 2018), presents a wide variation in body length (Lingnau et al. 2008; Pimenta et al. 2008; de Sá et al. 2015; Pimenta et al. 2015; Haddad et al. 2013), and includes large voiceless species (Giaretta & Aguiar Jr. 1998; Haddad et al. 2013; Augusto-Alves et al. 2018). We tested the following hypotheses: i) there is a negative allometric relationship between SVL and call frequencies among species of the genera *Hylodes* and *Crossodactylus*; ii) louder background stream noise leads to louder and higher-pitched advertisement calls (model species: *Hylodes phyllodes*); iii) the allometric relationship in *Hylodes* and *Crossodactylus* coupled with information of background noise explains the muteness of *Megaelosia* and *Phantasmarana* genera.

Methods

We compiled the snout-vent length (SVL) and dominant frequency of advertisement call (hereafter referred as dominant frequency) data from the literature (Table 1), complemented with new data for species with no information available, using a similar approach as referred by Tonini and collaborators (2020). To species with unavailable data from the literature, we measured the SVL (using a calliper of 0.01 mm precision) from individuals housed at Museu de Diversidade Biológica (MDBio), Universidade Estadual de Campinas (Unicamp) (Appendix S1) and extracted the dominant frequency from recordings deposited at Fonoteca Neotropical Jacques Vielliard (FNJV) (Appendix S2). We also gathered data from the

literature and the same recordings as used to access dominant frequency, to obtain fundamental frequency, aiming to find what measure would better represent the acoustic allometry; since, according to Gingras and collaborators (2013), fundamental frequency has a clearer relationship between body mass and length of the vocal folds. We followed the terminology and definitions presented by Köhler and collaborators (2017). Before analyses, we normalized all recordings removing DC offset (mean amplitude displacement from zero), centring on vertical and to the maximum amplitude of -1.0 dB, using Adobe Audition CC; and standardized then in a sample rate of 44.1 kHz and 16-bit resolution. These audios were analysed with Raven Pro 1.5 (Cornell Lab of Ornithology, Bioacoustics Research Program), using the following configuration: 70 % of brightness, 50 % of contrast, and Discrete Fourier Transform window size (DFT) of 512. To determine the fundamental frequency, we used the frequency spectrum function on Audacity® (Audacity Team) and got the first call peak frequency (that is, the first frequency peak with highest power).

We analysed the maximum frequency (frequency 95% function in Raven) of the water flow background noise by measuring ten points, each about one second long, between the advertisement calls of the same recordings used to access the dominant frequency (following the same standardization described above; table 1; Appendix S2).

We measured the sound pressure level (SPL) of 30 calling males of *Hylodes phyllodes* and 30 stream noise recordings (in moments when frogs were not calling), from municipality of Bertioga (Parque Estadual Serra do Mar, Núcleo Bertioga) and Picinguaba, in the municipality of Ubatuba (Parque Estadual Serra do Mar, Núcleo Picinguaba), with approximately 140 km straight-line distance between them, both in the state of São Paulo, southeast Brazil. Sampling was performed between January and March 2019. Avoiding measure of call SPL from same males, the individuals were marked using toe clipping, the tissues were deposited at TLFT tissue collection (TLFT 4441–98), at Universidade Estadual de Campinas (Unicamp), Brazil. We used these measurements as a proxy of how the background noise influences the sound production of hylodid torrent frogs. The advertisement call and the background noise peak SPLs were measured with an Instrutherm DEC-490 Sound Level Meter (dB range: 30–130 dB; time weighting: Fast; Frequency weighting: A), at approximately 0.7 m from these individuals or the stream. Prior to analysis, we converted the gain values of dB in linear scale, following the formula: $\text{dB}_{\text{linear}} = 10^{(\text{gain}/20)}$ (Pinheiro 2017).

Table 1. Snout-vent length (SVL) and dominant frequency of the advertisement call of the analysed species, and background noise maximum frequency (inferred by the frequency 95% function in Raven). Values are presented as mean \pm standard deviation (range; sample size). Sample sizes are number of individuals for which SVL was measured; number of calls followed by number of males recorded for dominant frequency of the advertisement call; and number of recordings for background noise. An asterisk indicates mean values based on range values extracted from other references.

Species	Male SVL	Reference	Dominant Frequency	Reference	Background noise 95%
<i>Crossodactylus caramaschii</i>	24.5 \pm 0.99 (21.7–25.8; 11)	Bastos & Pombal Jr. 1995	4.47 \pm 0.47 (3.01–4.91; 26; 8)	Present study	2.68 \pm 2.79 (0.09–7.15; 8)
<i>Crossodactylus cyclopinus</i>	23.7 \pm 0.4 (23.2–24.4; 9)	Nascimento et al. 2005	4.98 \pm 0.62 (3.49–5.45; 25; 1)	Nascimento et al. 2005	-
<i>Crossodactylus franciscanus</i>	21.3 \pm 0.61 (20.4–22.1; 7)	Pimenta et al. 2015	3.46 \pm 0.28 (2.34–4.03; 284; 1)	Pimenta et al. 2015	-
<i>Crossodactylus gaudichaudii</i>	26.3 \pm 2.75 (23.5–30.4; 21)	Pimenta et al. 2008	3.66 \pm 0.26 (3.27–3.96; 13; 2)	Present study	5.04 \pm 2.90 (2.07–8.70; 2)
<i>Crossodactylus schmidti</i>	23.3 \pm 1.37 (21.4–25; 7)	Pimenta et al. 2008	3.3 \pm 0.64 (2.02–4.28; 11; 7)	Caldart et al. 2011	-
<i>Crossodactylus trachystomus</i>	22.2 \pm 1.23 (18.7–25.1; 67)	Pimenta et al. 2015	3.89 \pm 0.52 (1.8–4.83; 86; 18)	Pimenta et al. 2015	-
<i>Crossodactylus weneri</i>	22.1 \pm 1.2 (20.9–25.4; 13)	Vidigal et al. 2018	3.3 \pm 0.2 (3–3.6; 79; 10)	Vidigal et al. 2018	5.34 \pm 3.08 (0.17–9.99)
<i>Hylodes amnicola</i>	26.7 \pm 0.94 (25.3–28.1; 9)	Pombal Jr. et al. 2002	5.65 \pm 0.55 (5.1–6.2; 12; 3)	Pombal Jr. et al. 2002	-
<i>Hylodes asper</i>	29.6 \pm 5.98 (24.2–38.4; 7)	Hartmann et al. 2010	3.8 \pm 1.13 (2.15–5.43; 12; 3)	Present study	3.54 \pm 0.25 (3.10–4.04; 3)
<i>Hylodes babax</i>	31.5 \pm 0.99 (29.9–33.2; 7)	Pirani et al. 2010	4.85 \pm 0.32 (4.31–5.42; 6; 3)	Pirani et al. 2010	-
<i>Hylodes caete</i>	32.3 \pm 0.88 (31.1–34; 16)	Malagoli et al. 2017	4.64 \pm 0.03 (3.93–5.06; 6; 6)	Malagoli et al. 2017	-
<i>Hylodes cardosoi</i>	40.4 \pm 2 (35.6–44.1; 32)	Lignau et al. 2008	5.19 \pm 0.33 (3.90–5.80; 13; 4)	Lignau et al. 2008	-

<i>Hylodes charadranaetes</i>	33 ± 1.8 (31.3–34.7; 2)	Heyer & Cocroft 1986*	4.49 ± 1.23 (2.50–5.34; 11; 2)	Present study	3.87 ± 1.36 (1.55–5.51; 2)
<i>Hylodes fredii</i>	34.4 ± 1 (32.8–36.7; 19)	Canedo & Pombal Jr. 2007	4.03 ± 0.33 (3.60–4.50; 2; -)	Canedo & Pombal Jr. 2007	-
<i>Hylodes glaber</i>	31.7 ± 4.7 (22.5–39.1; 17)	Present study	4.13 ± 0.23 (3.96–4.56; 38; 2)	Present study	1.86 ± 0.22 (1.55–2.32; 2)
<i>Hylodes heyeri</i>	36 ± 0.9 (33.9–37.9; 17)	Lingnau & Bastos 2007	4.22 ± 0.12 (3.90–4.61; 85; 17)	Lingnau & Bastos 2007	-
<i>Hylodes japi</i>	24.7 ± 0.76 (22.9–25.8; 12)	de Sá et al. 2015	6.20 ± 0.17 (5.80–6.60)	de Sá et al. 2015	-
<i>Hylodes lateristrigatus</i>	37.9 ± 1.25 (36.7–39.2; 2)	Heyer & Cocroft 1986*	4.92 ± 1.11 (1.89–6.37; 58; 5)	Present study	3.15 ± 2.39 (0.52–9.56; 5)
<i>Hylodes magalhaesi</i>	30.1 ± 1.4 (28.7–31.5; 2)	Silva & Benmaman, 2008*	5.29 ± 0.21 (4.91–5.68; 29; 7)	Present study	6.05 ± 2.86 (0.86–9.99; 7)
<i>Hylodes meridionalis</i>	39.4 ± 1.96 (6)	Furtado et al. 2019	3.88 ± 0.86 (1.64–4.91; 45; 7)	Present study	3.13 ± 2.03 (0.17–8.27; 7)
<i>Hylodes nasus</i>	33 ± 1.8 (29–36; 13)	Machado et al. 2016	4.38 ± 0.25 (3.70–4.74; 66; 5)	Present study	4.12 ± 1.71 (0.86–6.54; 5)
<i>Hylodes ornatus</i>	24.2 ± 1.3 (22.9–25.5; 2)	Silva & Benmaman 2008*	5.33 ± 0.17 (5.12–6.37; 47; 1)	Bilate et al. 2012	-
<i>Hylodes otavioi</i>	32.6 ± 1.17 (30.9–34; 5)	Sazima & Bokermann 1982*	4.33 ± 0.08 (4.22–4.74; 56; 2)	Present study	1.93 ± 0.11 (1.81–2.15; 2)
<i>Hylodes perere</i>	25.2 ± 0.91 (23.4–27.1; 38)	Silva & Benmaman, 2008	5.49 ± 0.69 (4.80–6.19; 2; -)	Silva & Benmaman 2008	-
<i>Hylodes perplicatus</i>	38.6 ± 1.18 (37.–39.8; 4)	Haddad et al. 2003	3.02 ± 0.1 (2.91–3.13; 5; 1)	Monteiro et al. 2014	-
<i>Hylodes phyllodes</i>	27.4 ± 0.82 (25.6–29.8; 58)	Augusto-Alves & Toledo, 2022	4.83 ± 0.34 (4.05–5.68; 1,617; 58)	Augusto-Alves & Toledo 2022	5.87 ± 3.95 (0.17–11.71; 10)
<i>Hylodes pipilans</i>	24.1 ± 0.8 (23.0–25.1; 9)	Canedo and Pombal Jr. 2007	5.01 ± 0.24 (4.65–5.34; 33; 2)	Present study	4.95 ± 1.57 (2.50–8.01; 2)
<i>Hylodes regius</i>	34.7 ± 0.98	de Sá et al. 2020	4.66 ± 0.16	de Sá et al. 2020	-

	(33.6–35.5; 3)		(4.41–4.91; 32; -)		
<i>Hylodes sazimai</i>	27.6 ± 0.63 (27.1–28.5; 3)	Haddad & Pombal Jr. 1995	4.35 ± 0.31 (4.13–5.17; 11; 3)	Present study	5.04 ± 1.46 (2.49–8.27; 3)
<i>Hylodes uai</i>	32.7 ± 0.86 (31.2–33.6; 8)	Nascimento et al. 2001	4.66 ± 0.4 (3.96–5.08; 25; 6)	Present study	7.19 ± 0.75 (5.08–7.83; 6)
<i>Hylodes vanzolinii</i>	29 (1)	Heyer 1982	Mute	Heyer 1982	-
<i>Megaelosia goeldii</i>	87.4 ± 4.45 (82.2–91.4; 6)	Vittorazzi et al. 2021	Mute	Giaretta et al. 1993	-
<i>Phantasmarana apuana</i>	87.2 ± 8.82 (78.0–97.2; 4)	Santos et al. 2011	Mute	Augusto-Alves et al. 2018	-
<i>Phantasmarana bocainensis</i>	101 (1)	Haddad et al. 2013	Mute	Giaretta et al. 1993	-
<i>Phantasmarana boticariana</i>	98.8 (1)	Present study	Mute	Giaretta & Aguiar Jr. 1998	-
<i>Phantasmarana lutzae</i>	90.4 ± 2.1 (88.3–92.5; 2)	Giaretta et al. 1993	Mute	Giaretta et al. 1993	-
<i>Phantasmarana massarti</i>	107 ± 6.57 (100.3–113.8; 2)	Giaretta et al. 1993	Mute	Giaretta et al. 1993	-

We used linear models to assess the relationship between dominant frequency and SVL and genera (*Hylodes* and *Crossodactylus*). We first ran a model including an interaction between the predictors and genera. Although this model was significant ($F_{(3,27)} = 6.83$, Adjusted $r^2: 0.37$, $P = 0.001$), the interaction was not ($P = 0.18$), and so we performed a backward selection, excluding the interaction and modelling dominant frequency as a function of SVL and genera. We performed the same models using fundamental frequency as the response variable and, since the results were very similar to those of dominant frequency and we had more data available for dominant frequency, we present the results of fundamental frequency in the supplemental material (Table S2). Additionally, we ran a linear regression between call and background noise SPLs for individuals of *H. phyllodes* (using linear data for SPL). In order to test for SVL differences in the three genera, we performed a Kruskal-Wallis, followed by a Dunn's post hoc test. Analyses were run in R version 3.6.3 (R Core Team 2019).

This study was approved by animal ethics committee of Unicamp (CEUA #4396-1), sampling permits were provided by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio, SISBio #63697-2), Comissão Técnico-Científica of the Instituto Florestal (COTEC #483/2018), and was registered at Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado (SisGen #AE9A0E0).

Results

We found that dominant frequency depends on both SVL and genus, and that mean dominant frequency in *Hylodes* is 1.37 higher than in *Crossodactylus* (Table 2; Figure 1A). Additionally, we found that the *Hylodes* linear regression line intersected the x-axis (i.e., at 0 Hz) at 98 mm in SVL, and in *Crossodactylus*, at 78.57 mm in SVL, within the body length range of *Megaelosia* and *Phantasmarana* genera: that varied between 78 and 113.8 mm (Figure 1 B). The estimated average of the maximum frequency water flow background noise for *Hylodes* was 4.23 ± 1.15 kHz and for *Crossodactylus* was 5.06 ± 0.47 kHz. We found a positive relationship between the SPL of *H. phyllodes* advertisement call and background stream noise ($F_{(1,28)} = 8.71$; Adjusted $r^2 = 0.21$; $P = 0.006$; Figure 2).

The SVL among the genera were different (Kruskal-Wallis test: $X^2 = 30.47$, $P < 0.001$). Individuals of *Megaelosia goeldii* (monotypic genus) had similar size as *Phantasmarana* spp. (Dunn's test, $Z = 0.54$, $P = 0.587$), together they were larger than *Hylodes* spp. (*Megaelosia*: Dunn's test, $Z = 2.82$, $P = 0.007$; *Phantasmarana*: Dunn's test, $Z = 3.29$, $P = 0.002$), and *Crossodactylus* spp. (*Megaelosia*: Dunn's test, $Z = 4.32$, $P < 0.001$; *Phantasmarana*:

Dunn's test, $Z = 4.67$, $P < 0.001$). Lastly, SVL of *Crossodactylus* spp. and *Hylodes* spp. also differed (Dunn's test, $Z = 2.53$, $P < 0.014$; Figure 1B).

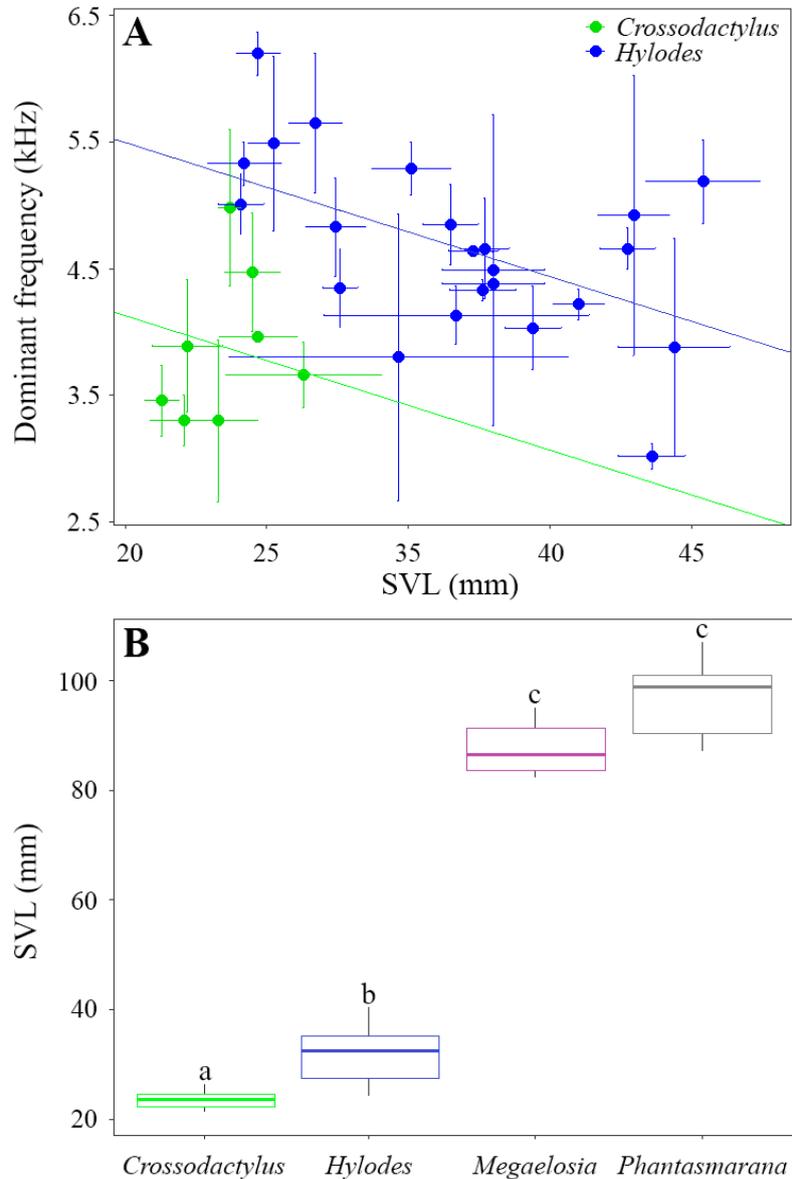


Figure 1. A) Relationship between mean dominant frequency and mean snout-vent length (SVL) of *Hylodes* and *Crossodactylus* species (with standard deviation). Line equations for *Hylodes*: Frequency = $-0.07 \cdot \text{SVL} + (5.5 + 1.37)$; and *Crossodactylus*: Frequency = $-0.07 \cdot \text{SVL} + 5.5$. B) SVL variation within the four hylodid genera [boxplot indicate median, upper and lower quartiles and upper and lower whiskers; boxplot of *Megaelosia* represents the SVL variation in *Megaelosia goeldii* (monotypic genus)].

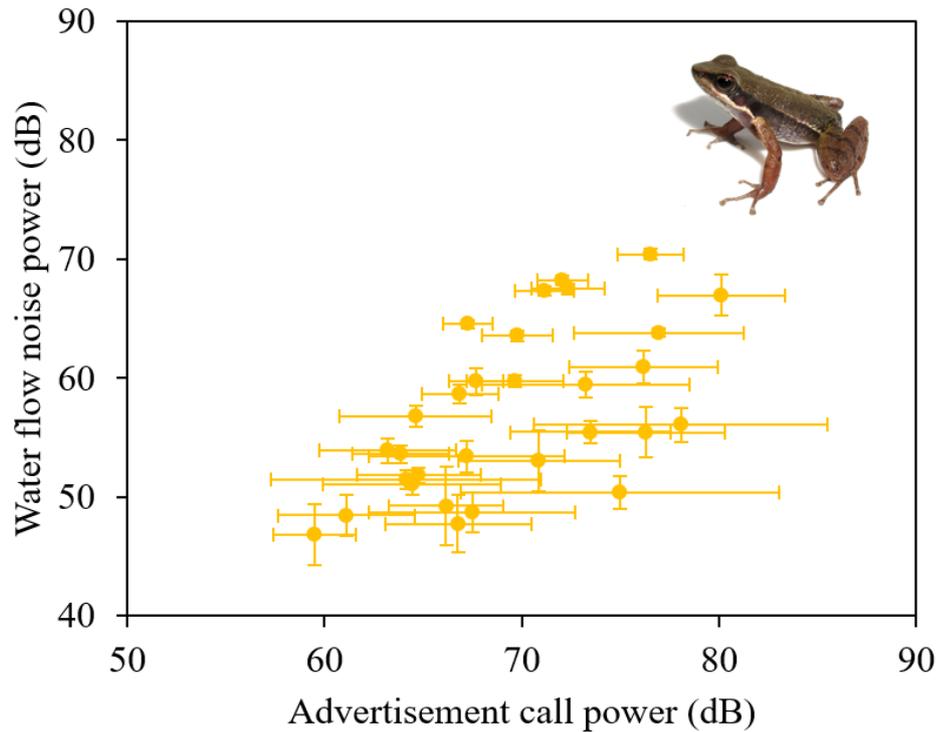


Figure 2. Relationship between water flow noise power and advertisement call power of *Hylodes phyllodes*. Bars represent the range. Data untransformed. Illustration is a picture of *H. phyllodes*.

Table 2. Results of general linear models testing for a linear relationship with dominant frequency as response variable, and body length (SVL) and genera (*Hylodes* and *Crossodactylus*) as predictors, with (top table) and without (bottom table) interaction between predictors. Significant coefficient estimates are presented in bold.

	Coefficients	Standard error	t	P
(Intercept)	1.22	3.23	0.38	0.71
SVL	0.11	0.14	0.82	0.42
Genera	5.89	3.33	1.77	0.09
SVL*Genera	-0.19	0.14	-0.36	0.18
$F_{(3, 27)} = 6.83$, Adjusted r^2 : 0.37, $P = 0.001$, $n = 31$				
(Intercept)	5.54	0.63	8.83	< 0.001
SVL	-0.07	0.03	-2.81	0.009
Genera	1.37	0.32	4.25	< 0.001
$F_{(2, 28)} = 9.04$, Adjusted r^2 : 0.35, $P < 0.001$, $n = 31$				

Discussion

The influence of environmental background noise on acoustic communication is complex. In many cases, it represents a barrier for animals who reproduce close to lotic environments, necessitating strategies to maintain communication effectiveness (Boeckle et al. 2009; Schwartz & Bee 2013; Caldart et al. 2016). Here, we evidenced an influence of water

flow noise from rivers and streams on the Hylodidae and individuals of *H. phyllodes*. In accordance with the general pattern among anurans reported by Tonini et al. (2020), we found that the dominant frequency depends on SVL, as there is a negative allometric relationship between body length and frequency in the genera *Hylodes* and *Crossodactylus*. Extending this result to the two other genera in the family, *Megaelasia*, and *Phantasmarana*, we suggest that if any species of *Megaelasia* or *Phantasmarana* could call, it would probably be very low-pitched due to its much larger body length and, consequently, acoustically masked by the stream noise.

Since SVL is related to vocal tract anatomy, such as larynx' volume (Narins & Smith 1986; Ryan 1988; McClelland et al. 1996; Suthers et al. 2006), and connected with dominant and fundamental frequencies, as we observed in our study, the acoustic communication in large-bodied amphibians that also evolved in noisy natural environments (such as *Megaelasia* and *Phantasmarana*) would be challenging. Based on the acoustic allometry observed, we infer that the muteness in the genera, *Megaelasia* and *Phantasmarana* could be driven by the environmental background noise. If on one hand, the background noise imposes a constraint to the acoustic communication of these species, on the other, frogs could be larger and consequently acquire different (trophic or spatial) niches, especially large streams that are not usually occupied by other rheophilic Neotropical anurans. Some other advantages may also be related to large body lengths. For example, larger SVLs are related to larger eggs or clutch sizes (Kusano & Hayashi 2002; Prado & Haddad 2005; Wells 2007; Hartmann et al. 2010). Besides, it may also be related to increased success in visual communication, since larger individuals may be more easily (e.g., at greater distances) detected in the environment by its conspecifics. Finally, it may also confer protection against predators, once larger animals have fewer potential predators (Vézina 1982; Cohen et al. 1993).

Species that communicate with sounds in environments with high background noise, such as hylodids, tend to develop strategies to overcome the background interference. *Crossodactylus* is exposed to environments with a higher maximum frequency noise (frequency 95%, in which all the frequency range below contains 95% of the sound energy) than *Hylodes*, which indicates that *Crossodactylus* species inhabit habitats with broader water flow noise frequencies. Besides, we found a positive relationship between *H. phyllodes* advertisement call frequencies and background SPL. In the Hylodidae family, this has been previously reported for *C. schmidti* (Caldart et al. 2016) and represents one more strategy for intraspecific communication effectiveness, influenced by the background noise. Such acoustic strategies can be combined with visual and tactile communication modes, widespread in the family and

frequently in a multimodal way, both for reproductive and agonistic purposes (Weygoldt & Carvalho-e-Silva 1992; Haddad & Giaretta 1999; Hödl & Amézquita 2001; Narvaes & Rodrigues 2005; Lingnau et al. 2008; Caldart et al. 2014; de Sá et al. 2016; Malagoli et al. 2017; Furtado et al. 2019).

Although we have not tested this in our study, the presence of calls composed by rhythmically separated short notes with narrow frequency bands was considered a convergent characteristic in comparative studies between birds (*Lochmias nematura*, *Phylloscopus* sp. and *Scoteria naevia*) and amphibians (*Hylodes* spp. in Brazil and *Nanorana* spp. in Nepal) that dwell in torrent waters, avoiding masking by the stream background noise (Dubois & Martens 1984; Vielliard & Cardoso 1996).

We were unable to apply comparative methods and analysis incorporating phylogenetic signals (e.g., phylogenetic generalized least squares; PGLS), because even the most comprehensive phylogenies (Pyron & Wiens 2011; Pyron 2014; Grant et al. 2017; Vittorazzi et al. 2021) did not include most Hylodidae species tested here. Adding this analysis would imply reducing substantially the data for each genus. However, our comparisons within the genus level are still relevant and bring light into the acoustic allometry of these torrent frogs. We also used a combination of data taken from the literature and from individuals and recordings deposited in scientific collections. Nonetheless, this data collection approach has been used previously in broad studies (e.g., Tonini et al. 2020) and represents an outlet for working with groups with sparse data, such as several of the species included here.

Finally, we provide information that extends the known influence of the background streams noise as a phenotypic constraint, and to the presence of louder advertisement calls in *H. phyllodes*, as a strategy for intraspecific communication effectiveness in rivers and streams. Such a hypothesis may be tested with other stream-dweller anurans all over the globe. Besides, although *Hylodes* and *Crossodactylus* have differences in morphology and natural history, we found the same acoustic allometry, which supports the hypothesis that in much larger and phylogenetically close related frogs (*Megaelosia* and *Phantasmarana*) the lack of the ability to emit advertisement call could be an extraordinary evolutionary solution to inhabit noisy environments.

Acknowledgements

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SUPPLEMENTARY MATERIAL

Table S1. Sound pressure level (SPL) of the *Hylodes phyllodes* advertisement call and background water flow noise. Values are presented as mean \pm standard deviation (range; number of SPL sampled). We analysed one call and one silence period (to evaluated background noise) by individual, and the sound level meter (Instrutherm DEC-490 Sound Level Meter) was configured to sample a sample by 0.1 second.

Male	Locality	Sound pressure level (dB)	
		Advertisement call	Background water flow
1	Bertioga	78.06 \pm 7.45 (64.3 – 89.4; 14)	56.06 \pm 1.43 (55 – 61; 26)
2	Bertioga	69.75 \pm 1.8 (66.8 – 73; 11)	63.53 \pm 0.42 (63.1 – 65.3; 31)
3	Bertioga	59.48 \pm 2.09 (55.1 – 62; 12)	46.77 \pm 2.57 (44 – 52.8; 22)
4	Bertioga	73.49 \pm 4.06 (65 – 78.4; 16)	55.43 \pm 0.97 (54.8 – 59.8; 39)
5	Bertioga	63.18 \pm 3.47 (56.6 – 69; 16)	53.86 \pm 1.04 (52.2 – 55.9; 32)
6	Bertioga	64.13 \pm 6.86 (54.1 – 73.7; 12)	51.47 \pm 0.8 (50.3 – 53; 29)
7	Bertioga	63.87 \pm 2.44 (58 – 67.4; 16)	53.57 \pm 0.76 (52.9 – 56.8; 40)
8	Bertioga	69.66 \pm 2.46 (64.3 – 73.4; 15)	59.76 \pm 0.49 (59.2 – 61.9; 59)
9	Bertioga	64.43 \pm 4.53 (56.2 – 71.3; 12)	51.02 \pm 0.86 (50.3 – 54.9; 42)
10	Bertioga	67.68 \pm 1.36 (64.2 – 69.7; 17)	59.69 \pm 1.17 (58.7 – 63.6; 26)
11	Bertioga	72.07 \pm 1.30 (70.2 – 73.9; 7)	68.2 \pm 0.36 (67.5 – 69.4; 51)
12	Bertioga	67.28 \pm 1.26 (65.5 – 70.6; 26)	64.52 \pm 0.33 (64 – 66.3; 67)
13	Bertioga	71.14 \pm 1.48 (68.3 – 73.7; 14)	67.36 \pm 0.4 (66.3 – 69.4; 207)
14	Bertioga	66.86 \pm 1.94 (62.8 – 70.6; 29)	58.61 \pm 0.78 (57.9 – 62.3; 41)
15	Bertioga	72.34 \pm 1.84 (68.9 – 75.2; 15)	67.49 \pm 0.47 (66.7 – 68.5; 34)
16	Picinguaba	66.76 \pm 3.71 (57.7 – 72.8; 19)	47.73 \pm 2.43 (45.6 – 55.8; 34)
17	Picinguaba	64.77 \pm 3.15 (55.8 – 67.6; 19)	51.81 \pm 0.64 (51.2 – 54.6; 41)
18	Picinguaba	64.62 \pm 3.85 (57.4 – 70.1; 19)	56.8 \pm 0.89 (55.8 – 59.9; 26)
19	Picinguaba	76.15 \pm 3.76 (65.5 – 83.9; 79)	60.92 \pm 1.39 (59 – 64.2; 13)
20	Picinguaba	70.86 \pm 4.1 (57.6 – 77.4; 44)	52.99 \pm 2.55 (47.7 – 56.8; 48)
21	Picinguaba	76.29 \pm 4.03 (67.5 – 83.5; 29)	55.4 \pm 2.12 (51.5 – 61.8; 38)
22	Picinguaba	61.11 \pm 3.49 (53.4 – 65.4; 16)	48.43 \pm 1.7 (46.2 – 52.6; 54)
23	Picinguaba	66.16 \pm 2.9 (61.1 – 71; 28)	49.22 \pm 3.3 (46.3 – 57.9; 21)
24	Picinguaba	80.12 \pm 3.23 (71 – 83.1; 39)	66.95 \pm 1.73 (64.8 – 70.4; 19)
25	Picinguaba	73.22 \pm 5.26 (65.2 – 82.5; 12)	59.44 \pm 1.05 (57.7 – 62.1; 25)
26	Picinguaba	67.49 \pm 5.22 (56.2 – 73.7; 15)	48.69 \pm 1.64 (46.8 – 54.3; 34)
27	Picinguaba	67.23 \pm 4.96 (57.1 – 78; 18)	53.38 \pm 1.35 (51.3 – 56.2; 35)
28	Picinguaba	76.94 \pm 4.31 (66.3 – 83.4; 44)	63.8 \pm 0.36 (63.2 – 64.8; 23)
29	Picinguaba	74.98 \pm 8.07 (55.7 – 86.9; 22)	50.39 \pm 1.39 (48.6 – 54.2; 23)
30	Picinguaba	76.52 \pm 1.66 (72.2 – 78.2; 36)	70.4 \pm 0.46 (69.9 – 71.8; 31)
Total		69.55 \pm 5.31 (59.48 – 80.11; 30)	57.12 \pm 6.81 (46.76 – 70.4; 30)

Table S2. Results of general linear models testing for a linear relationship with fundamental frequency as response variable, and body length (SVL) and genera (*Hylodes* and *Crossodactylus*) as predictors, with (top table) and without (bottom table) interaction between predictors. Significant coefficient estimates are presented in bold.

	Coefficients	Standard error	t	P
(Intercept)	1.99	5.22	0.38	0.71
SVL	-0.02	0.21	-0.08	0.94
Genera	3.22	5.29	0.61	0.55
SVL*Genera	-0.08	0.21	-0.38	0.72
F _(3, 16) = 6.16, Adjusted r ² : 0.45, P = 0.005, n = 20				
(Intercept)	3.89	0.66	5.92	< 0.001
SVL	-0.09	0.03	-3.74	0.002
Genera	1.28	0.31	4.11	< 0.001
F _(2, 17) = 9.67, Adjusted r ² : 0.48, P < 0.002, n = 20				

Appendix S1. Voucher number of examined specimens (museum acronym ZUEC-AMP: Amphibian Collection of the Museu de Diversidade Biológica (MDBio), Unicamp).

Hylodes glaber: ZUEC-AMP 739–41, 843, 7997, 10731–7, 10740, 10742, 10744–6;
Phantasmarana boticariana: ZUEC-AMP 11843.

Appendix S2. Catalogue number of analysed files (FNJV: Fonoteca Neotropical Jacques Vielliard, MDBio, Unicamp).

Crossodactylus caramaschii: FNJV 12963–12964, 32698, 33325–33327; 33543, 33932;
Crossodactylus gaudichaudii: FNJV 11787, 31905;
Hylodes asper: FNJV 31847, 32109–32110;
Hylodes charadranaetes: FNJV 11876, 44275;
Hylodes glaber: FNJV 31788, 32072;
Hylodes lateristrigatus: FNJV 8622, 14283, 31991, 32144, 44142;
Hylodes magalhaesi: FNJV 10436, 31889, 44972–44976;
Hylodes meridionalis: FNJV 11882–11884; 12937; 33052; 33057; 44965;
Hylodes nasus: FNJV 11878, 11880–11881, 31761, 31850;
Hylodes otavioi: FNJV 14281–14282;
Hylodes phyllodes: FNJV 45485–45542;
Hylodes pipilans: FNJV 33853–33854;
Hylodes sazimai: FNJV 11873–11875;
Hylodes uai: FNJV 34072–34075, 34077, 34585.

CHAPTER II

COMMUNICATION ACROSS MULTIPLE SENSORY MODES: QUANTIFYING THE RICH BEHAVIOURAL REPERTOIRE OF A NEOTROPICAL TORRENT FROG

**Comunicação através de múltiplos modos sensoriais: quantificando o rico
repertório comportamental de um sapo de corredeira neotropical**

Guilherme Augusto-Alves & Luís Felipe Toledo

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Communication across multiple sensory modes: quantifying the rich behavioural repertoire of a Neotropical torrent frog

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Abstract

Anuran communication involves different channels of signal transmission, including acoustic, chemical, seismic, tactile, and visual stimuli. If emitted in combination, the components of the different channels form the multimodal communication, which can be important to reinforce, complement, or transfer fundamental information. This is especially key for species that dwell in noisy environments, such as *Hylodes phyllodes*. This rheophilic frog species has a complex behavioural repertoire, including acoustic and visual signals. In this study, we quantified and characterized the multimodal communication of this species. We identified and characterized advertisement, territorial, and encounter calls. Additionally, we compared the advertisement calls from the same males when emitted with one or both vocal sacs expanded and found that they differed in temporal and spectral parameters. *Hylodes phyllodes* performed 16 visual displays, which varied among individuals and populations. We elucidate that visual signalling is easily quantifiable and could be used to compare individuals, populations, and species, as typically done with anuran acoustic signals.

Keywords: Anura; Hylodidae; *Hylodes phyllodes*; multimodal communication; agonistic interaction; diurnal anuran; acoustic, tactile, visual signalling.

Introduction

Anuran communication is beautiful and rich. Communication using sounds is the most common signal transmission mode used by anurans (Wells 2007; Starnberger et al. 2014; Folly & Hepp 2019). This fact is reflected in the disproportional number of bioacoustics studies produced, mainly related to reproductive vocalizations (e.g., Pombal Jr. et al. 1995; Forti et al. 2019; Bastos et al. 2021), compared to other forms of communication. Male advertisement calls attract females and signal males' readiness to defend territories. The calls allow conspecific receivers to access information on traits of the emitters, such as body size and physical conditions (Ryan 1980; Forester & Czarnowsky 1985). However, the acoustic repertoire of frogs and toads is not limited to one type of call. Recent reviews listed 14 call types in four different overarching categories: reproductive, aggressive, defensive, and related to feeding (Toledo et al. 2015; Köhler et al. 2017).

In addition, the behavioural repertoire of frogs is composed by several other modes of signal transmission. Although less common (or at least less reported in the literature), frogs also communicate by chemical (Byrne & Keogh 2007; Poth et al. 2012; Starnberger et al. 2013), seismic/vibrational (Narins 1990; Lewis et al. 2001; Caldwell et al. 2010), and tactile signals (Bourne et al. 2001; Brunetti et al. 2014; de Sá et al. 2016; de Sá et al. 2018; Zornosa-Torres & Toledo 2019). Furthermore, they communicate by visual signalling, which is mostly present in species that have diurnal activity or breeding behaviour linked to environments with intense background noise, such as streams, cascades, and waterfalls (Pombal et al. 1994; Haddad & Giaretta 1999; Hirschmann & Hödl 2006; Preininger et al. 2009; Furtado et al. 2019). Communication across multiple sensory modes, either simultaneously or alternated, is called multimodal communication (de Luna et al. 2010; Starnberger et al. 2014a; 2014b). Recently, studies of anuran communication using a multimodal approach have increased, based both on experimental and observational perspectives (Grafe & Wanger 2007; Grafe et al. 2012; Preininger et al. 2009; Furtado et al. 2016; Jacobs et al. 2017; Furtado et al. 2019). Most of these studies evidence that two or more signal modes may transmit redundant information (reinforce information), provide complementary information, or transmit a fundamental signal for the intraspecific interaction (de Luna et al. 2010; Taylor & Ryan 2013; Laird et al. 2016).

This information reinforces that multimodal communication can be important to rheophilic frogs. The background noise produced by water flow represents an acoustic barrier for species that dwell in lotic environments (Boeckle et al. 2009; Schwartz & Bee 2013; Caldart et al. 2016). Thus, communication using more than one signalling mode could be important to transfer information from a sender to a receiver and may help to overcome environmental noise

interference. This is the case for the species of the Atlantic Forest torrent-frog family Hylodidae, which deals with high background noise from rushing water. The family is composed by four genera (Vittorazzi et al. 2021): *Megaelosia* and *Phantasmarana*, which are mute (but see Muscat et al., 2020), and at least *Phantasmarana apuana* use their contrasting vocal sacs for visual communication (Augusto-Alves et al. 2018); and *Crossodactylus* and *Hylodes*, which exhibit rich behavioural repertoires, including acoustic, visual, and tactile signals (Weygoldt & Carvalho-e-Silva 1992; Haddad & Giaretta 1999; Narvaes & Rodrigues 2005; Caldart et al. 2014; de Sá et al. 2016; Furtado et al. 2019).

As most species of the genus *Hylodes*, our focal species, *Hylodes phyllodes* advertise for mating in cascading streams (Hatano et al. 2002; Hartmann et al. 2006), which generate significant background noise. Part of the behavioural repertoire of *H. phyllodes* has been described, including acoustic and visual signals (Hartmann et al. 2005; 2006), but some behaviours still lack description. Thus, considering the vast behavioural repertoire of *H. phyllodes*, we aimed to: i) quantify and characterize the multimodal communication from two different populations of this species; ii) describe unreported acoustic, visual, and tactile signals, indicating the context of emission, whenever possible; and iii) describe agonistic interactions observed while sampling their multimodal signals.

Methods

Data sampling

We carried out the study in two different locations of the Atlantic Forest, in the municipality of Bertioga (Parque Estadual Serra do Mar, Núcleo Bertioga) and in Picinguaba, in the municipality of Ubatuba (Parque Estadual Serra do Mar, Núcleo Picinguaba). Both study locations are in the state of São Paulo, southeast Brazil, with approximately 140 km straight-line distance between them.

To assess the behavioural repertoire of *H. phyllodes*, we installed cameras in front of actively calling individuals. The cameras remained recording for approximately 30 minutes (GoPro Hero 3+ Black Edition, Panasonic HDC-HS80, and Sony DCR-SR47). The species presents high site fidelity (Magnan-Neto et al. 2016); when they move away from camera view, generally they quickly return. For individuals that were disturbed due to our initial approach, we waited until the focal male resumed calling and then started the data collection. After the video recording, we recorded the call from the same focal male (ZOOM H4n PRO digital recorder with its built-in microphones) for about three minutes at approximately 70 cm distance. Some individuals fled away when we approached for the audio recording; nevertheless, their

videos were included in the analyses, totalling 58 individuals with audio recordings (32 from Bertiooga and 26 from Picinguaba) and 78 individuals with video recordings (36 from Bertiooga and 42 from Picinguaba). All media files were deposited at the Audio-visual Collection [Fonoteca Neotropical Jacques Vielliard (FNJV) and Video Collection (ZUEC-VID)] of the Museu de Diversidade Biológica (MDBio), Unicamp (FNJV 45485–542; ZUEC-VID 818–94), following previous recommendations (Dena et al., 2020). This study was approved by the Instituto Chico Mendes de Conservação da Biodiversidade (SISBio #63697-2), Comissão Técnico-Científica do Instituto Florestal (COTEC #483/2018), university animal ethics committee (CEUA #4983/2018), and registered at the Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado (SISGen # AE9A0E0).

Media analyses

Videos were edited using Adobe Premiere PRO 2020, trimming off the sections when the focal individuals left from camera frame. Videos were fully analysed, searching for all frog movements, gestures or postures that could represent visual or tactile signals. The recordings were watched in slow-motion (at 0.30x), when the movements needed to be described in greater detail. Prior to audio analyses, all recordings were normalized (peak -1dB) and standardized at a sampling of rate of 44.1 kHz, and resolution of 16 bits, using Adobe Audition 2020. Then, we analysed these calls in Raven 1.6.1 PRO (using 60 % contrast, 60 % brightness, and 512 DFT). Calls were characterized by describing the following parameters: call duration; inter-call interval; notes per call; note duration; inter-note interval; call rate; dominant frequency (peak frequency function in Raven); minimum frequency (frequency 5% function in Raven); maximum frequency (frequency 95% function in Raven); frequency bandwidth (BW 90% function in Raven) (Figure S1 details the selection limits; measurements are presented as mean \pm standard deviation (range; sample size). Additionally, we analysed call emissions that were made with only one vocal sac extended separate from those with both sacs as well as different call types according to Toledo et al. (2015) and Köhler et al. (2017). These audios were extracted from videos using Adobe Premier PRO 2020 and analysed with the same method as the audio recordings. The note-centred terminology of Köhler et al. (2017) was used. We followed the nomenclature of visual signals previously proposed by Hödl & Amézquita (2001), Hartmann et al. (2005), Caldart et al. (2014), and de Sá et al. (2016), with exception of one visual signal, described here.

Statistical analyses

To visually evaluate the advertisement call variation between populations, we performed a Principal Component Analysis, using the package *FactoMineR* (Lê et al. 2008) to run the analysis and *factoextra* (Kassambara & Mundt 2016) to construct the graphic representation.

To compare the advertisement call emitted using one or both vocal sacs, we ran non-parametric analyses, since our samples were not normally distributed. We tested the correlation among call parameters based on Spearman's correlation matrix with $|r| > 0.7$. We did not evaluate the trait 'notes per call' and 'frequency bandwidth' due to high collinearity with 'call duration' and 'dominant frequency', respectively. We compared the call traits (call duration, note duration, inter-note interval, dominant frequency, minimum frequency, and maximum frequency) between calls emitted with one or two vocal sacs extended from same individuals, by means of a Wilcoxon Signed Rank Exact Test, using the function *wilcox.test*. All analyses were implemented in R version 4.1.0 (R Core Team 2021).

Results

Acoustic repertoire

We detected three different call types emitted by *Hylodes phyllodes* males.

A) Advertisement call. *Hylodes phyllodes* advertisement call (Figure 1A and 1B) was composed of a sequence of tonal notes with harmonics in both populations. The dominant frequency was on the third visible harmonic [4.83 ± 0.341 (4.05 – 5.68) kHz]. The spectral and temporal parameters presented similar values in both populations (Table 1). A variation in the advertisement call was observed in one individual. In addition to the most frequent note – named Note A_A: dominant frequency (DF) = 4.78 ± 0.17 (4.13 – 5.17) kHz; note duration (ND) = 39.9 ± 4.2 (26.9 – 56.5; 674) ms – this male also emitted pure tonal notes, with lower frequency, and without harmonics – named Note A_B: DF = 3.15 ± 0.22 (2.07 – 3.96) kHz, ND = 22.8 ± 3.7 (15.5 – 34.1; 47) ms; (Figure 1C). The principal component analysis of the acoustic parameters showed no cluster formation between population (Figure 2; Table S2). Advertisement call parameters for each individual male are listed in Table S3.

B) Territorial call. This call was emitted by four different males and is composed by two different notes, named T_A and T_B. Note T_A was shorter and had higher frequency than T_B (Figure 1D; Table 2). Generally, the call is composed of the emission of two T_A notes followed by one T_B note (n = 16; 84.2 % of the calls). However, we also observed the emission of three T_A notes followed by one T_B (n = 2; 10.5 % of the calls), and one T_A followed by one

T_B ($n = 1$; 5.3 % of the calls). Another male emitted similar vocalizations, but only with emission of T_B notes (14 calls).

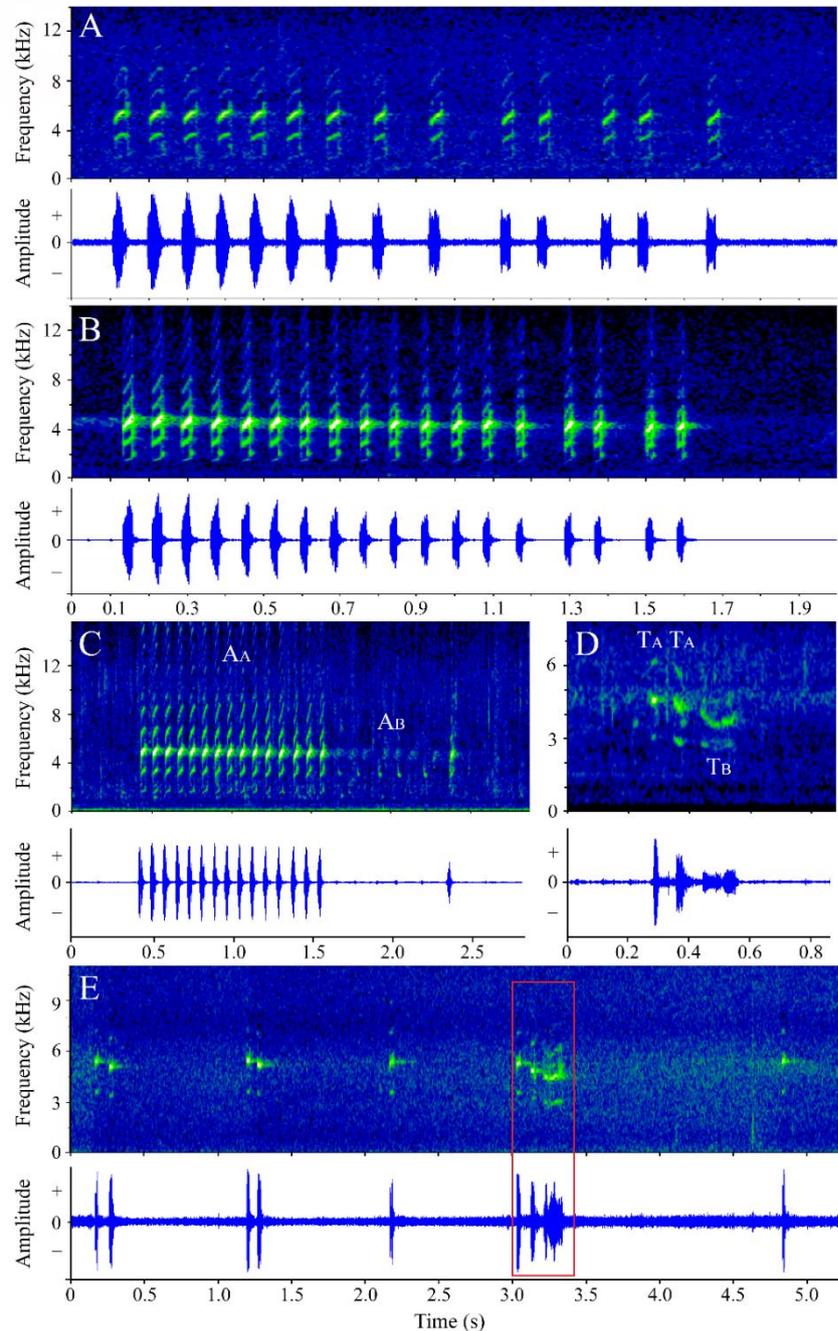


Figure 1. Spectrogram (top) and waveform (bottom) of the advertisement call of *Hylodes phyllodes* recorded in Bertioga (A) and Pinguaba, Ubatuba (B), both in the state of São Paulo, Brazil. Spectrogram (top) and waveform (bottom) of the advertisement call variation emitted by one male (FNJV 45531) in Pinguaba (C). Besides the emission of the common notes (note A), this male emitted pure tonal notes, in lower frequency (note B). Spectrogram (top) and waveform (bottom) of the territorial call (D), this call is composed of two different notes, note A is shorter and emitted in a higher pitch than note B. Spectrogram (top) and waveform (bottom) of a sequence of encounter calls of *H. phyllodes* (E). Red rectangle represents a territorial call emitted in the sequence of encounter calls.

Table 1. Advertisement calls spectral and temporal parameters of *Hylodes phyllodes*. Values are presented as mean \pm standard deviation (range; sample size).

	Bertioga	Picinguaba	Both populations
Call duration (s)	1.85 \pm 1.3 (0.24 – 18.31; 917)	2.68 \pm 2.32 (0.44 – 21.18; 700)	2.21 \pm 1.87 (0.24 – 21.18; 1,617)
Notes per call (n)	17.03 \pm 10.72 (3 – 160; 917)	28.29 \pm 22.76 (6 – 214; 700)	21.99 \pm 17.9 (3 – 214; 1,617)
Inter-call interval (s)	4.41 \pm 2.25 (1.45 – 29.55; 883)	4.49 \pm 3.74 (0.44 – 59.63; 674)	4.44 \pm 2.99 (0.44 – 59.63; 1,557)
Note duration (ms)	39.89 \pm 7.26 (8.4 – 77.8; 15,602)	36.75 \pm 8.14 (4.5 – 65.3; 19,805)	38.13 \pm 7.92 (4.5 – 77.8; 35,407)
Inter-note interval (ms)	71.69 \pm 34.53 (13.4 – 293.6; 14,649)	58.93 \pm 31.03 (14 – 356.3; 19,106)	64.47 \pm 33.2 (13.4 – 356.3; 33,755)
Call rate (call/m)	175.17 \pm 34.32 (77.84 – 239.78; 32)	188.7 \pm 38.34 (112.35 – 272.32; 26)	181.23 \pm 36.8 (77.84 – 272.32; 58)
Dominant frequency (kHz)	4.9 \pm 0.32 (4.13 – 5.68; 917)	4.73 \pm 0.34 (4.05 – 5.51; 700)	4.83 \pm 0.34 (4.05 – 5.68; 1,617)
Minimum frequency (kHz)	4.26 \pm 0.30 (3.36 – 4.91; 697)	3.99 \pm 0.50 (2.50 – 4.82; 516)	4.15 \pm 0.42 (2.50 – 4.91; 1,213)
Maximum frequency (kHz)	5.25 \pm 0.19 (4.65 – 6.03; 697)	5.06 \pm 0.28 (4.13 – 5.77; 516)	5.17 \pm 0.23 (4.13 – 6.03; 1,213)
Frequency bandwidth (kHz)	0.99 \pm 0.29 (0.34 – 2.07; 697)	1.08 \pm 0.47 (0.43 – 2.24; 516)	1.03 \pm 0.38 (0.34 – 2.24; 1,213)

C) Encounter call. This call was registered during the two male-male short-distance interactions. We observed simple calls, that had one tonal note with harmonics, and compose calls, with a sequence of these notes, normally (98.3 % of the observations) ranging from 1 to 4 notes; however, calls with 5 or 6 notes were also registered in 1.7 % of the observations [mean 2.37 ± 1 (1 – 6; 357) notes/call; Figure 1E; Table 2].

Some call traits differed from advertisement call emitted with one or two vocal sacs expanded. When calling with one vocal sac, males emitted shorter notes and lower pitched minimum and maximum frequencies (Wilcoxon Signed Rank Exact Test: note duration $P = 0.004$; minimum frequency $P = 0.027$; maximum frequency $P = 0.039$). Other temporal and spectral parameters did not differ (for all parameters $P > 0.7$) (Table S4 for spectral and temporal parameters of advertisement call, when emitted using one or two vocal sacs).

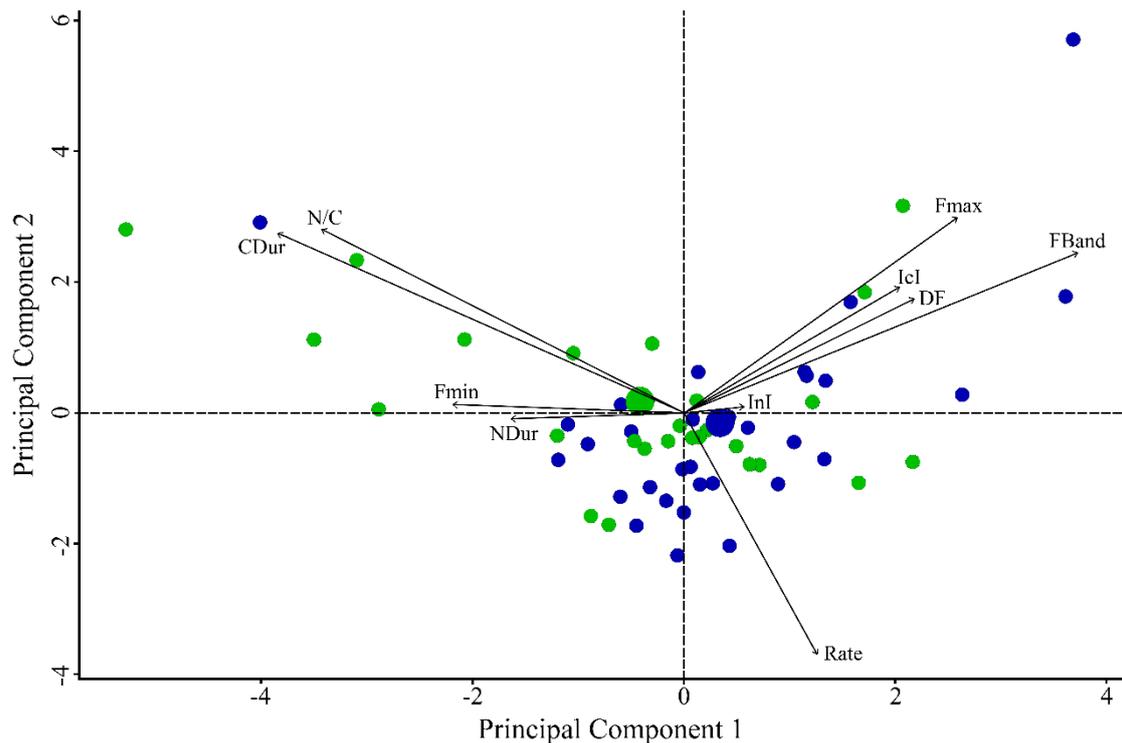


Figure 2. Principal component analysis biplot based on advertisement call parameters of the *Hyldodes phyllodes* from Bertioga (blue) and Picinguaba (green). Small circles represent each analysed specimens, while big circles represent the variation for each population. The length and direction of arrows correspond to each adjusted call parameter on the first two principal components. The two firsts principal components had 45.8 % of the advertisement call variability (PCA 1 = 25.9 %; PCA 2 = 19.9 %). Abbreviations: CDur = call duration; N/C = notes per call; IcI = inter-call interval; NDur = note duration; InI = inter-note interval; Rate = call rate; DF = dominant frequency; Fmin = minimum frequency; Fmax = maximum frequency; FBand = frequency bandwidth.

Visual repertoire

We analysed 39:05 hours of videos from 78 different *H. phyllodes* males. Individuals performed 16 different visual displays in both populations (for visual displays in each population see Table 3), divided into three categories: i) limbs displays (n = 9); ii) posture displays (n = 5); iii) mouth and throat displays (n = 2). The description, emission frequency, and variation of all displays are provided in Table S5 (Video S1 exemplifies all visual displays).

i) Limbs displays

Arm lifting (n = 598 displays; 66 males): Rapid up and down arm movement. Individuals performed this display with the left or right arms. This signal was emitted often (84.6 % of the males), in different contexts, including male-male agonistic interactions, males stimulated by calls from other nearby males, or without any apparent stimulus.



Figure 3. Individual of *Hylodes phyllodes* emitting advertisement call with only one lateral vocal sac. Note the translucent vocal sacs reflecting the sun light and contrasting with its body and the environment.

Leg lifting (n = 348 displays; 49 males): Rapid up and down leg movement. Individuals performed this display with the left or right legs. This display was emitted often (62.8 % of the males), in different contexts, including male-male agonistic interactions, males stimulated by calls from other nearby males, or without any apparent stimulus.

Two limbs lifting (n = 53 displays; 11 males): Rapid up and down movement of two limbs concomitantly or alternatively. Three variations were observed for this display: a) lifting of one arm and one leg concomitantly, which was performed using limbs on alternate sides (n = 23 displays; 6 males); b) lifting of both legs concomitantly (n = 11 displays; 4 males); c) lifting of both legs alternatively (n = 19 displays; 5 males).

Arm waving (n = 17 displays; 3 males): Rapid up and down arc movement with the arm, passing the limb in front of the head, which differentiates this movement from arm lifting. We classify arm waving display when the arm clearly performed an arc movement in front of the head. Individuals performed this display with left or right arm.

Table 2. Spectral and temporal parameters of the territorial and encounter calls of *Hylodes phyllodes*. Values are presented as mean \pm standard deviation (range; sample size).

	Territorial call		
	Bertioga (2 males)	Picinguaba (2 males)	Both populations (4 males)
Call duration (ms)	272.3 \pm 55 (175.9 – 357.1; 12)	291.9 \pm 44.1 (260.1 – 365.6; 7)	279.5 \pm 53.7 (175.9 – 365.6; 19)
Notes per call (n)	1.91 \pm 0.26 (1 – 2; 12)	2.28 \pm 0.45 (2 – 3; 7)	2.05 \pm 0.39 (1 – 3; 19)
Note A duration (ms)	27.9 \pm 7.8 (10.6 – 43.9; 23)	37.2 \pm 11.9 (8.2 – 55.9; 16)	31.7 \pm 10.7 (8.2 – 55.9; 39)
Note B duration (ms)	122.9 \pm 29.3 (70.1 – 178.5; 12)	101.7 \pm 14.2 (88 – 128.9; 7)	115.1 \pm 26.9 (70.1 – 178.5; 19)
Inter-note interval (ms)	49.5 \pm 18.01 (2.6 – 68.3; 23)	46.1 \pm 9 (32.1 – 62.8; 16)	48.1 \pm 15.1 (2.6 – 68.3; 39)
Call dominant frequency (kHz)	5.01 \pm 0.31 (4.39 – 5.43; 12)	5.04 \pm 0.45 (4.56 – 5.77; 7)	5.02 \pm 3.78 (4.39 – 5.77; 19)
Note A dominant frequency (kHz)	4.96 \pm 0.42 (3.79 – 5.51; 23)	5.32 \pm 0.85 (4.13 – 7.49; 16)	5.11 \pm 0.66 (3.79 – 7.49; 39)
Note B dominant frequency (kHz)	4.34 \pm 0.41 (3.62 – 4.91; 11)	4.53 \pm 0.6 (3.62 – 5.25; 7)	4.43 \pm 0.49 (3.62 – 5.25; 19)
Minimum frequency (kHz)	3.93 \pm 0.6 (2.84 – 4.56; 12)	3.79 \pm 0.38 (3.35 – 4.65; 7)	3.88 \pm 0.55 (2.84 – 4.65; 19)
Maximum frequency (kHz)	5.55 \pm 0.39 (4.39 – 6.03; 12)	5.38 \pm 0.46 (4.65 – 5.86; 7)	5.48 \pm 0.44 (4.39 – 6.03; 19)
Frequency bandwidth (kHz)	1.61 \pm 0.67 (0.95 – 2.76; 12)	1.59 \pm 0.5 (0.77 – 2.06; 7)	1.34 \pm 0.62 (0.27 – 2.67; 19)
	Encounter call		
	Bertioga (2 males)	Picinguaba (3 males)	Both populations (5 males)
Call duration (ms)	81.8 \pm 66.9 (21.2 – 346.5; 80)	170.5 \pm 77.2 (17.7 – 435.7; 277)	150.2 \pm 83.6 (1.77 – 435.7; 357)
Notes per call (n)	1.52 \pm 0.74 (1 – 4; 80)	2.61 \pm 0.93 (1 – 6; 277)	2.37 \pm 1 (1 – 6; 357)
Note duration (ms)	34.64 \pm 14.72 (13.1 – 127; 79)	51.2 \pm 18.2 (20.6 – 135.6; 691)	44.16 \pm 13.53 (8.2 – 135.6; 770)
Inter-note interval (ms)	51.07 \pm 20.7 (12.2 – 117.7; 43)	33.2 \pm 15.8 (2.1 – 213.6; 444)	34.8 \pm 17.1 (2.1 – 213.6; 487)
Dominant frequency (kHz)	4.91 \pm 0.48 (3.7 – 5.68; 80)	5.24 \pm 0.35 (2.84 – 5.86; 277)	5.17 \pm 0.41 (2.84 – 5.86; 357)
Minimum frequency (kHz)	4.37 \pm 0.65 (2.67 – 5.25; 80)	4.71 \pm 0.62 (2.76 – 5.68; 277)	4.63 \pm 0.64 (2.67 – 5.68; 357)
Maximum frequency (kHz)	5.37 \pm 0.38 (3.87 – 6.03; 80)	5.48 \pm 0.32 (4.22 – 6.63; 277)	5.46 \pm 0.33 (3.87 – 6.63; 357)
Frequency bandwidth (kHz)	1 \pm 0.79 (0.17 – 3.27; 80)	0.77 \pm 0.65 (0.17 – 3.62; 277)	0.82 \pm 0.69 (0.17 – 3.62; 357)

Table 3. Visual displays observed for *Hylodes phyllodes*. Values presented as total observations (number of males).

	Bertioga	Picinguaba	Both populations
Limbs displays			
Arm lifting	315 (34)	283 (32)	598 (66)
Leg lifting	147 (24)	203 (25)	350 (49)
Toe flagging	102 (4)	209 (9)	311 (13)
Foot flagging	10 (4)	50 (11)	60 (15)
Two limbs lifting	21 (6)	32 (5)	53 (11)
Arm waving	4 (1)	13 (2)	17 (3)
Two-legged kicking	10 (7)	2 (2)	12 (9)
Foot shaking	2 (1)	12 (7)	14 (8)
Hand shaking	1 (1)	7 (3)	8 (4)
Postural displays			
Upright posture	7 (5)	6 (5)	13 (10)
Truncated walking	1 (1)	8 (2)	9 (3)
Head down	-	4 (1)	4 (1)
Body raising	2 (2)	1 (1)	3 (3)
Body lowering	-	2 (1)	2 (1)
Mouth and throat displays			
Throat display	47 (11)	22 (10)	69 (21)
Mouth opening	15 (2)	10 (3)	25 (5)

Hand shaking (n = 8 displays; 4 males): Rapid agitate movement of the hand, performed with left or right hand.

Foot shaking (n = 14 displays; 8 males): Rapid agitate movement of the foot. We observed individuals performing this display with left or right foot.

Foot flagging (n = 60 displays; 15 males): Slowly raising one leg in a semicircle and slowly returning to the original position. The complete movement may take few seconds. Males performed a sequence of several foot-flagging display with alternative legs, but also performed a single display. Foot-flagging displays were observed in agonistic interactions between males (n = 14 displays; 3 males) or in response to calls from other males (n = 46 displays; 12 males).

Toe flagging (n = 311 displays; 13 males): Up and down movement of one or more toes without lifting the foot from the floor. The toes can move synchronously, in a wave shape or independently, and can be synchronous between the toes of both feet or use only one. This display may be performed without a previously movement (n = 197 displays; 9 males) or immediately after a lateral movement, in which males shift the body all the way to the side (n = 114 displays; 10 males). This signalling was observed in all short distance agonistic interactions.

Two-legged kicking (n = 12 displays; 9 males): Rapid movement of both legs up and down concurrently, as if the individual kicks backward in the air. Kicks were displayed either once or several times in quick succession.

ii) Postural displays

Upright posture (n = 13 displays; 10 males): Elevation of only the anterior part of the body through the arm extension, exposing the gular region. The gular portion is whitish, well covered with many irregular black blotches, which contrast with other body parts and the environment (Figure 3).

Truncated walking (n = 9 displays; 3 males): Elevation of the body by extending limbs and walking slowly; the movements are synchronous between one arm and one leg, always on opposite sides. This display was observed in short distance agonistic interactions.

Body lowering (n = 2 displays; 1 male): Lowering the body, touching the entire ventral region on the floor. This was a rare display, only performed by one male in a short-distance interaction (we could not determine whether it was an agonistic or courtship interaction).

Body raising (n = 3 displays; 3 males): Elevation of the body by the extension of the legs. This was a rare visual display.

Head down (n = 4 displays; 1 male): Moving the body quickly forward, touching the head on the ground and returning to the previous position. This signalling was performed only by one individual in a short-distance interaction, and it was not possible to identify if the interaction was with another male or a female. This display is novel for the family.

iii) Mouth and throat displays

Mouth opening (n = 25 displays; 5 males): Opening and closing of the mouth. The individual can remain with the mouth open for a few seconds. This display was observed in male-male short-distance interaction and in response to calls from other males. We also observed males open their mouth after eating, probably related to swallowing process (n = 42 displays; 6 males), and these cases were not included in the visual display frequencies table but are identified in Table S4.

Throat display (n = 69 displays; 21 males): Exposure of the translucent vocal sacs (see Figures 3 and 4), which contrasts with its body and the environmental. We characterize three variations of the throat display: a) inflating and deflating vocal sacs without emitting audible sound (n = 27 displays; 9 males); b) call emission (advertisement or encounter call) using one of the vocal sacs (n = 40 displays; 11 males; Figure 3); c) maintaining both vocal sacs inflated more than three seconds after the end of the call (n = 2 displays; 2 males).

Male-male agonistic interaction

We observed two agonistic interactions (i.e., continuum of behavioural adaptations related to aggressiveness) one in each population and both were multimodal, including acoustic, visual, and tactile signals. In the agonistic interaction recorded at Bertioga (ZUEC-VID 824), the intruder male (referred as male 1) approached male 2 and emitted encounter calls and visual signals (arm lifting and mouth opening). When male 1 was in close proximity to the resident, male 2, it started emitting advertisement calls and performed foot-flagging displays. Male 2 approached male 1 and stopped at a distance of about 10 cm. At this stage, male 1 remained with its back to male 2, performing toe flagging and emitting encounter and territorial calls inflating only one vocal sac. Unilateral vocal sac inflations were directed to the visual field of male 2 (Figure 4A). After a period of 1:30 min, male 2 approached closer to male 1, touching the back (tactile signal) of male 1, which turned around immediately and ceased calling. The males remained in this position for 2:50 min, and during this period both males performed several toe-flagging displays (male 1: $n = 61$; male 2: $n = 12$). Afterwards the males were back-to-back, and they maintained physical contact and performed toe-flagging displays (Figure 4B; for 4:50 min). Subsequently, male 1 suddenly left the area, and male 2 remained quiet for 2:10 min and then emitted encounter and territorial calls. During this short-distance interaction males emitted more visual signals (69.8 %) than acoustic ones (30.2 %).

In the agonistic interaction recorded in Picinguaba (ZUEC-VID 867), our observation started when both males were already in physical contact (Figure 4C). They remained in this position for 2:40 min performing toe-flagging displays. Then male 3 categorized as the intruder (because male 3 left and entered the signalling site several times), touched the resident's leg (male 4), which responded with two leg-lifting displays, which consequently moved away male 3. Male 3 emitted an advertisement call and approach another time, touching male 4. This physical contact lasted for approximately one min, during this time both males performed toe flagging. Then, male 3 left and male 4 continued to perform toe flagging. At a distance of about 30 cm, both males emitted several acoustic and visuals displays: male 3 emitted advertisement and encounter calls and did foot flagging; male 4 emitted advertisement and encounter calls, truncated walking, and toe flagging. After 2 min, male 3 approached again, constantly touching male 4, (Figure 4D). Male 3 touched the leg of male 4 with its hand and the back of male 4 with its gular region, while male 4 remained facing backward and motionless. Subsequently, male 3 emitted advertisement calls and performed toe flagging. After 2:20 min, male 3 moved away and out of camera view, but it was possible to hear its advertisement and encounter calls, and male 4 remained at the same location emitting

visuals displays (toe flagging, foot flagging, leg lifting, and arm lifting), advertisement and encounter calls. After 11 min, male 3 again jumped close to male 4, and male 3 touched its leg with its hand, and its back with its gular region, and emitting advertisement calls. Two min later male 3 left the area and did not return in the next 9 min (until the end of the recording). In this short-distance interaction males emitted more acoustic signals (60.5 %) than visual displays (39.5 %). And totalling both interactions here described, during short-distance agonistic interactions, the males emitted a similar proportion of calls (55 %) and visual displays (45 %).

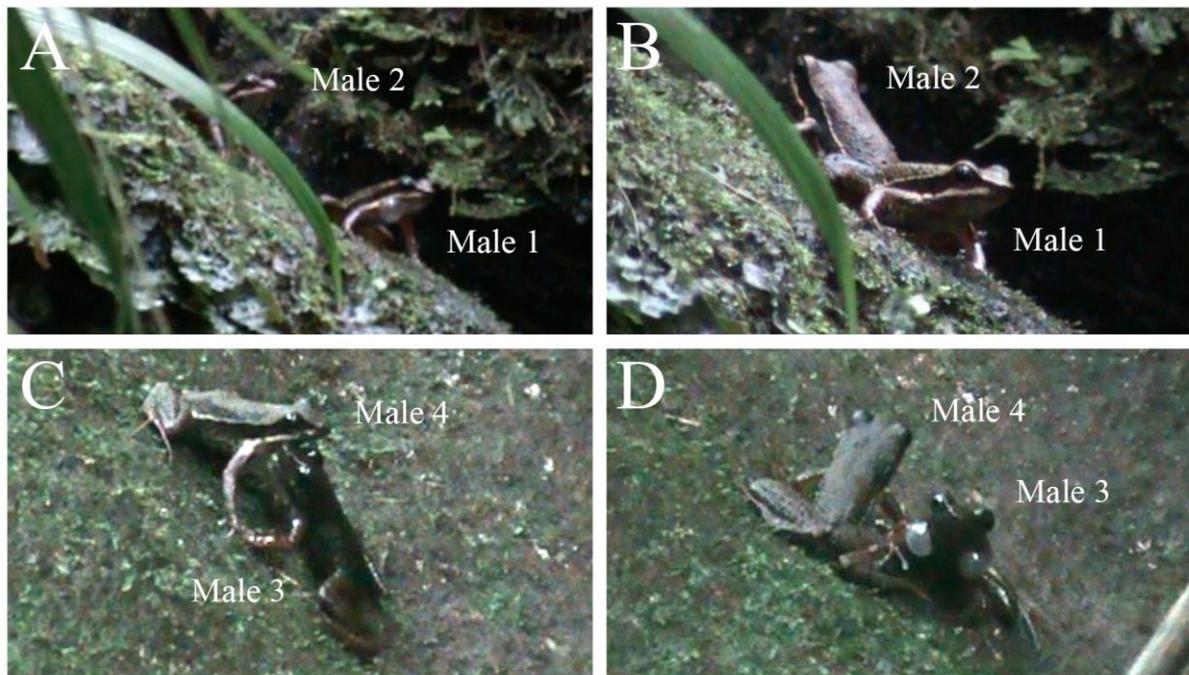


Figure 4. Agonistic interaction between *Hylodes phyllodes* males. A and B are the same individuals from Bertioga (ZUEC-VID 824): A) male 1 emitting encounter call with only one lateral vocal sac (visual field of male 2); B) tactile communication between both males; during this period, both males performed several ‘toe flagging’ displays. C and D are the same individuals from Picinguaba, Ubatuba (ZUEC-VID 867): C) tactile communication between both males; D) emission of acoustic and tactile signals concomitantly by male 3, during the aggressive interaction.

Discussion

The behavioural repertoire of *Hylodes phyllodes* was already known to be vast (Hartmann et al. 2005; 2006), but our investigation shows that it is richer and more complex than previously reported. The frogs emitted four call types, three of which are reported in this study and the formerly described courtship call (Hartmann et al. 2006). *Hylodes phyllodes* performed 18 visual displays and tactile stimuli. Of the 16 visual displays characterized here, 11 were previously reported [toe flagging, leg stretching, foot flagging, mouth opening, throat

display, body lowering, body raising, upright posture, jump display, arm lifting, and leg lifting (Hartmann et al. 2005; Furtado et al. 2019)]. We identified six additional displays previously only reported in other hylodids [arm waving, hand shaking, foot shaking, two-legged kicking, two limbs lifting, and truncated walking (Forti & Castanho 2012; Caldart et al. 2014; de Sá et al. 2016; Furtado et al. 2019)]. Additionally, we describe for the first time the head down movement, a novelty for the species, family, and probably anurans. This display seems to be rare as it was observed four times in only one interaction, in which we were unable to detect if it was related to reproduction or aggression. Additional observational/experimental studies are necessary to gain a better understanding of the function and context in which the head down movement is exhibited. Only two previously reported displays, jump display and head snaking (Hartmann et al. 2005; Furtado et al. 2019), were not observed in this study.

Following our aim of quantifying and characterizing the *H. phyllodes* repertoire, we tried to distinguish the displays with as much details as possible. This generated some differences from previous studies, for instance: behaviours such as arm lifting and leg lifting, or hand shaking and foot shaking (as we report) are generally reported as limbs lifting or limbs shaking, respectively (Hödl & Amezcua 2001; Caldart et al. 2014; de Sá et al. 2016). Separating each into two different displays allowed us to show differences in emission frequencies; for example, arm lifting was more frequent than leg lifting. In addition, this approach could be important to compare interspecific differences. For example, in *H. cardosoi* and *H. japi* only arm lifting was reported (Forti & Castanho 2012; de Sá et al. 2016). Furthermore, we created subcategories to better characterize some displays, as in the case of two limbs lifting and throat display.

Arm lifting and leg lifting were the most frequent displays in both populations. These are probably the predominant visual displays used as advertisement signals with similar function as the advertisement call. Hence, these behaviours could be emitted without prior detection of mates or competitors and could be intended to attract females or repel males. We hypothesize that this form of advertisement can reinforce the information carried by advertisement calls. Such reinforcement would be especially relevant in environments with noise pollution, as those they inhabit. This was also observed in a playback experiment with *Staurois parvus*, a Bornean rheophilic ranid, which increases foot flagging and decreases advertisement call emission depending on background noise intensity (Grafe & Tony 2017).

Although vocal sacs are linked primarily with the frogs vocal activity (see Starnberger et al. 2014b and Köhler et al. 2017 for a summary of call emission process), they are also important for other communication modes including visual, seismic, and chemical

(Rosenthal et al. 2004; Hirschmann & Hödl 2006; Forti & Encarnação 2012; Starnberger et al. 2013; 2014b). For hylodids, vocal sacs can be a secondary visual cue during calling activity (Heyer et al. 1990; Haddad & Giaretta 1999). Furthermore, new observations elucidate that these frogs are able to pulsate lateral vocal sacs without audible sound emission, also as a specific visual signal [*H. japi* (de Sá et al. 2016); *H. meridionalis* (Furtado et al. 2019); *H. phyllodes* (Hartmann et al. 2005; present study)]. The use of vocal sacs without sound emission was also reported in another hylodid genus [*Phantasmarana apuana* (Augusto-Alves et al. 2018)]. Besides this pulsate of vocal sacs without sound emission, we characterized two other variations of throat display – the maintenance of both vocal sacs inflated after a call emission and inflation/pulsation of only one lateral vocal sac. The ability to pulse just one of the vocal sacs was reported for *H. japi* (de Sá et al. 2016) and *H. meridionalis* (Furtado et al. 2019), and based on our observation, we suggest that this is a common behaviour within the genus *Hylodes*, and perhaps among other hylodids.

Heyer et al. (1990) wrote referring to *H. phyllodes*, “males call with either one or both vocal sacs”. Now, we expanded that information finding that *H. phyllodes* is able of emit advertisement and encounter calls with one or both lateral vocal sacs (Figure 3 and 4; Table S4). We also found that calling with one vocal sac modified the acoustic signal. Although it did not affect the dominant frequency, which could imply that most basic information is preserved (such as body size), these divergences may indicate that males can transmit other specific information to nearby conspecifics. Additionally, pulsating one vocal sac represents a way of transmitting a directional visual signal to close-range individuals (de Sá et al. 2016). However, using just one vocal sac for acoustic and/or visual displays have been neglected. The anatomical mechanism which allows the asymmetric inflation/deflation of vocal sacs, is already known in Hylodidae (Elias-Costa et al. 2017). This mechanism is convergent among hylodids and non-related ranids genera *Staurois*, *Huia*, *Meristogenys*, and *Amolops* (Elias-Costa & Faivovich 2019). Although this has only been recorded before for *Staurois guttatus* (Elias-Costa & Faivovich 2019), this behaviour may be frequent in stream-dwelling anurans.

All three call types we recorded have been previously described (Hartmann et al. 2006), but the encounter call was referred to as *canto de intervalo de briga* (fighting interval call). This call was emitted in short-distance agonist interactions (Hartmann et al. 2006; present study), and for this reason we reassigned it as an encounter call (Toledo et al. 2015). Although this call was observed during agonistic interactions, sequences of this call were also heard before the males started to emit advertisement calls, after a silent period. We made few recordings in this context, but it was a behaviour frequently heard in the field. Additionally, we

described a variation of an advertisement call, with presence of a second note type. This new register is uncommon, it was emitted just by one male. This call variation could be related with intrinsic characteristics of the individual that emitted it and not change the basic information transmitted, or it could transmit a specific rare signal to nearby conspecifics.

Territorial defence using multiple sensory channels is known for several anurans (e.g., Hirschmann & Hödl 2006; Narins et al. 2003; Preininger et al. 2013). The acoustic and visual signals during agonistic interactions are also common for hylodids (Forti & Castanho 2012; Caldart et al. 2014; de Sá et al. 2016; de Sá et al. 2018; Furtado et al. 2019), except in the voiceless species, for which only visual signalling has been reported (Augusto-Alves et al. 2018). Our results showed that some visual displays are performed mainly in territorial contexts. For example, foot flagging and toe flagging were emitted in short-distance agonistic interactions only. Both of these, plus truncated walking, mouth opening, and throat display, were more frequent when there was another male near or calling nearby. During short-distance agonistic interactions, the number of visual displays increased, and our results showed a similar proportion between acoustic and visual signals in this context. This proportion is also similar to that found for *H. japi* (with 53.5 % of acoustic and 46.5 % of visual displays; de Sá et al. 2016), demonstrating a consonance in the use of acoustic and visual signals in agonistic interactions between these species, and probably within the group. Besides acoustic and visual signals, during the male-male short-distance interactions, we observed some tactile stimuli, similar to what was observed for *H. meridionalis* (de Sá et al. 2018). Through these stimuli, together with acoustic and visual close-range signals, males may avoid physical combats. Despite frequently observed for hylodid species (de Sá et al. 2016; Augusto-Alves et al. 2018; Furtado et al. 2019), fights should be the last strategy used by males during territory defence, because they cost energy and can generate body injuries in frogs (Pombal Jr. et al. 1998; Candaten et al. 2020).

Detailed descriptions of acoustic repertoire, mainly the advertisement call, are common in anuran communication studies (Costa & Toledo 2013; Forti et al. 2019; Bovolon et al. 2020). This is useful, for instance, to delimitate cryptic species (Köhler et al. 2017; Andrade et al. 2020; Lima et al. 2020) but also aims to assess some intraspecific variation of call traits (Ryan & Wilczynski 1991; Gasser et al. 2009) and the influence on abiotic (Navas & Bevier 2001; Lingnau & Bastos 2007) and biotic factors (Kelleher et al. 2021; Madelaire et al. 2013) of call variation. However, the use of a similar approach for visual or other alternative communication modes is still incipient. Here, we quantified the multimodal communication of

H. phyllodes. The different call types were parameterized, and the rich visual repertoire was characterized.

We then demonstrated that visual signals are quantifiable. Therefore, we propose that for anurans groups which present high visual communication rate, such as the Hylodidae family, these signals should be included in studies aimed at understanding the behavioural variation between individuals, populations, or species, as usually done with acoustic signals.

Acknowledgments

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SUPPLEMENTARY MATERIAL

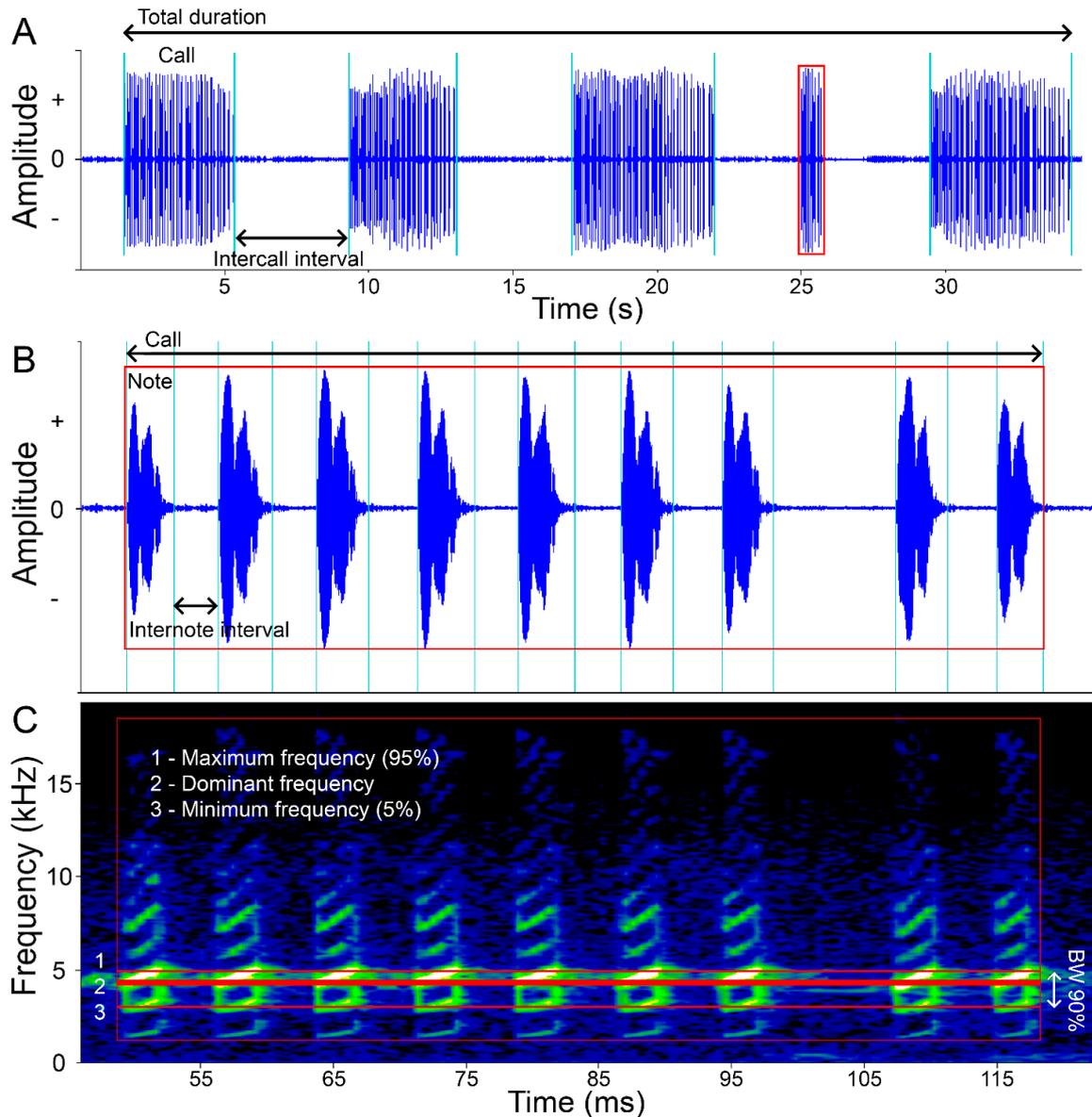


Figure S1. Demonstration of how we performed the selection of each parameter during the sounds analyses. The parameters of time were measured in the waveform, while the spectral parameters were measured in the spectrogram. A) we measured call duration selecting from the beginning of the first note to the end of the last one. Inter-call interval represents the measure between two calls of a same call group. The call rate was the number of calls within one minute. B) We considered note as a call subunit with 100% of amplitude modulation. Notes per call were the number of these subunits in each call, and note duration was the duration from the beginning to the end of each note. Inter-note interval was the time between two notes. C) For spectral parameters, we select the call completely in the spectrogram and the measurements were automatically available from the software: dominant frequency (peak frequency function in Raven); minimum frequency (frequency 5% function in Raven); maximum frequency (frequency 95% function in Raven); and frequency bandwidth (BW 90% function in Raven).

Table S1. Catalogue numbers of media analysed. Audios and videos were deposited at Audiovisual Collection [Fonoteca Neotropical Jacques Vielliard (FNJV) and Video Collection], Museu de Diversidade Biológica (MDBio), Universidade Estadual de Campinas (Unicamp), Campinas, SP, Brazil. An asterisk represents two males observed in the same video.

Male ID	Field number	Audio voucher	Video voucher
1	GH001	FNJV 45485	ZUEC-VID 818
2	GH002	FNJV 45486	ZUEC-VID 819
3	GH003	FNJV 45487	ZUEC-VID 820
4	GH004	FNJV 45488	ZUEC-VID 821
5	GH005	FNJV 45489	ZUEC-VID 822
6	GH006	FNJV 45490	ZUEC-VID 823
7	GH007	FNJV 45491	ZUEC-VID 824
8	GH008	FNJV 45492	ZUEC-VID 825
9	GH009	FNJV 45493	ZUEC-VID 826
10	GH010	FNJV 45494	ZUEC-VID 827
11	GH011	FNJV 45495	ZUEC-VID 828
12	GH012	FNJV 45496	ZUEC-VID 829
13	GH013	FNJV 45497	ZUEC-VID 830
14	GH014	FNJV 45498	ZUEC-VID 831
15	GH015	FNJV 45499	ZUEC-VID 832
16	GH016	FNJV 45500	ZUEC-VID 833
17	GH017	FNJV 45501	ZUEC-VID 834
18	GH018	FNJV 45502	ZUEC-VID 835
19	GH035	FNJV 45503	ZUEC-VID 836
20	GH036	FNJV 45504	ZUEC-VID 837
21	GH037	FNJV 45505	ZUEC-VID 838
22	GH038	FNJV 45506	ZUEC-VID 839
23	GH039	FNJV 45507	ZUEC-VID 840
24	GH040	FNJV 45508	ZUEC-VID 841
25	GH041	FNJV 45509	ZUEC-VID 842
26	GH042	FNJV 45510	ZUEC-VID 843
27	GH043	FNJV 45511	ZUEC-VID 844
28	GH044	FNJV 45512	ZUEC-VID 845
29	GH045	FNJV 45513	ZUEC-VID 846
30	GH046	FNJV 45514	ZUEC-VID 847
31	GH047	FNJV 45515	ZUEC-VID 848
32	GH049	FNJV 45516	ZUEC-VID 849
33	GH019	FNJV 45517	ZUEC-VID 850
34	GH020	FNJV 45518	ZUEC-VID 851
35	GH021	FNJV 45519	ZUEC-VID 852
36	GH022	FNJV 45520	ZUEC-VID 853
37	GH023	FNJV 45521	ZUEC-VID 854
38	GH024	FNJV 45522	ZUEC-VID 855
39	GH025	FNJV 45523	ZUEC-VID 856
40	GH026	FNJV 45524	ZUEC-VID 857
41	GH027	FNJV 45525	ZUEC-VID 858

42	GH028	FNJV 45526	ZUEC-VID 859
43	GH029	FNJV 45527	ZUEC-VID 860
44	GH030	FNJV 45528	ZUEC-VID 861
45	GH031	FNJV 45529	ZUEC-VID 862
46	GH032	FNJV 45530	ZUEC-VID 863
47	GH033	FNJV 45531	ZUEC-VID 864
48	GH034	FNJV 45532	ZUEC-VID 865
49	GH048	FNJV 45533	ZUEC-VID 866
50	GH050	FNJV 45534	ZUEC-VID 867*
51	GH051	FNJV 45535	ZUEC-VID 868
52	GH052	FNJV 45536	ZUEC-VID 869
53	GH053	FNJV 45537	ZUEC-VID 870
54	GH054	FNJV 45538	ZUEC-VID 871
55	GH055	FNJV 45539	ZUEC-VID 872
56	GH056	FNJV 45540	ZUEC-VID 873
57	GH059	FNJV 45541	ZUEC-VID 874
58	GH060	FNJV 45542	ZUEC-VID 875
59	RVB001	not recorded	ZUEC-VID 876
60	RVB002	not recorded	ZUEC-VID 877
61	RVB003	not recorded	ZUEC-VID 878
62	RVB004	not recorded	ZUEC-VID 879
63	RVP001	not recorded	ZUEC-VID 880
64	RVP002	not recorded	ZUEC-VID 881
65	RVP003	not recorded	ZUEC-VID 882
66	RVP004	not recorded	ZUEC-VID 883
67	RVP005	not recorded	ZUEC-VID 884
68	RVP006	not recorded	ZUEC-VID 885
69	RVP007	not recorded	ZUEC-VID 886
70	RVP008	not recorded	ZUEC-VID 887
71	RVP009	not recorded	ZUEC-VID 888
72	RVP010	not recorded	ZUEC-VID 889
73	RVP011	not recorded	ZUEC-VID 890
74	RVP012	not recorded	ZUEC-VID 891
75	RVP013	not recorded	ZUEC-VID 892
76	RVP014	not recorded	ZUEC-VID 893
77	RVP015	not recorded	ZUEC-VID 894
78	RVP016	not recorded	ZUEC-VID 867*

Table S2. Summary of the principal component importance and axis loading on advertisement call parameters of the *Hylodes phyllodes* from Bertioga and Picinguaba.

	PC1	PC2	PC3
Variance	2.585	1.990	1.855
Proportion of variance	25.848	19.904	18.547
Cumulative	25.848	45.752	64.299
Loadings			
Call duration	-0.764	0.547	0.025
Notes per call	-0.681	0.558	-0.029
Inter-call interval	0.408	0.385	-0.438
Note duration	-0.324	0.018	0.274
Inter-note interval	0.102	0.016	0.494
Call rate	0.245	-0.72	0.425
Dominant frequency	0.433	0.347	0.575
Minimum frequency	-0.435	0.026	0.752
Maximum frequency	0.516	0.595	0.506
Frequency bandwidth	0.744	0.488	-0.095

Table S3. Advertisement call spectral and temporal parameters from all individuals of *Hylodes phyllodes* analyzed. Values are presented as mean \pm standard deviation (range; sample size). On the site column, B represents individuals from Bertioiga and P represents individuals from Picinguaba, Ubatuba. An asterisk indicates individuals that we excluded from the analyses for minimum frequency, maximum frequency, and frequency bandwidth. This was necessary because we were not able to separate the influence of background noise from water flow and/or vehicle traffic from the call analyzed for these parameters.

Male ID	Site	Call duration (s)	Notes per call (n)	Inter-call interval (s)	Note duration (ms)	Inter-note interval (ms)	Call rate	Dominant frequency (kHz)	Minimum frequency (kHz)	Maximum frequency (kHz)	Frequency bandwidth (kHz)
1	B	1.88 \pm 0.66 (0.62 - 5.13; 48)	17.19 \pm 4.75 (7 - 38; 48)	1.76 \pm 0.33 (1.45 - 3.28; 47)	37.53 \pm 4.53 (23.6 - 54.2; 810)	77.56 \pm 38.97 (35.1 - 293.6; 761)	16.65	4.80 \pm 0.16 (4.48 - 5.25; 48)	4.43 \pm 0.05 (4.31 - 4.56; 48)	5.37 \pm 0.07 (5.25 - 5.51; 48)	0.94 \pm 0.07 (0.86 - 1.12; 48)
2	B	1.55 \pm 0.77 (0.40 - 4.74; 36)	14.42 \pm 6.23 (4 - 39; 36)	3.54 \pm 0.66 (2.86 - 6.32; 34)	51.18 \pm 6.16 (30.8 - 64.8; 525)	57.78 \pm 26.64 (13.4 - 193.5; 483)	12.06	5.12 \pm 0.29 (4.56 - 5.51; 36)	4.33 \pm 0.17 (3.53 - 4.56; 36)	5.41 \pm 0.07 (5.25 - 5.51; 36)	1.08 \pm 0.17 (0.86 - 1.89; 36)
3	B	1.46 \pm 0.23 (0.60 - 1.86; 29)	14.86 \pm 1.48 (12 - 18; 29)	3.54 \pm 0.65 (2.84 - 5.31; 28)	37.53 \pm 3.9 (29 - 51.5; 420)	65.61 \pm 31.04 (27.3 - 163.2; 397)	12.29	4.85 \pm 0.19 (4.65 - 5.25; 29)	4.53 \pm 0.19 (3.62 - 4.74; 29)	5.29 \pm 0.1 (4.99 - 5.43; 29)	0.76 \pm 0.23 (0.43 - 1.81; 29)
4	B	1.76 \pm 0.58 (0.92 - 3; 30)	18.37 \pm 4.98 (11 - 30; 30)	3.4 \pm 0.5 (2.41 - 4.64; 29)	40.46 \pm 4.67 (27.4 - 54.9; 551)	56.11 \pm 27.24 (21.7 - 173.5; 491)	11.98	4.64 \pm 0.16 (4.39 - 5.17; 30)	4.42 \pm 0.08 (4.31 - 4.56; 30)	5.28 \pm 0.08 (5.17 - 5.43; 30)	0.86 \pm 0.06 (0.77 - 0.95; 30)
5	B	0.99 \pm 0.16 (0.81 - 1.46; 19)	10.47 \pm 1.46 (9 - 15; 19)	8.11 \pm 6.14 (3.99 - 29.55; 18)	27.12 \pm 6.05 (8.4 - 38.1; 199)	73.51 \pm 33.62 (35.1 - 176.8; 180)	6.89	4.73 \pm 0.16 (4.56 - 4.99; 19)	4.11 \pm 0.09 (3.87 - 4.31; 19)	4.93 \pm 0.14 (4.65 - 5.08; 19)	0.82 \pm 0.08 (0.69 - 0.95; 19)
6	B	1.28 \pm 0.14 (1.04 - 1.66; 30)	13.47 \pm 1.36 (11 - 17; 30)	3.41 \pm 0.43 (2.66 - 4.4; 29)	38.23 \pm 3.1 (30.1 - 48.4; 404)	58.93 \pm 24.81 (21.9 - 142.4; 373)	13.06	5.03 \pm 0.11 (4.65 - 5.08; 30)	3.93 \pm 0.16 (3.36 - 4.22; 30)	5.37 \pm 0.05 (5.25 - 5.43; 30)	1.44 \pm 0.15 (1.12 - 1.89; 30)
7	B	0.99 \pm 0.17 (0.17 - 0.52; 20)	8.45 \pm 1.16 (5 - 10; 20)	3.04 \pm 0.4 (2.44 - 3.85; 19)	48.34 \pm 5.26 (21.8 - 62.6; 169)	77.76 \pm 30.02 (28.8 - 190.3; 149)	15.42	4.97 \pm 0.21 (4.74 - 5.34; 20)	4.43 \pm 0.08 (4.22 - 4.56; 20)	5.43 \pm 0.06 (5.25 - 5.51; 20)	1 \pm 0.08 (0.86 - 1.2; 20)
8	B	1.60 \pm 0.26 (1.16 - 2.17; 30)	14.5 \pm 1.71 (12 - 19; 30)	4.95 \pm 1.41 (3.62 - 9.53; 29)	38.32 \pm 3.84 (29.1 - 54.8; 435)	75.41 \pm 38.1 (32.9 - 246; 404)	9.37	5.22 \pm 0.09 (5.08 - 5.34; 30)	4.38 \pm 0.28 (3.44 - 4.56; 30)	5.38 \pm 0.04 (5.34 - 5.43; 30)	0.99 \pm 0.3 (0.77 - 1.98; 30)
9	B	1.27 \pm 0.27 (0.55 - 1.85; 30)	12.8 \pm 2.2 (7 - 18; 30)	4.19 \pm 0.88 (3.23 - 6.92; 29)	39.14 \pm 4.34 (27.5 - 50.1; 398)	64.73 \pm 32.67 (29.9 - 172.9; 367)	10.77	4.54 \pm 0.29 (4.22 - 5.34; 30)	3.95 \pm 0.28 (3.36 - 4.31; 30)	5.27 \pm 0.14 (5.08 - 5.51; 30)	1.32 \pm 0.34 (0.95 - 2.07; 30)
10	B	1.83 \pm 0.53 (0.65 - 2.66; 30)	18.8 \pm 5.01 (8 - 26; 30)	4.03 \pm 0.65 (2.81 - 5.72; 29)	34.42 \pm 3.74 (12.6 - 45.7; 564)	64.68 \pm 31.94 (30.1 - 251.8; 533)	10.51	4.65 \pm 0 (4.65 - 4.65; 30)	3.83 \pm 0.31 (3.39 - 4.31; 30)	5.04 \pm 0.06 (4.91 - 5.17; 30)	1.29 \pm 0.27 (0.77 - 1.64; 30)
11	B	1.52 \pm 0.21 (1.13 - 2.04; 28)	13.82 \pm 1.71 (11 - 18; 28)	3.96 \pm 1.16 (2.83 - 7.73; 26)	38.99 \pm 4.61 (28.1 - 52.6; 386)	75.11 \pm 36.18 (37.2 - 193.6; 357)	11.26	5 \pm 0.11 (4.82 - 5.25; 28)	4.69 \pm 0.07 (4.56 - 4.91; 28)	5.21 \pm 0.05 (5.17 - 5.34; 28)	0.52 \pm 0.09 (0.34 - 0.69; 28)
12	B	2.25 \pm 0.63 (1.14 - 3.49; 30)	19 \pm 4.81 (11 - 29; 30)	5.84 \pm 3.86 (2.11 - 22.07; 29)	35.82 \pm 4.9 (25.3 - 52.2; 570)	85.64 \pm 38.8 (39.3 - 238.8; 539)	7.58	5.22 \pm 0.08 (5.08 - 5.34; 30)	4.38 \pm 0.1 (4.22 - 4.65; 30)	5.31 \pm 0.05 (5.25 - 5.43; 30)	0.92 \pm 0.08 (0.77 - 1.03; 30)
13	B	2.52 \pm 1.13 (1.15 - 6.17; 30)	20.53 \pm 8.02 (13 - 47; 30)	4.58 \pm 1.63 (2.53 - 9.74; 29)	39.53 \pm 3.36 (10.3 - 48.3; 616)	85.36 \pm 34.92 (44.8 - 190.5; 586)	8.62	4.62 \pm 0.04 (4.56 - 4.65; 30)	4.18 \pm 0.26 (3.36 - 4.39; 30)	4.95 \pm 0.08 (4.82 - 5.08; 30)	0.77 \pm 0.24 (0.6 - 1.46; 30)
14*	B	2.2 \pm 0.69 (1.2 - 3.81; 30)	20.43 \pm 5.29 (12 - 32; 30)	4.08 \pm 0.78 (2.84 - 5.87; 29)	38.15 \pm 4.05 (17 - 51; 613)	71.9 \pm 33.56 (31.5 - 182.1; 582)	9.58	5.32 \pm 0.16 (4.48 - 5.43; 30)	3.56 \pm 0.39 (3.27 - 4.39; 30)	5.44 \pm 0.08 (5.34 - 5.86; 30)	1.88 \pm 0.42 (1.03 - 2.58; 30)
15*	B	1.02 \pm 0.26 (0.24 - 1.45; 30)	10.73 \pm 2.29 (3 - 15; 30)	4.88 \pm 1.39 (3.27 - 8.65; 29)	37.7 \pm 4.25 (16.3 - 47.8; 322)	61.55 \pm 22.56 (35 - 161.4; 292)	10.43	4.87 \pm 0.16 (4.56 - 4.99; 30)	3.32 \pm 0.08 (3.1 - 3.53; 30)	5.08 \pm 0.07 (4.99 - 5.17; 30)	1.76 \pm 0.1 (1.55 - 1.89; 30)
16	B	1.51 \pm 0.19 (1.15 - 1.92; 30)	14.87 \pm 1.5 (12 - 18; 30)	3.59 \pm 0.62 (3.13 - 6.57; 29)	34.9 \pm 3.86 (25.2 - 51.8; 446)	69.25 \pm 32.26 (32.7 - 189.2; 416)	12	4.79 \pm 0.21 (4.22 - 4.91; 30)	4.05 \pm 0.07 (3.96 - 4.13; 30)	4.93 \pm 0.04 (4.82 - 4.99; 30)	0.88 \pm 0.05 (0.77 - 0.95; 30)
17*	B	0.9 \pm 0.11 (0.68 - 1.15; 30)	10.03 \pm 1.22 (8 - 13; 30)	4.73 \pm 1.77 (2.92 - 11.17; 29)	30.22 \pm 3.67 (18.8 - 39.9; 301)	64.27 \pm 27.4 (34.1 - 158.6; 271)	10.95	5.06 \pm 0.17 (4.82 - 5.68; 30)	3.53 \pm 0.068 (3.44 - 3.62; 30)	5.98 \pm 0.58 (5.68 - 7.41; 30)	2.45 \pm 0.62 (2.07 - 3.96; 30)

18*	B	1.82 ± 0.4 (1.15 - 2.62; 30)	19.5 ± 3.68 (13 - 28; 30)	2.93 ± 0.54 (2.14 - 4.03; 29)	33.84 ± 3.86 (23 - 44; 585)	59.63 ± 28.11 (27.2 - 158.1; 555)	12.88	5.42 ± 0.41 (4.74 - 5.68; 30)	3.44 ± 0.03 (3.36 - 3.53; 30)	7.07 ± 0.15 (6.63 - 7.41; 30)	3.63 ± 0.15 (3.19 - 3.96; 30)
19*	B	1.77 ± 0.23 (1.09 - 2.28; 30)	15.34 ± 1.89 (10 - 20; 30)	5.1 ± 0.56 (4.31 - 6.39; 29)	40.92 ± 4.35 (27.9 - 54.6; 460)	77.95 ± 35.62 (30.6 - 189.8; 432)	8.95	4.93 ± 0.10 (4.74 - 5.08; 30)	3.46 ± 0.13 (3.19 - 3.79; 30)	5.55 ± 0.33 (5.17 - 6.37; 30)	2.09 ± 0.37 (1.64 - 2.93; 30)
20	B	2.56 ± 0.74 (1.24 - 3.84; 24)	22.42 ± 5.33 (13 - 32; 24)	5.71 ± 1.41 (2.25 - 10.73; 23)	38.56 ± 5.56 (25.2 - 62.8; 538)	78.53 ± 39.07 (26.1 - 236.1; 514)	7.46	4.39 ± 0 (4.39 - 4.39; 24)	4.21 ± 0.02 (4.13 - 4.22; 24)	5.01 ± 0.07 (4.91 - 5.25; 24)	0.8 ± 0.06 (0.69 - 1.03; 24)
21	B	2.38 ± 0.51 (1.22 - 3.37; 30)	17.4 ± 3.06 (11 - 24; 30)	3.49 ± 0.83 (2.6 - 6.92; 29)	59.06 ± 4.65 (40.2 - 77.8; 522)	81.77 ± 38.36 (25.3 - 236; 492)	10.42	4.75 ± 0.15 (4.48 - 4.91; 30)	4.48 ± 0.07 (4.39 - 4.65; 30)	5.32 ± 0.06 (5.25 - 5.43; 30)	0.85 ± 0.04 (0.77 - 0.95; 30)
22	B	1.81 ± 0.35 (0.79 - 2.47; 30)	14.43 ± 1.94 (8 - 18; 30)	3.28 ± 0.63 (2.52 - 5.6; 29)	41.39 ± 3.68 (21 - 50.7; 433)	89.15 ± 34.65 (45.7 - 192.1; 403)	12.02	4.31 ± 0.22 (4.13 - 4.74; 30)	4.02 ± 0.23 (2.84 - 4.22; 30)	5.06 ± 0.07 (4.91 - 5.34; 30)	1.04 ± 0.27 (0.86 - 2.5; 30)
23	B	1.06 ± 0.15 (0.07 - 1.27; 30)	11.13 ± 1.28 (8 - 13; 30)	4.6 ± 0.98 (3.31 - 7.56; 29)	46.76 ± 6.42 (30.3 - 58.5; 334)	52.12 ± 29.03 (21 - 176.7; 304)	10.89	4.59 ± 0.27 (4.39 - 5.34; 30)	4.05 ± 0.09 (3.79 - 4.22; 30)	5.34 ± 0.05 (5.25 - 5.43; 30)	1.29 ± 0.08 (1.21 - 1.55; 30)
24*	B	1.19 ± 0.18 (0.74 - 1.55; 30)	11.73 ± 1.44 (8 - 14; 30)	6.23 ± 3.39 (4.58 - 23.5; 29)	32.86 ± 5.04 (20.7 - 43; 352)	72.76 ± 27.98 (46.7 - 197.2; 322)	8.31	4.54 ± 0.08 (4.48 - 4.65; 30)	3.29 ± 0.13 (3.1 - 3.53; 30)	4.94 ± 0.09 (4.82 - 5.08; 30)	1.65 ± 0.17 (1.29 - 1.89; 30)
25*	B	3.06 ± 0.67 (2.05 - 4.73; 22)	26 ± 4.66 (19 - 37; 22)	7.76 ± 3.39 (4.99 - 20.8; 21)	36.3 ± 2.7 (22.9 - 47.1; 572)	83.33 ± 34.79 (45.8 - 197.6; 550)	5.73	5.21 ± 0.07 (5.17 - 5.34; 22)	4.6 ± 0.1 (4.39 - 4.74; 22)	8.81 ± 1.3 (6.8 - 10.77; 22)	4.2 ± 1.31 (2.24 - 6.37; 22)
26	B	1.44 ± 0.24 (0.69 - 1.86; 30)	13.97 ± 1.91 (8 - 17; 30)	6.76 ± 3.42 (4.89 - 22.67; 29)	38.96 ± 3.48 (27.6 - 46.9; 416)	69 ± 25.62 (40.9 - 166.2; 386)	7.51	5.08 ± 0 (5.08 - 5.08; 30)	3.65 ± 0.1 (3.44 - 3.88; 30)	5.27 ± 0.03 (5.25 - 5.34; 30)	1.62 ± 0.11 (1.38 - 1.81; 30)
27*	B	2.71 ± 1.28 (1.09 - 7.25; 17)	21.59 ± 7.76 (10 - 48; 17)	4.22 ± 1.33 (2.44 - 8.07; 16)	35.25 ± 3.37 (29.7 - 61.2; 367)	93.02 ± 32.28 (40.7 - 213.8; 350)	8.96	5.24 ± 0.13 (4.99 - 5.34; 17)	3.94 ± 0.34 (3.36 - 4.74; 17)	6.26 ± 1.06 (5.34 - 8.53; 17)	2.32 ± 1.16 (1.12 - 4.91; 17)
28	B	1.87 ± 0.52 (0.74 - 3; 25)	15.2 ± 4 (7 - 24; 25)	6.03 ± 1.57 (4.44 - 9.72; 24)	43.51 ± 3.13 (23 - 51.8; 371)	87.72 ± 43.81 (40.3 - 252.4; 346)	7.83	4.82 ± 0 (4.82 - 4.82; 25)	4.34 ± 0.12 (3.88 - 4.56; 25)	5.01 ± 0.04 (4.91 - 5.08; 25)	0.66 ± 0.1 (0.43 - 1.03; 25)
29	B	7.54 ± 4.74 (0.91 - 18.31; 19)	66.68 ± 0.04 (10 - 160; 19)	3.35 ± 1.62 (2.17 - 9.73; 18)	46.06 ± 3.98 (35 - 57.3; 1,267)	67.56 ± 31.49 (24.2 - 192.1; 1,248)	5.6	4.81 ± 0.03 (4.74 - 4.82; 19)	4.37 ± 0.06 (4.31 - 4.47; 19)	5.29 ± 0.18 (5.17 - 5.94; 19)	0.92 ± 0.16 (0.77 - 1.55; 19)
30	B	2.31 ± 0.95 (1.17 - 5.11; 30)	22.8 ± 7.09 (14 - 43; 30)	4.8 ± 2.04 (2.66 - 11.79; 29)	38.41 ± 5.39 (18.5 - 55.5; 684)	64.83 ± 31.81 (18.5 - 221.4; 654)	8.64	4.84 ± 0.04 (4.74 - 4.91; 30)	4.5 ± 0.11 (4.31 - 4.74; 30)	5.41 ± 0.15 (5.25 - 6.03; 30)	0.91 ± 0.12 (0.77 - 1.46; 30)
31	B	1.37 ± 0.23 (0.53 - 1.65; 30)	13.53 ± 1.91 (7 - 16; 30)	4.93 ± 1.1 (3.87 - 8.61; 29)	46.18 ± 4.85 (31.6 - 58.3; 406)	57.54 ± 24.84 (19.4 - 116.7; 376)	9.76	5.23 ± 0.09 (4.82 - 5.34; 30)	4.37 ± 0.1 (4.13 - 4.56; 30)	5.39 ± 0.05 (5.25 - 5.43; 30)	1.02 ± 0.07 (0.86 - 1.12; 30)
32	B	2.04 ± 0.45 (1.32 - 3.25; 30)	18.87 ± 3.45 (13 - 27; 30)	3.74 ± 0.95 (2.82 - 7.42; 29)	38.24 ± 3.65 (22.8 - 51.8; 566)	72.54 ± 34.24 (33.1 - 192.5; 536)	10.6	5.23 ± 0.13 (4.56 - 5.34; 30)	4.58 ± 0.17 (3.88 - 4.74; 30)	5.56 ± 0.1 (5.43 - 5.94; 30)	0.98 ± 0.24 (0.77 - 1.98; 30)
33	P	1.8 ± 0.35 (1.13 - 2.71; 30)	15.3 ± 2.92 (11 - 24; 30)	5.22 ± 1.86 (3.26 - 11.29; 29)	27.94 ± 4.52 (10 - 43.3; 458)	94.82 ± 45.28 (45.4 - 285.1; 428)	8.745	4.9 ± 0.04 (4.82 - 4.99; 30)	4.42 ± 0.46 (3.27 - 4.65; 30)	5.22 ± 0.14 (5.08 - 5.86; 30)	0.79 ± 0.58 (0.52 - 2.58; 30)
34*	P	1.66 ± 0.15 (1.19 - 1.95; 30)	18.63 ± 1.6 (14 - 21; 30)	4.6 ± 0.61 (3.76 - 6.74; 29)	28.16 ± 2.52 (20 - 36.1; 559)	63.21 ± 19.25 (45.7 - 174.5; 529)	9.82	4.37 ± 0.06 (4.31 - 4.56; 30)	3.07 ± 0.07 (2.76 - 3.19; 30)	4.86 ± 0.08 (4.56 - 4.99; 30)	1.78 ± 0.04 (1.72 - 1.81; 30)
35	P	1.65 ± 0.28 (0.53 - 2.13; 30)	19.8 ± 2.77 (8 - 23; 30)	4.22 ± 2.05 (2.88 - 11.37; 29)	24.99 ± 5.76 (10.3 - 40.2; 594)	59.81 ± 31.17 (27.8 - 159.5; 564)	10.46	4.57 ± 0.21 (4.05 - 4.91; 30)	3.48 ± 0.6 (2.41 - 4.39; 30)	5 ± 0.15 (4.48 - 5.17; 30)	1.53 ± 0.55 (0.69 - 2.58; 30)
36	P	6.23 ± 3.24 (1.59 - 16.92; 29)	67.93 ± 33.3 (19 - 178; 29)	1.79 ± 0.35 (1.32 - 3.22; 28)	32.51 ± 3.05 (23 - 43.2; 1,970)	58.79 ± 25.28 (30.8 - 211.6; 1,941)	7.54	5.22 ± 0.14 (4.99 - 5.34; 29)	4.45 ± 0.07 (4.31 - 4.56; 29)	5.27 ± 0.06 (5.17 - 5.34; 29)	0.82 ± 0.05 (0.69 - 0.86; 29)
37	P	2.06 ± 0.36 (1.26 - 2.64; 30)	22.57 ± 3.4 (15 - 28; 30)	5.45 ± 1.35 (3.43 - 8.04; 29)	32.71 ± 5.58 (9 - 43.9; 678)	60.2 ± 28.58 (32.7 - 172.6; 648)	8.19	4.29 ± 0.26 (4.05 - 4.82; 30)	3.48 ± 0.28 (2.93 - 3.96; 30)	4.75 ± 0.2 (4.13 - 5.08; 30)	1.27 ± 0.28 (0.95 - 1.89; 30)
38	P	5.54 ± 2.3 (0.71 - 11.2; 30)	59.53 ± 23.89 (9 - 114; 30)	3.05 ± 0.95 (1.74 - 5.6; 29)	32.44 ± 3.45 (24.3 - 47.5; 1786)	60.58 ± 30.87 (26.9 - 356.3; 1756)	7.06	4.39 ± 0.07 (4.31 - 4.74; 30)	4.24 ± 0.05 (4.13 - 4.31; 30)	4.87 ± 0.06 (4.82 - 4.99; 30)	0.63 ± 0.04 (0.6 - 0.69; 30)
39	P	1.4 ± 0.26	16.3 ± 2.77	5.98 ± 4.61	31.9 ± 6.17	56.05 ± 26.49	8.35	4.41 ± 0.07	3.39 ± 0.38	4.92 ± 0.17	1.52 ± 0.24

		(0.5 - 1.83; 30)	(7 - 22; 30)	(2.7 - 24.17; 29)	(4.5 - 44.2; 489)	(30.2 - 192.4; 459)		(4.39 - 4.74; 30)	(2.76 - 4.13; 30)	(4.56 - 5.17; 30)	(1.03 - 1.89; 30)
40	P	2.3 ± 0.4 (1.11 - 3.07; 30)	24.47 ± 3.6 (14 - 31; 30)	2.37 ± 0.26 (1.89 - 3.03; 29)	31.3 ± 4.46 (10.5 - 42.6; 734)	64.72 ± 30.66 (29.6 - 195; 704)	13.05	4.64 ± 0.02 (4.56 - 4.65; 30)	3.94 ± 0.08 (3.79 - 4.13; 30)	4.76 ± 0.06 (4.65 - 4.91; 30)	0.82 ± 0.05 (0.77 - 0.95; 30)
41*	P	3.58 ± 0.91 (2.03 - 6.59; 30)	37.23 ± 8.2 (23 - 64; 30)	3.01 ± 0.51 (2.31 - 4.89; 29)	38.31 ± 4.69 (21.5 - 62; 1,117)	57.7 ± 30.1 (15.2 - 186.7; 1,088)	9.23	5.03 ± 0.28 (4.65 - 5.34; 30)	3.58 ± 0.21 (3.36 - 4.39; 30)	5.41 ± 0.06 (5.25 - 5.51; 30)	1.83 ± 0.25 (0.95 - 2.07; 30)
42	P	1.19 ± 0.16 (0.88 - 1.48; 30)	13.43 ± 1.41 (11 - 16; 30)	3.51 ± 0.56 (2.62 - 4.83; 29)	33.3 ± 4.9 (18.2 - 46.2; 403)	58.31 ± 31.76 (27.2 - 155.2; 373)	13.08	4.9 ± 0.24 (4.65 - 5.17; 30)	3.19 ± 0.15 (2.93 - 3.53; 30)	5.20 ± 0.06 (5.08 - 5.25; 30)	2.01 ± 0.11 (1.72 - 2.15; 30)
43*	P	1.1 ± 0.14 (0.88 - 1.32; 30)	14.23 ± 1.45 (12 - 16; 30)	3.98 ± 0.86 (2.96 - 6.91; 29)	23.57 ± 3.41 (11.9 - 32.2; 427)	56.35 ± 22.46 (35.8 - 169.2; 397)	12.08	4.89 ± 0.27 (4.31 - 5.17; 30)	2.93 ± 0.11 (2.67 - 3.1; 30)	5.14 ± 0.14 (4.91 - 5.77; 30)	2.21 ± 0.14 (2.07 - 2.84; 30)
44	P	1.6 ± 0.23 (0.84 - 2.11; 30)	20.23 ± 2.44 (13 - 26; 30)	4.71 ± 2.84 (2.95 - 15.9; 29)	42.23 ± 4.75 (24.7 - 51.1; 607)	38.03 ± 28.15 (14.7 - 177.8; 577)	9.73	4.81 ± 0.19 (4.74 - 5.51; 30)	4.39 ± 0.22 (3.62 - 4.65; 30)	5.35 ± 0.15 (5 - 5.5; 30)	0.96 ± 0.11 (0.69 - 1.38; 30)
45	P	8.12 ± 5.28 (2.45 - 21.18; 24)	82.68 ± 51.73 (28 - 214; 24)	2.81 ± 0.43 (2.16 - 4.43; 21)	44.98 ± 4.28 (31.7 - 61.4; 1,820)	52.81 ± 29.33 (16.8 - 275.4; 1,798)	5.54	4.57 ± 0.02 (4.56 - 4.65; 24)	4.23 ± 0.05 (4.13 - 4.31; 24)	4.89 ± 0.06 (4.74 - 5; 24)	0.66 ± 0.04 (0.6 - 0.69; 24)
46	P	2.33 ± 0.33 (1.77 - 3.04; 30)	25.1 ± 3.13 (20 - 32; 30)	2.91 ± 0.28 (2.49 - 3.64; 29)	32.59 ± 6.07 (13.3 - 49.3; 753)	61.79 ± 40.72 (16.5 - 232.4; 723)	11.67	5.05 ± 0.41 (4.48 - 5.34; 30)	4.3 ± 0.09 (4.13 - 4.48; 30)	5.37 ± 0.08 (5.17 - 5.51; 30)	1.07 ± 0.06 (0.95 - 1.21; 30)
47	P	2.9 ± 1.58 (1.09 - 8.26; 30)	30.23 ± 13.6 (14 - 76; 30)	3.03 ± 0.78 (1.9 - 5.85; 29)	34.63 ± 5.22 (12.4 - 49.9; 905)	62.3 ± 34.61 (28 - 282.5; 875)	10.3	4.89 ± 0.08 (4.74 - 4.99; 30)	4.44 ± 0.19 (3.62 - 4.65; 30)	5.13 ± 0.17 (4.99 - 6.03; 30)	0.69 ± 0.33 (0.43 - 2.41; 30)
48	P	1.53 ± 0.31 (0.44 - 1.89; 21)	18.62 ± 3.52 (6 - 22; 21)	11.99 ± 14.47 (4.53 - 59.63; 20)	36.45 ± 6.02 (12.6 - 50; 391)	47.73 ± 18.51 (25.3 - 137.2; 370)	4.63	4.83 ± 0.26 (4.48 - 5.08; 21)	3.08 ± 0.07 (2.93 - 3.19; 21)	5.16 ± 0.15 (4.82 - 5.6; 21)	2.08 ± 0.14 (1.89 - 2.67; 21)
49	P	1.51 ± 0.31 (1.09 - 2.61; 24)	17.04 ± 3.15 (13 - 28; 24)	5.89 ± 2.6 (4.21 - 15.21; 23)	50.91 ± 6.06 (31.2 - 63.3; 409)	39.58 ± 22.09 (14 - 127; 385)	8.37	4.99 ± 0.17 (4.74 - 5.34; 24)	3.94 ± 0.35 (3.36 - 4.56; 24)	5.32 ± 0.27 (5.17 - 6.55; 24)	1.38 ± 0.5 (0.86 - 3.19; 24)
50	P	3.17 ± 0.97 (1.19 - 5.32; 21)	33.66 ± 10.03 (13 - 54; 21)	5.14 ± 1.62 (2.24 - 7.41; 20)	37.16 ± 3.93 (25.9 - 52.4; 707)	58.11 ± 28.18 (18.7 - 213.4; 686)	7.44	4.86 ± 0.07 (4.74 - 4.91; 21)	4.3 ± 0.13 (3.96 - 4.56; 21)	5.32 ± 0.07 (5.17 - 5.51; 21)	1.02 ± 0.12 (0.86 - 1.38; 21)
51	P	6.78 ± 3.49 (1.04 - 17.62; 16)	4.96 ± 0.05 (4.91 - 5.08; 16)	2.99 ± 0.88 (0.44 - 4.29; 15)	49.71 ± 4.43 (34.3 - 65.3; 971)	62.15 ± 37.41 (23.8 - 250.4; 955)	6.26	4.2 ± 0.06 (4.13 - 4.31; 16)	4.2 ± 0.06 (4.13 - 4.31; 16)	4.96 ± 0.05 (4.91 - 5.08; 16)	0.76 ± 0.04 (0.69 - 0.86; 16)
52	P	1.95 ± 0.63 (0.87 - 2.61; 7)	19.57 ± 5.07 (11 - 25; 7)	16.43 ± 6.84 (7.3 - 26.61; 6)	37.84 ± 4.38 (21.8 - 51.6; 137)	64.66 ± 33.57 (29 - 216.1; 130)	3.74	5.09 ± 0.27 (4.82 - 5.43; 7)	3.68 ± 0.44 (3.27 - 4.65; 7)	5.59 ± 0.12 (5.43 - 5.77; 7)	1.91 ± 0.38 (1.12 - 2.34; 7)
53*	P	4.9 ± 1.63 (1.48 - 9.82; 30)	46.47 ± 13.87 (17 - 89; 30)	3.34 ± 0.56 (2.76 - 5.95; 29)	39.53 ± 4.61 (22.1 - 62.9; 1,394)	66.62 ± 28.8 (26.4 - 282.2; 1,364)	7.37	4.53 ± 0.17 (4.22 - 5.08; 30)	3.67 ± 0.27 (3.19 - 4.22; 30)	5.01 ± 0.08 (4.82 - 5.17; 30)	1.34 ± 0.31 (0.77 - 1.98; 30)
54	P	1.57 ± 0.23 (1.04 - 1.97; 23)	15.13 ± 1.75 (11 - 18; 23)	5.4 ± 1.05 (4.24 - 8.19; 22)	39.4 ± 2.92 (25.7 - 47.8; 348)	67.83 ± 19.76 (41.8 - 133.4; 325)	8.9	4.91 ± 0.32 (4.22 - 5.08; 23)	4.04 ± 0.21 (3.36 - 4.31; 23)	5.08 ± 0.08 (4.82 - 5.17; 23)	1.04 ± 0.14 (0.86 - 1.46; 23)
55	P	1.57 ± 0.25 (0.98 - 2.13; 30)	17.13 ± 2.33 (12 - 22; 30)	5.15 ± 3.83 (3.46 - 24.98; 29)	39.36 ± 4.25 (25.7 - 54.1; 514)	54.84 ± 29.66 (26 - 232; 484)	9.16	5.06 ± 0.29 (4.48 - 5.25; 30)	4.4 ± 0.18 (3.87 - 4.82; 30)	5.29 ± 0.06 (5.08 - 5.34; 30)	0.89 ± 0.16 (0.52 - 1.46; 30)
56	P	1.54 ± 0.23 (0.59 - 1.87; 30)	16.17 ± 2.31 (7 - 19; 30)	5.1 ± 1.35 (3.89 - 9.92; 29)	39.66 ± 6.08 (11.2 - 56.3; 485)	58.24 ± 27.91 (28.1 - 195.5; 455)	9.13	4.3 ± 0.03 (4.22 - 4.31; 30)	3.7 ± 0.26 (2.67 - 4.05; 30)	4.43 ± 0.08 (4.31 - 4.74; 30)	0.72 ± 0.21 (0.52 - 1.64; 30)
57*	P	2 ± 0.293 (1.43 - 2.74; 28)	21.11 ± 2.58 (16 - 28; 28)	4.82 ± 1.33 (3.56 - 8.8; 27)	34.42 ± 4.24 (22.7 - 46.6; 591)	62.12 ± 27.89 (35.5 - 219.1; 563)	9.03	4.84 ± 0.19 (4.13 - 4.91; 28)	2.78 ± 0.06 (2.67 - 2.84; 28)	4.95 ± 0.05 (4.82 - 5; 28)	2.17 ± 0.03 (2.15 - 2.24; 28)
58*	P	1.77 ± 0.2 (1.15 - 2.12; 29)	19.24 ± 1.89 (13 - 22; 29)	5.08 ± 0.58 (4.22 - 6.31; 28)	50.76 ± 6.48 (22 - 62.4; 558)	43.21 ± 24.66 (19.5 - 133.6; 529)	8.97	4.38 ± 0.2 (4.31 - 5.08; 29)	3.08 ± 0.04 (3.01 - 3.1; 29)	5.02 ± 0.1 (4.82 - 5.17; 29)	1.94 ± 0.08 (1.89 - 2.07; 29)

Table S4. Spectral and temporal parameters of the advertisement calls emitted using one or both vocal sacs by the same individual of *Hylodes phyllodes*. Values are presented as mean \pm standard deviation (range; sample size).

Male ID	Vocal sacs	Call duration (s)	Notes per call (n)	Note duration (ms)	Inter-note interval (ms)	Dominant frequency (kHz)	Minimum frequency (kHz)	Maximum frequency (kHz)	Frequency bandwidth (kHz)
35	1	1.51 \pm 0.13 (1.32 - 1.6; 3)	21.6 \pm 2.62 (18 - 24; 3)	25.8 \pm 5.64 (12.2 - 38.9; 65)	45.07 \pm 18.14 (25.2 - 136.4; 62)	4.48 (3)	4.05 \pm 0.07 (3.96 - 4.13; 3)	4.85 \pm 0.08 (4.74 - 4.91; 3)	0.8 \pm 0.04 (0.77 - 0.86; 3)
35	2	1.36 \pm 0.17 (1.07 - 1.55; 7)	18.28 \pm 1.48 (15 - 20; 7)	32.5 \pm 9.17 (11.5 - 49.3; 141)	51.81 \pm 33.8 (17.8 - 176.3; 134)	4.56 (7)	4.34 \pm 0.06 (4.22 - 4.39; 7)	4.97 \pm 0.04 (4.91 - 4.99; 7)	0.63 \pm 0.06 (0.52 - 0.69; 7)
39	1	1.69 (1)	21 (1)	35.32 \pm 5.73 (21.4 - 46; 21)	46.6 \pm 19.06 (27.3 - 97; 20)	4.48 (1)	3.79 (1)	4.82 (1)	1.03 (1)
39	2	1.51 \pm 0.18 (1.21 - 1.86; 7)	17.86 \pm 1.88 (15 - 21; 7)	40.88 \pm 4.54 (23.8 - 50.9; 125)	45.57 \pm 22.49 (21.5 - 116.1; 118)	4.67 \pm 0.17 (4.48 - 4.82; 7)	4.27 \pm 0.06 (4.22 - 4.39; 7)	4.98 \pm 0.116 (4.91 - 5.25; 7)	0.71 \pm 0.06 (0.69 - 0.86; 7)
42	1	1.12 \pm 0.16 (0.96 - 1.28; 2)	14.5 \pm 1.5 (13 - 16; 2)	32.05 \pm 6.7 (17.9 - 43.2; 29)	48.67 \pm 19.85 (29.4 - 109.5; 27)	4.65 (2)	3.74 \pm 0.39 (3.36 - 4.13; 2)	5.3 \pm 0.21 (5.08 - 5.51; 2)	1.55 \pm 0.6 (0.97 - 2.15; 2)
42	2	1.61 \pm 0.16 (1.4 - 1.92; 7)	17.28 \pm 1.48 (15 - 20; 7)	48.24 \pm 5.38 (35.8 - 65.9; 121)	46.51 \pm 32.45 (12.3 - 135.2; 114)	4.73 (7)	4.32 \pm 0.08 (4.22 - 4.48; 7)	5.22 \pm 0.07 (5.08 - 5.34; 7)	0.89 \pm 0.06 (0.86 - 1.03; 7)
44	1	2.62 (1)	33 (1)	37.75 \pm 6.71 (25.4 - 53.4; 33)	42.68 \pm 24.62 (19 - 112.7; 32)	4.91 (1)	3.19 (1)	5.08 (1)	1.89 (1)
44	2	1.97 \pm 0.29 (1.58 - 2.4; 7)	24.14 \pm 3.27 (20 - 29; 7)	47.02 \pm 5.66 (22.6 - 57.5; 169)	35.57 \pm 27.32 (7.4 - 137.7; 162)	4.69 \pm 0.16 (4.48 - 4.91; 7)	4.39 \pm 0.05 (4.31 - 4.48; 7)	5.36 \pm 0.06 (5.25 - 5.43; 7)	0.97 \pm 0.04 (0.95 - 1.03; 7)
47	1	2.15 \pm 0.16 (1.98 - 2.31; 2)	29 \pm 3 (26 - 32; 2)	33.35 \pm 9.36 (15.4 - 55.5; 57)	43.29 \pm 21.9 (8.4 - 130.9; 55)	4.65 \pm 0.09 (4.56 - 4.74; 2)	4.13 \pm 0.09 (4.05 - 4.22; 2)	4.99 \pm 0.17 (4.82 - 5.17; 2)	0.86 \pm 0.26 (0.6 - 1.19; 2)
47	2	1.64 \pm 0.26 (1.22 - 2.06; 7)	19.86 \pm 2.69 (15 - 24; 7)	39.67 \pm 4.44 (30.9 - 53.8; 138)	45.43 \pm 31.63 (18.5 - 244.7; 131)	4.7 \pm 0.06 (4.65 - 4.82; 7)	4.43 \pm 0.08 (4.31 - 4.56; 7)	5.06 \pm 0.04 (4.99 - 5.08; 7)	0.63 \pm 0.09 (0.52 - 0.77; 7)
21	1	1.99 (1)	18 (1)	42.28 \pm 8.63 (24.9 - 57.2; 18)	72.54 \pm 33.43 (34.43 - 128.4; 17)	4.99 (1)	4.22 (1)	5.25 (1)	1.03 (1)
21	2	2.35 \pm 0.36 (1.86 - 3.03; 7)	20.43 \pm 2.82 (17 - 26; 7)	47.88 \pm 5.08 (34.8 - 62.2; 143)	69.1 \pm 35.34 (26.1 - 174.1; 136)	4.96 \pm 0.14 (4.65 - 5.08; 7)	4.47 \pm 0.07 (4.39 - 4.56; 7)	5.39 \pm 0.04 (5.34 - 5.43; 7)	0.92 \pm 0.06 (0.86 - 1.03; 7)
23	1	1.03 (1)	12 (1)	41.29 \pm 4.99 (32.7 - 49.8; 12)	46.29 \pm 13.5 (31.5 - 75.9; 11)	4.91 (1)	4.39 (1)	5.08 (1)	0.69 (1)
23	2	0.88 \pm 0.07 (0.74 - 0.96; 7)	9.14 \pm 0.64 (8 - 10; 7)	51.39 \pm 5.48 (31.9 - 65.4; 64)	48.47 \pm 25.31 (14 - 118.3; 57)	4.56 (7)	4.19 \pm 0.04 (4.13 - 4.33; 7)	5.26 \pm 0.05 (5.17 - 5.34; 7)	1.07 \pm 0.04 (1.03 - 1.12; 7)
56	1	0.97 (1)	11 (1)	43.5 \pm 4.4 (37.4 - 52.6; 11)	48.74 \pm 7.75 (39.3 - 61.6; 10)	4.56 (1)	3.96 (1)	4.65 (1)	0.69 (1)
56	2	2.1 \pm 0.45 (1.1 - 2.55; 7)	19.71 \pm 3.69 (12 - 24; 7)	51.57 \pm 6.29 (31.4 - 68.6; 138)	56.72 \pm 34.74 (18.7 - 144.4; 131)	4.06 \pm 0.32 (3.79 - 4.56; 7)	3.84 \pm 0.19 (3.7 - 4.22; 7)	4.58 \pm 0.11 (4.48 - 4.74; 7)	0.74 \pm 0.1 (0.52 - 0.86; 7)
69	1	1.64 \pm 0.12 (1.49 - 1.79; 3)	16.33 \pm 1.7 (14 - 18; 3)	39.24 \pm 4.72 (25.5 - 53.6; 49)	63.93 \pm 25.26 (27.8 - 136.7; 46)	4.74 (3)	3.1 \pm 0.07 (3.01 - 3.19; 3)	5.08 \pm 0.07 (4.99 - 5.17; 3)	1.98 \pm 0.07 (1.89 - 2.07; 3)
69	2	1.85 \pm 0.52 (0.84 - 2.62; 7)	17.14 \pm 4.12 (9 - 23; 7)	45.87 \pm 5.4 (33.7 - 68; 120)	65.99 \pm 28.68 (23.1 - 160.9; 113)	5.08 (7)	3.26 \pm 0.24 (3.1 - 3.79; 7)	5.18 \pm 0.03 (5.17 - 5.25; 7)	1.92 \pm 0.21 (1.46 - 2.07; 7)
Total	1	1.62 \pm 0.46 (0.96 - 2.62; 15)	19.73 \pm 6.54 (11 - 33; 15)	34.42 \pm 8.76 (12.2 - 57.2; 295)	49.85 \pm 23.79 (8.4 - 136.7; 280)	4.67 \pm 0.16 (4.48 - 4.99; 15)	3.78 \pm 0.46 (3.01 - 4.39; 15)	5.01 \pm 0.12 (4.65 - 5.51; 15)	1.23 \pm 0.56 (0.6 - 2.15; 15)
Total	2	1.7 \pm 0.51 (0.74 - 3.03; 63)	18.21 \pm 4.65 (8 - 29; 63)	44.73 \pm 8.15 (12.6 - 68.6; 1145)	50.67 \pm 31.9 (7.4 - 244.7; 1082)	4.67 \pm 0.3 (3.79 - 5.08; 63)	4.17 \pm 0.38 (3.1 - 4.56; 63)	5.11 \pm 0.25 (4.48 - 5.43; 63)	0.94 \pm 0.39 (0.52 - 2.07; 63)

Table S5. Visual signalling emission by each male of *Hylodes phyllodes*. On the locality column, B represents individuals from Bertioiga and P represents individuals from Picinguaba, Ubatuba. On the two limbs lifting column: lifting of one arm and one leg concomitantly (a); lifting of both legs concomitantly b); lifting of both legs alternatively (c). On the throat display column: inflating and deflating both vocal sacs without emitting audible sound (a); call emission using only one of the vocal sacs (b); keeping both vocal sacs inflated after the end of the call (c). An asterisk indicates individuals that were observed open their mouth after eating, probably related to the swallowing process. These cases were not included on visual displays frequencies tables in the manuscript, but they are indicated here.

Male ID	Site	Total visual signalling	Limbs displays									Postural displays					Mouth and throat displays	
			Arm lifting	Leg lifting	Two limbs lifting	Hand shaking	Foot shaking	Foot flagging	Arm waving	Toe flagging	Two-legged kicking	Truncated walking	Upright posture	Body lowering	Body raising	Head down	Mouth opening	Throat display
1	B	25	12	11	-	-	-	-	-	-	2	-	-	-	-	-	-	-
2	B	39	28	9	-	-	-	2	-	-	-	-	-	-	-	-	-	-
3	B	31	7	20	-	-	-	-	-	-	-	-	-	-	-	-	-	4(a)
4	B	44	11	-	-	-	-	-	-	17	1	-	1	-	-	-	14	-
5	B	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	B	72	64	6	-	-	-	-	-	-	2	-	-	-	-	-	-	-
7	B	74	3	6	-	-	-	1	-	61	1	-	1	-	-	-	1	-
8	B	32	21	7	-	-	-	-	-	-	-	-	3	-	-	-	-	1(a)
9	B	14	10	3	-	-	-	-	-	-	-	1	-	-	-	-	-	-
10	B	53	42	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	B	6	2	1	-	-	-	-	-	-	-	-	1	-	-	-	-	2(b)
12	B	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	B	13	6	3	-	-	-	-	4	-	-	-	-	-	-	-	-	-
14	B	28	13	13	-	-	-	-	-	-	1	-	-	-	-	-	-	1(a)
15	B	2	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	B	49	12	23	11(c)	-	-	3	-	-	-	-	-	-	-	-	-	-
17	B	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	B	15	7	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	B	5	4	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	B	2	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	B	10	9	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	B	3	1	-	2(c)	-	-	-	-	-	-	-	-	-	-	-	-	-
23	B	12	5	1	2(c)	-	-	-	-	-	-	-	1	-	1	-	9*	2(a)
24	B	4	2	-	-	-	-	-	-	-	-	-	-	-	-	-	1*	2(a/c)
25	B	3	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	B	13	11	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1(a)
27	B	1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-

67	P	3	-	-	-	-	-	1	-	-	-	-	1	-	1	-	-	-
68	P	9	7	1	-	-	-	-	-	-	1	-	-	-	-	-	-	-
69	P	10	2	3	-	-	-	-	-	-	-	-	-	-	-	-	26*	5(b)
70	P	3	1	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-
71	P	23	2	1	-	-	-	8	-	6	-	6	-	-	-	-	-	-
72	P	26	3	2	1(a)	-	-	11	-	9	-	-	-	-	-	-	-	-
73	P	11	8	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
74	P	79	44	32	2(a)	-	1	-	-	-	-	-	-	-	-	-	-	-
75	P	25	4	1	-	-	-	8	-	12	-	-	-	-	-	-	-	-
76	P	11	2	2	-	-	-	4	-	3	-	-	-	-	-	-	-	-
77	P	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
78	P	47	5	-	-	-	-	2	-	39	-	-	-	-	-	-	-	1(a)

Video S1. Visual displays which compose the behavioural repertoire of *Hylodes phyllodes*: arm lifting (00:05; ZUEC-VID 851); leg lifting (00:25; ZUEC-VID 891); two limbs lifting (00:38; ZUEC-VID 825 and 840); arm waving (01:17; ZUEC-VID 825); hand shaking (01:29; ZUEC-VID 875); foot shaking (01:47; ZUEC-VID 875); foot flagging (02:02; ZUEC-VID 865); toe flagging (02:09; ZUEC-VID 865); two-legged kicking (02:33; ZUEC-VID 844); upright posture (02:56; ZUEC-VID 825); truncated walking (03:14; ZUEC-VID 888); body raising (03:35; ZUEC-VID 849); body lowering (03:47; ZUEC-VID 850); head down (04:00; ZUEC-VID 850); mouth opening (04:13; ZUEC-VID 886); and throat display (04:21; ZUEC-VID 825, 849 and 886).

SECOND SECTION.

Body color traits as visual cues in a multimodal context

Content:

Chapter III. Augusto-Alves, G., Höbel, G. & Toledo, L. F. Geographic variation in acoustic and visual cues and their potential to signal body condition in the Brazilian treefrog, *Boana albomarginata*.

Chapter IV. Augusto-Alves, G., Feagles, O. S., Toledo, L. F. & Höbel, G. Visual cues do not function in a multimodal signaling context for mate attraction in Eastern Gray Treefrogs.

CHAPTER III

GEOGRAPHIC VARIATION IN ACOUSTIC AND VISUAL CUES AND THEIR POTENTIAL TO SIGNAL BODY CONDITION IN THE BRAZILIAN TREEFROG, *Boana albomarginata*

**Variação geográfica nos sinais acústicos e visuais e seus potenciais para sinalizar a
condição corporal em *Boana albomarginata*.**

Guilherme Augusto-Alves, Gerlinde Höbel & Luís Felipe Toledo

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Geographic variation in acoustic and visual cues and their potential to signal body condition in the Brazilian treefrog, *Boana albomarginata*

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Abstract

Anuran communication is largely based on acoustic signals, but different sensory modes are also widespread, including visual communication using body color traits as a way of signaling. The Brazilian treefrog, *Boana albomarginata*, has a complex behavioral repertoire presenting several call types and performing gestures as visual signals. This species has a greenish body color with orange patches on the flanks and thighs, patches that become visible when males are in calling posture or performing visual signals, suggesting that they might use the color of these patches as visual cues. We sampled males from seven populations, using call recordings and photographs to access individual call and color traits. We demonstrate that there is variation in color and call properties across populations. Additionally, we observe variation in the relationship between color traits and call properties in different populations, revealing that only two populations exhibit a significant correlation between color and call traits. Further, while call properties and color traits were not related with individual body size, they were associated with body condition. The results indicate a universal pattern across populations for call properties, wherein males in better condition consistently displayed lower-pitched calls, longer call durations, and shorter intervals between calls. Regarding color traits, males in better condition in four out of the seven evaluated populations exhibited larger orange patch sizes, lower orange hue values, and higher hue contrasts. Although we observed some level of relation among color, call, and body traits, there is not a universal pattern across all populations.

Keywords: animal body color; animal communication; anurans; bioacoustics; multimodal communication; visual signaling.

Introduction

Body color traits can serve a range of functions, and thus evolve under both natural and sexual selection (Bell and Zamudio 2012; Dunn et al. 2015; Heinen-Kay et al. 2015). Body color can increase an individual's survival and reproductive success by providing a tool for protection, thermoregulation, and social behavior (Cuthill et al. 2017; Endler and Mappes 2017). Coloration used for visual signaling in social interactions is well documented across taxa [e.g., arthropods (Robertson and Monteiro 2005), fishes (Amundsen and Forsgren 2001), amphibians (Vásquez and Pfennig 2007), birds (Hill 1991), and mammals (Waite et al. 2003)]. Moreover, color traits for intraspecific communication are used in a range of different social contexts, among them: male-male agonistic interaction (Bruinje et al. 2019), mate attraction (Dubuc et al. 2014), and parent-offspring communication (Avilés and Parejo 2013).

Regardless of the sensory modality (i.e., visual, auditory, etc.), communication often involves more than one behavioral component. In multicomponent communication/signals, only aspects of the same sensory modality are involved (Candolin 2003; Hebets and Papaj 2005; Partan and Marler 2005). For example, in a visual signal distinct body portions with distinct coloration traits may provide information for receivers (Jones et al. 2017; Ferrer et al. 2021), or in an acoustic signal, receivers may use temporal and spectral features to assess emitter traits (Vignal and Kelley 2007). There are differences on the propagation and perception of acoustic and visual signals, and sending the information by different sensory modes may be beneficial because it increases the probability of accurate information transfer. Acoustic signals can be transmitted over longer distances (Forrest 1994; Velásquez et al. 2018), and function in a variety of contexts ranging from identification of conspecifics (Höbel and Gerhardt 2003; Köhler et al. 2017; Freitas and Toledo 2021), to assessment of the emitter's traits and position in short- and long-range interactions (Toledo et al. 2015; Köhler et al. 2017). Visual signals on the other hand are most effective at shorter distances, and function mainly for close-range assessment of the precise location and condition of the emitter (Giasson and Haddad 2007; Robertson and Greene 2017). Likewise, each communication mode has different challenges. Acoustic signals can be influenced by abiotic noise, such as wind, rain, flowing water (Lengagne and Slater 2002; Boeckle et al. 2009; Luther and Gentry 2013), anthropogenic noise (Parris et al. 2009), and intra- or interspecific biotic noise (i.e., frog choruses; Gerhardt and Klump 1988; Bee and Micheyl 2008). Similarly, detection of visual signals may be difficult in visually complex environments, such as windblown vegetation in the background or under insufficient illumination (Fleishman 1992; Ord et al. 2007; Ord and Stamps 2008).

Acoustic signaling is the predominant mode of communication used to mediate intraspecific interactions in anurans (Arch and Narins 2009; Chen and Wiens 2020), but they communicate using several other sensory modes [chemical (Poth et al. 2012), seismic/vibrational (Caldwell et al. 2010), visual (Augusto-Alves et al. 2018), and tactile signals (Augusto-Alves and Toledo 2022)]. Because light intensity contributes to the transmission of the visual signal, visual communication was linked mostly with diurnal species. However, frogs have well-adapted vision under low light intensity (Gomez et al. 2009, 2010; Kelber et al. 2017; Yovanovich et al. 2017; Robertson et al. 2022), which allows them to communicate by visual signals such as gestures, postures, or secondary visual cues [such as vocal sac color and movements during call activity (Rosenthal et al. 2004; Starnberger et al. 2014)]. Frogs also have the capacity of color discrimination (Gomez et al. 2010; Kelber et al. 2017; Yovanovich et al. 2017; Robertson et al. 2022), permitting communication by body color traits (Vásquez and Pfennig 2007).

The behavioral components of a species, such as calls and visual cues, can exhibit geographic variability. This is often observed in anuran calls (Smith and Hunter 2005; Amezcuita et al. 2009; Lee et al. 2016; Tessarolo et al. 2016), but also in the phenotype of body color and patterns (Amezcuita et al. 2009; Robertson et al. 2009). Numerous hypotheses have been proposed to account for the geographic variation. These hypotheses encompass subjects such as geological history, sexual selection, genetic isolation, variations in environments, morphology, as well as countless others (Pröhl et al. 2007; Amezcuita et al. 2009; Jang et al. 2011). Species with extensive distribution may experience different selective pressures on their call and color patterns, and combined with geographic isolation, might manifest diverse patterns across populations (Jang et al. 2011; Röhr et al. 2020).

Boana albomarginata is endemic to the Atlantic rainforest, widely distributed on the Brazilian coastal region, and found on the mainland of the state of Paraíba (northernmost limit) to the state of Santa Catarina (southernmost limit) (Carnaval et al. 2009; Frost 2023), as well as some coastal islands (Bittencourt-Silva and Silva 2013; Rebouças et al. 2020). The species has a greenish color pattern almost in the entire body, with exceptions of the flanks and thighs, which have orange patches (Fig. 1). They have a complex behavioral repertoire, composed of different call types – ‘advertisement call’, ‘distress call’, and ‘aggressive call’ (Giasson and Haddad 2006; Toledo and Haddad 2009; Furtado and Nomura 2014) – and visual displays, including ‘body raising’ and ‘limb lifting’ (Hartmann et al. 2005; Giasson and Haddad 2006; Furtado and Nomura 2014) which expose the orange parts of the body that are generally hidden when the frogs are not active. These visual displays can be observed during close

interactions between males or in the presence of nearby calling males, and they are linked with male-male agonistic interactions (Hartmann et al. 2005; Giasson and Haddad 2006).

Bright color patches on the thighs and/or flanks of cryptic frogs, which are only temporarily exposed, are frequently associated with defensive behaviors, acting as visual cues to alert predators (Williams et al. 2000; Toledo and Haddad 2009; Loeffler-Henry et al. 2023). Two observations suggest that *B. albomarginata*'s orange patches might also constitute a visual signal for conspecifics: first, there is sexual dimorphism, with males showing more pronounced coloration (Appendix I). Second, visual displays that expose the orange patches are common during short-distance interactions with conspecifics (Hartmann et al. 2005; Giasson and Haddad 2006; Furtado and Nomura 2014).

Here, we conducted a multi-population study examining the call and color traits of the Brazilian treefrog *B. albomarginata* to elucidate the potential presence of geographic variation in these traits across populations. Furthermore, we predicted that the orange patches on the thigh are correlated with call properties and body measurements. In this way, both call and color might serve as honest signals of male quality in this treefrog.

Methods

Study site and data sampling

In total, we sampled 170 *Boana albomarginata* males from seven sites in the Brazilian states of São Paulo (SP) and Rio de Janeiro (RJ), including mainland sites and islands (Fig. 2): Angra dos Reis (RJ, 22°56'12"S, 44°24'21"W; 29 males); Bertioga (SP, 23°48'55"S, 46°02'38"W; 30 males); Gipóia Island (RJ, 23°02'16"S, 44°21'19"W; 10 males); Itacuruçá Island (RJ, 22°56'20"S, 43°53'51"W; 13 males); Marambaia Island (RJ, 23°03'42"S, 43°59'01"W; 27 males); Picinguaba (SP, 23°21'35"S, 44°50'59"W; 30 males); and Seropédica (RJ, 22°45'05"S, 43°40'56"W; 31 males). We conducted one field expedition for each locality, with 3-4 days dedicated to sampling. Data sampling occurred from November 2018 to March 2019.



Fig. 1 Male of *Boana albomarginata*. Note the greenish color pattern almost in the entire body, with exceptions of the flanks and internal portion of the thighs. Photography by Luís Felipe Toledo.

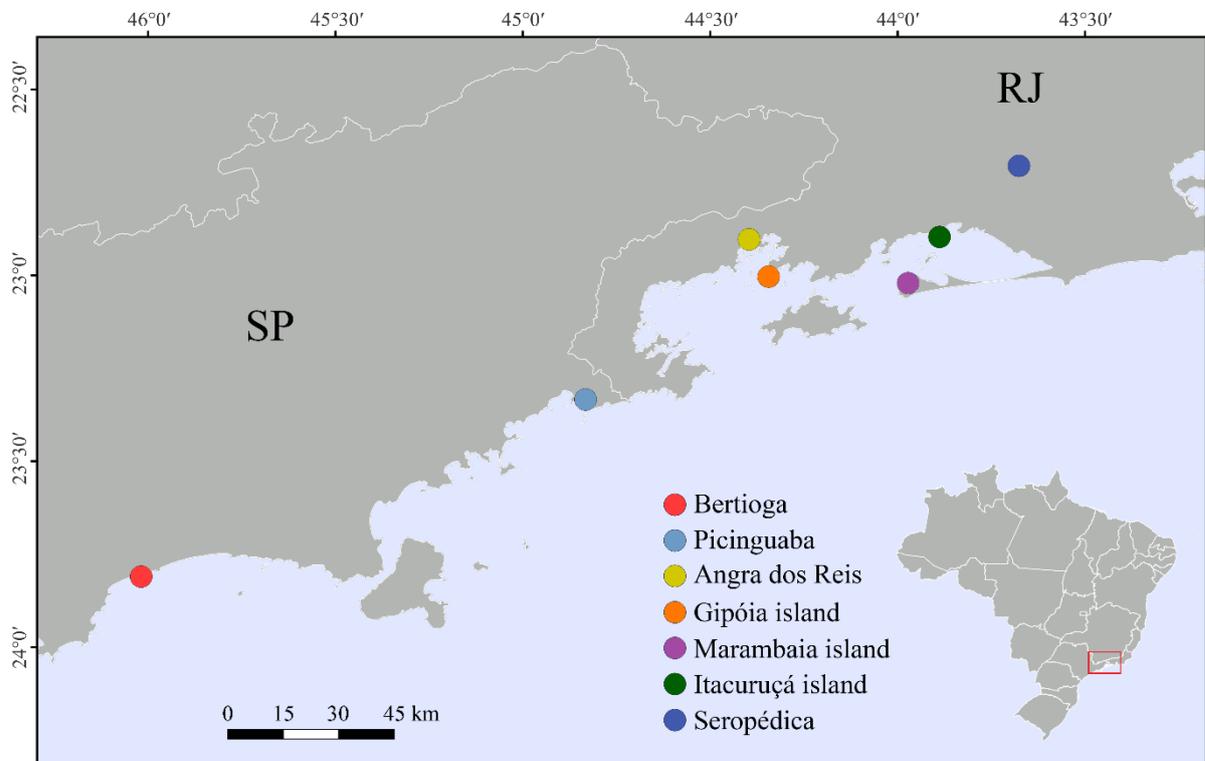


Fig. 2 Schematic map showing the distribution of the sampled populations in the present study. Figure legend was organized geographically from the West to East.

We only sampled sexually mature males that were actively participating in a breeding chorus. Males were located through their call activity. First, we recorded the advisement call of each male for approximately three minutes (ZOOM H4n PRO and a Marantz PMD 661 MKII digital recorders with their built-in microphones). The recordings were made approximately 1 m away from the calling male, horizontally aligned with the individual, and under red lighting. Subsequently, we collected the individuals for measuring their snout-vent length (SVL; caliper 0.1 mm of precision), and weight (field scale 0.1 g of precision). These measurements were used to calculate the scaled mass index (SMI; Peig and Green 2009) as an indicator of body condition. Lastly, frogs were photographed (Nikon D3200) dorsally, exposing the orange patches in the dorsal portion of the thighs. All photographs were taken using a color standard grey 18 % reflectance card in the background, for posterior light correction, and saved as a RAW file format. After that, the individual was toe-clipped and released at the same spot where it was captured for avoiding pseudoreplication. The toes clippings were preserved in 100% ethanol and deposited in the TLFT tissue collection at Universidade Estadual de Campinas (Unicamp; TLFT 4501–609; 4611–8; 4620–4; 4626–73). Air temperature was measured during sampling with a HOBO data logger (Onset Computer Corporation). Audio files were deposited at Fonoteca Neotropical Jacques Vielliard (FNJV), Museu de Diversidade Biológica (MDBio), Unicamp (FNJV 43831–939; 43941–53; 43955–83; 43985–4003), following previous recommendations (Dena et al. 2020). This study was approved by the Instituto Chico Mendes de Conservação da Biodiversidade (SISBio #63697-2), Comissão Técnico-Científica, Instituto Florestal (COTEC #483/2018), ethics committee of Unicamp (CEUA #4983/2018), and was registered at the Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado (SISGen #AE9A0E0).

Color analyses

Boana albomarginata individuals have orange patches in the femoral regions, contrasting with greenish body color (Fig. 3a). We characterized hue and relative size of the orange patches in relationship to the entire thigh. All images were imported into the ImageJ software (Schneider et al. 2012) with the plugin MICA Toolbox 2.2.2 (Troscianko and Stevens 2015). For each photograph, we generated a Multispectral Image calibrated (.mspec) based on the standard grey card reference (Troscianko and Stevens 2015). Then, we selected two 7^2 pixels as our regions of interests (ROIs), being one in the middle of the orange patch, and another out of the patch, in the leg green portion (Fig. 3b). From the ROIs we accessed reflectance in the red-green-blue (RGB) color system, on the scale of 0-255 for each channel, and RGB were used

to obtain hue values. We calculated the hue contrast (ΔH) of the orange patch (H_o) related to the thigh green portion (H_g) as: $\Delta H = (H_o - H_g) / (H_o + H_g)$ [based on a ‘brightness contrast’ calculation (Fleishman and Persons 2001)]. ImageJ was also used to determine the relative size of the orange patch. First, carefully, we selected all the thigh contour and measured the number of pixels of the selection, subsequently, we did the same procedure with the patch (Fig. 3c). To calculate the relative size of the orange patch, we divided the patch area by the total thigh area and multiplied by 100.

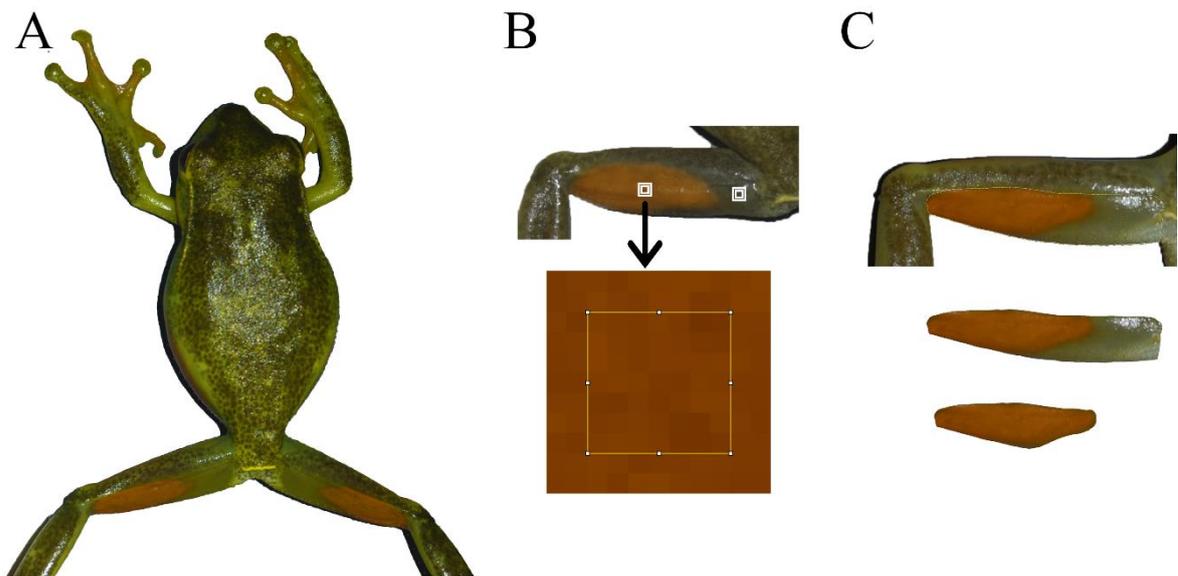


Fig. 3 A) *Boana albomarginata* presents orange patches in the femoral regions that contrasts with greenish body color. B) for hue analysis, we selected two regions of interests (ROIs - white squares: one in the middle of the orange patch; and another out of the patch, in the leg green portion). Each selected ROI had 7^2 pixels, of which, we extracted red-green-blue reflectance information. C) demonstration of how we calculated the relative size between thigh and orange patch. We selected the thigh portion that contains the orange patch, after, we selected the orange patch (relative size was calculated by dividing the total thigh area by the patch area and multiplied by 100).

Audio analyses

Prior to audio analyses, all recordings were standardized at a sampling rate of 44.1 kHz, and resolution of 16 bits, and normalized (peak -1 dB), using Adobe Audition 2020. We analyzed these calls in Raven 1.6.1 PRO (Center for Conservation Bioacoustics 2019), with the following configuration: 60 % contrast, 60 % brightness, 512 DFT, window type Hann. Individuals emit advertisement calls as simple units (single calls) or in call series of two or more calls rhythmically emitted (Giasson and Haddad 2006; Fig. S1). In order to standardize we only included single calls in this study [call-centered approach as indicated (Köhler et al. 2017)]. We analyzed on average (\pm SD) 8.26 ± 2.4 (2–10) calls per individual (Table S1).

During the acoustic analyses, we noticed that the call harmonic which contains the dominant frequency (frequency with the highest sound energy) changes according with the analyzed call; this happens among different individuals, but also among different calls from same male. Thus, for spectral analysis we selected separately the two harmonics, instead of one selection for the entire call (Fig. S1 shows details of the selection limits). Calls were characterized by the following properties: call duration; pulses per call; intercall interval; call period (the call and the silence period subsequently); first harmonic dominant frequency (peak frequency function in Raven); first harmonic minimum frequency (frequency 5% function in Raven); first harmonic maximum frequency (frequency 95% function in Raven); second harmonic dominant frequency; second harmonic minimum frequency; second harmonic maximum frequency; dB difference (Second harmonic dB FS – first harmonic dB FS; delta power dB FS function in Raven). Several temporal properties were temperature-dependent, and therefore, values for these traits were temperature corrected to 25.4 °C (mean temperature of our sample; Table S2 for temperature information for each population) using the results of a linear regression of the call variable on temperature before further statistical analyses. Using the same method, we corrected the spectral properties of the call based on the body size of the individuals.

Statistical analyses

Because traits constituting a signal (color or call, respectively) are frequently correlated, we conducted a Principal Component Analysis (PCA) to generate smaller numbers of uncorrelated variables. For color, we conducted a PCA that included four color traits (orange ROI hue, green ROI hue, contrast hue, and patch relative size). For advertisement calls, we conducted another PCA that included 11 call traits (call duration, pulses per call, intercall interval, call period, first harmonic dominant frequency, first harmonic minimum frequency, first harmonic maximum frequency, second harmonic dominant frequency, second harmonic minimum frequency, second harmonic maximum frequency, and dB difference). We used the resulting PC scores in subsequent analyses. All analyses were run using JMP PRO v. 11.1.0 (SAS Institute Inc., 2015). Figures were built in R version 3.6.3 (R Core Team 2019).

Color and call geographic variation – To test for geographic variations in color and call traits between *Boana albomarginata* populations, we fitted analyses of variance (ANOVA) using the products from both PCAs (color and call, respectively). Then, we ran *post hoc* Tukey tests.

Relationship between patch color and call traits – We used standard least squares models to assess the relationship between the orange color patch and call traits; we focused on

the orange leg patch because we did not expect the green based color of the frogs to act as a potential visual signal. We fitted the models with the data of the principal component summarizing variation in the orange leg patch (PC1_C) as response. As predictor we included the principal component summarizing variation in calls [either spectral (PC1_A) or temporal (PC2_A)], population, and call*population (either PC1_A*population or PC2_A*population).

Relationship of color patch and call traits with male body measures – We used standard least squares models to assess the relationship of putative signal traits (orange color patch and call traits, respectively) with body length (SVL) and body condition (SMI). We fitted different models using orange patch traits (PC1_C from color PCA), spectral (PC1_A from advertisement call PCA), and temporal (PC2_A from call PCA) properties as response variables, and SVL, SMI, population, SVL*population, SMI*population as predictors, in all models. We included both SVL and SMI in the same model, since they are uncorrelated descriptors ($|r| < 0.7$) of male morphology and energy reserves.

Results

The PCA of color traits returned two principal components with eigenvalues greater than 1 (PC1_C = 2.17, PC2_C = 1.21). Together they account for 84.5 % of the variation. PC1_C loaded primarily with relative patch size, orange hue and contrast hue. PC2_C loaded primarily with green hue (Table 1).

Table 1 Factor loading on the first two principal components, which together are responsible for 84.5% of variation in *Boana albomarginata* color traits.

factor	PC1 _C	PC2 _C
patch relative size	-0.4674	-0.2831
hue orange	0.6286	0.1946
hue green	-0.0638	0.8826
hue contrast	0.6183	-0.3208

The PCA of advertisement call traits returned two principal components with eigenvalues greater than 1 (PC1_A = 5.37, PC2_A = 2.43). Together they account for 70.7 % of the variation. PC1_A loaded primarily with spectral properties (first harmonic dominant frequency, first harmonic minimum frequency, first harmonic maximum frequency, second harmonic dominant frequency, second harmonic minimum frequency, and second harmonic maximum frequency). PC2_A loaded primarily with temporal and power properties (call duration; pulses per call; intercall interval; call period; dB difference) (Table 2).

Table 2 Factor loading on the first two principal components, which together are responsible for 70.7% of variation in *Boana albomarginata* advertisement call properties.

factor	PC1_A	PC2_A
call duration	0.10942	-0.401
pulses per call	0.14546	-0.3589
call period	-0.0888	0.50259
intercall interval	-0.0885	0.5043
dB difference	0.17796	-0.3068
first harmonic minimum frequency	0.36941	0.19826
first harmonic maximum frequency	0.39272	0.1298
first harmonic dominant frequency	0.38439	0.18639
second harmonic minimum frequency	0.39715	-0.0598
second harmonic maximum frequency	0.39383	0.10036
second harmonic dominant frequency	0.41011	0.06912

Geographic variation in color and advertisement call properties

In total, we evaluated 170 *Boana albomarginata* males from seven sites (see Table S3 for mean values for color, call and body size/condition traits for each population). We document significant geographic variation in all color and call traits: Orange patch size and color (PC1_C: $F_{(6,147)} = 9.64$, $P < 0.001$; Fig. 4a); green body portion (PC2_C: $F_{(6,147)} = 7.22$, $P < 0.001$; Fig. 4b); spectral advertisement call properties (PC1_A: $F_{(6,162)} = 34.87$, $P < 0.001$; Fig. 4c), and temporal advertisement call properties (PC2_A: $F_{(6,162)} = 4.91$, $P < 0.001$; Fig. 4d).

Relationship between orange patch color and call traits

At the species level, we detected an association between the orange patch traits and temporal call properties (Table 3; bottom) but not spectral call properties (Table 3; top). There was also substantial geographic variation in advertisement call properties, as well as in the relationship between the orange color patch and advertisement call properties, indicated by significant call (PC1_A or PC2_A)*population interaction terms (Table 3).

The slopes of the regressions between spectral call properties and orange color patch varied between - 0.40 and + 0.76 for different populations (Fig. 5a, Table S4). A population-based post-hoc analysis showed that the relationship was statistically significant for two populations: Pinguaba ($r^2 = 0.31$, $P = 0.002$) and Seropédica ($r^2 = 0.22$, $P = 0.007$); both showed positive slopes indicating that males with lower-frequency calls had more pronounced orange patches (larger relative size, higher contrast hue value, and lower orange hue value). On the other hand, no relationship between orange patches and spectral properties was observed for the other five populations (All $P > 0.05$).

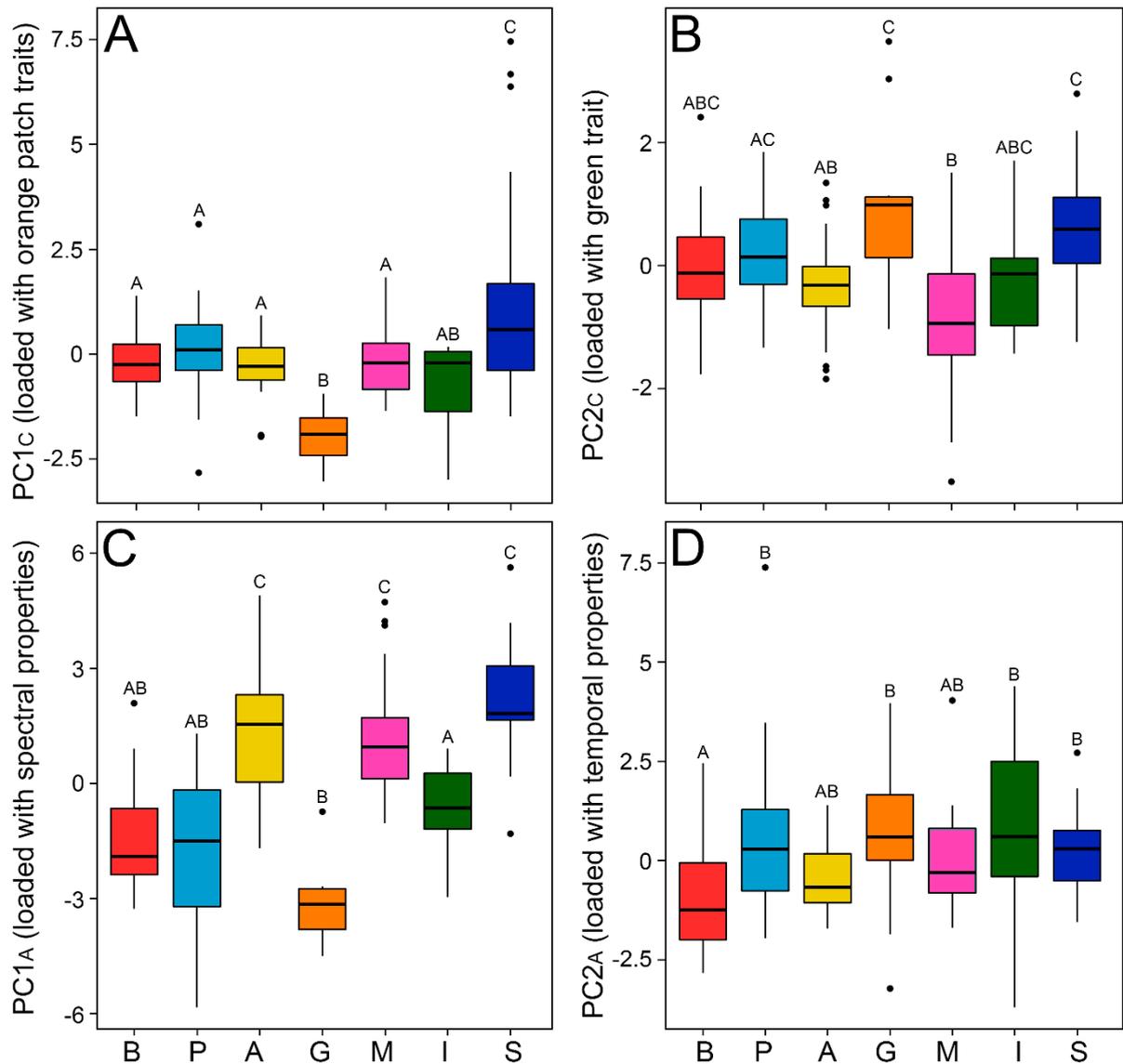


Fig. 4 Geographic variation in color and call traits. A) orange patch traits (PC1_C of the PCA set with color traits). B) green body portion trait (PC2_C of the PCA set with color traits). C) spectral properties (PC1_A of the PCA set with call proprieties). D) temporal properties (PC2_A of the PCA set with call proprieties). Boxplots indicate median, upper, and lower quartiles, and upper and lower whiskers. Boxplot was ordered geographically from the westmost to eastmost population. Abbreviation of populations: B = Bertioaga; P = Pinguaba; A = Angra dos Reis; G = Gipóia Island; M = Marambaia Island; I = Itacuruçá Island; S = Seropédica.

The slopes of the regressions between temporal call properties and orange color patch varied between - 0.16 and + 0.96, with most populations showing relatively flat slopes (Fig. 5b, Table S4). This model returned a significant call term (Table 3), suggesting a general pattern of association between the temporal call properties of the advertisement call and the orange patch. However, visual inspection of the associated graph (Fig. 5b) suggests that this result is likely due to one population with very steep slope (other populations showed relatively flat slopes). In fact, the population-based post-hoc analysis showed that the regression was

statistically significant only for one population: Seropédica ($r^2 = 0.17$, $P = 0.02$). Here, males with longer duration/more pulses per call, and shorter intervals between calls had more pronounced orange patches (larger relative size, higher contrast hue value, and lower orange hue value). For the other six populations, we did not find relationship between orange patches and temporal properties (All $P > 0.05$).

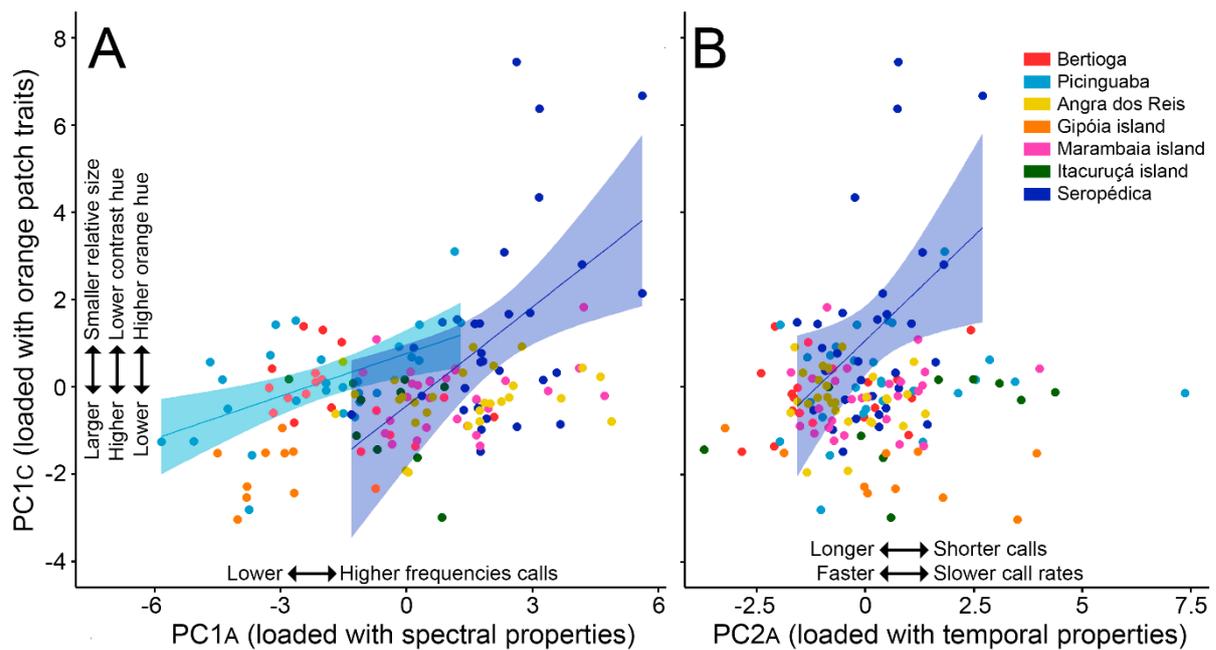


Fig. 5 Relationship between color and call traits. A) In two populations spectral call traits and orange color traits were related; males with more pronounced color patches (lower PC1_C scores) had lower frequency calls (lower PC1_A scores). B) In one population temporal call traits and orange color traits were related; males with more pronounced color patches (lower PC1_C scores) had longer calls/more pulses per call, and shorter intervals between calls (lower PC2_A scores). In Seropédica we observed some individuals with exceptionally high color PC scores. To examine whether they biased our results, we ran the analyses with (Table 3) and without (Table S5) them. Results were very similar, and accordingly, we present data from the entire dataset. Figure legend was organized geographically from the westmost to eastmost population.

Relationship of color patch and call traits with male body measures

Both spectral and temporal call properties were associated with body condition (SMI; Table 4): males with better body condition had lower frequency calls (Fig. 6a), longer duration/more pulses per call, and shorter intervals between calls (Fig. 6b). There was geographic variation in call properties, significant effect of population. By contrast, body length (SVL) was not associated with advertisement call traits (Table 4).

Table 3 Results of the standard least squares models showing the relation of orange patch traits (loaded in the PC1C of the PCA with color traits - patch relative size, orange hue, and contrast hue) with acoustic properties. PC1A of the PCA with call traits was loaded by spectral properties (first harmonic dominant frequency, first harmonic minimum frequency, first harmonic maximum frequency, second harmonic dominant frequency, second harmonic minimum frequency, and second harmonic maximum frequency) and PC2A with temporal properties (call duration; pulses per call; intercall interval; call period, and dB difference). Bold font indicates significant *P*-values.

Spectral call traits (PC1 _A)				
factor	DF	Sum of squares	F	<i>P</i>
PC1 _C	1,6	1.779	1.275	0.261
population	1,6	21.811	2.605	0.02
PC1 _C *population	1,6	33.052	3.948	0.001
Temporal call traits (PC2 _A)				
factor	DF	Sum of squares	F	<i>P</i>
PC1 _C	1,6	8.131	5.284	0.023
population	1,6	82.317	8.916	<0.001
PC1 _C *population	1,6	24.744	2.68	0.017

Table 4 Results of the standard least squares models showing the relation of signals with males' traits. Abbreviations: SVL – Snout-Vent Length; SMI – Scaled Mass Index. Bold font indicates significant *P*-values.

Call spectral properties (PC1 _A)				
factor	DF	Sum of squares	F	<i>P</i>
SVL	1,6	1.584	1.114	0.293
SMI	1,6	32.913	23.156	<0.001
population	1,6	103.133	12.094	<0.001
SVL*population	1,6	12.902	1.513	0.178
SMI*population	1,6	12.716	1.491	0.185
Call temporal properties (PC2 _A)				
factor	DF	Sum of squares	F	<i>P</i>
SVL	1,6	3.584	1.779	0.184
SMI	1,6	19.233	9.548	0.002
population	1,6	63.954	5.291	<0.001
SVL*population	1,6	24.813	2.053	0.062
SMI*population	1,6	7.324	0.606	0.725
Orange patch color traits (PC1 _C)				
factor	DF	Sum of squares	F	<i>P</i>
SVL	1,6	0.548	0.483	0.488
SMI	1,6	10.694	9.422	0.003
population	1,6	13.75	2.019	0.067
SVL*population	1,6	5.963	0.876	0.515
SMI*population	1,6	25.024	3.675	0.002

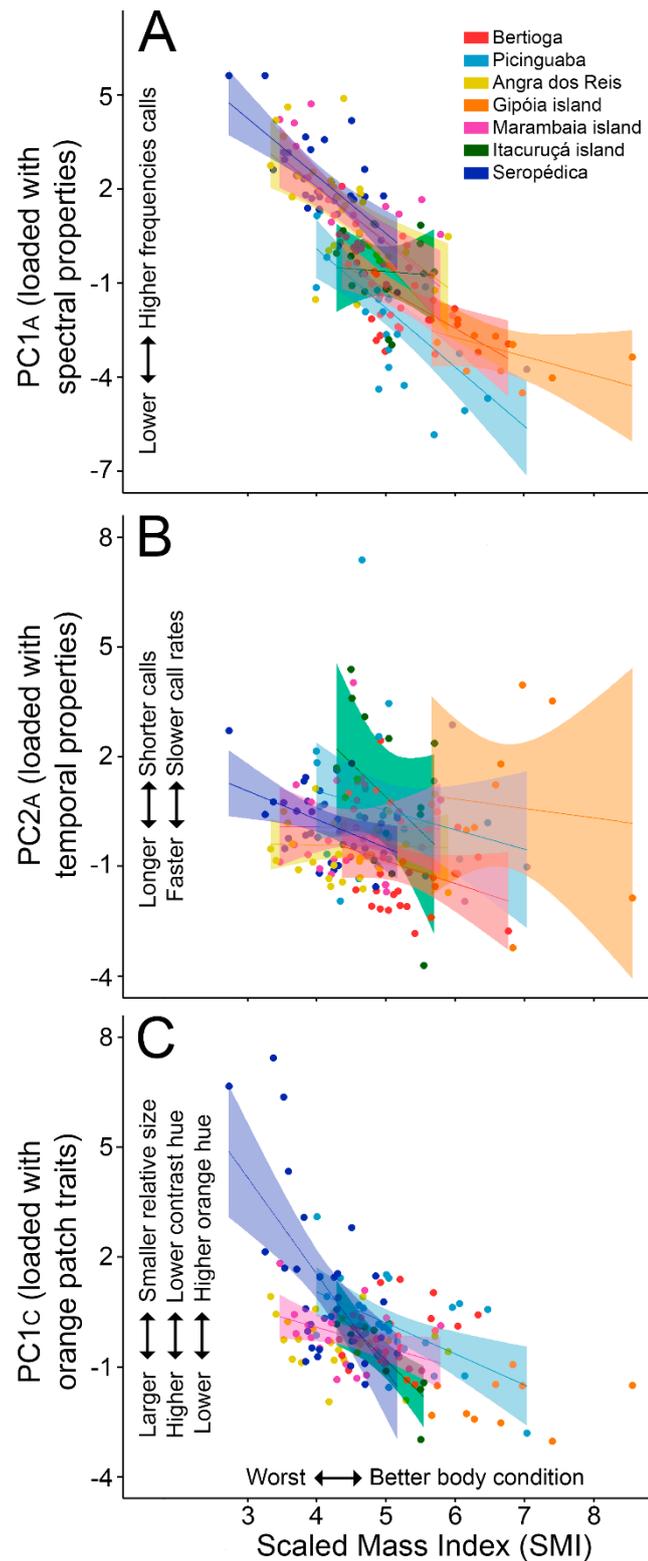


Fig. 6 Relationship between color and call traits with body condition (SMI: Scaled Mass Index). A) Males with better body condition (higher SMI) had lower frequency calls (lower PC1_A scores). B) Males with better body condition (higher SMI) had longer and faster call rate (lower PC2_A scores). C) In 4 populations, males in better condition (higher SMI) had more pronounced orange patches (lower PC1_C scores). Trend line and confidence intervals indicate populations with significant relationships. Figure legend was organized geographically from the westmost to eastmost population.

Orange patch size and color was also associated with body condition: males in better condition had more pronounced orange patches (larger relative size, higher contrast hue, and lower orange hue) (Table 4; Fig. 6c). Again, there was geographic variation in how closely the orange patch was associated with body condition (Table S6). The slopes of the regressions between orange color patch and body condition varied between -2.64 and $+0.52$. Most populations showed negative slopes (Fig. 6c; Table S7), and the regression was statistically significant for 4 populations: Itacuruçá island ($r^2 = 0.64$, $P = 0.005$); Marambaia island ($r^2 = 0.17$, $P = 0.034$); Picinguaba ($r^2 = 0.31$, $P = 0.002$); Seropédica ($r^2 = 0.39$, $P < 0.001$); other three populations $P > 0.05$. By contrast, body length (SVL) was not associated with orange patch traits (Table 4).

Discussion

We conducted a multi-population study examining call and color traits of the Brazilian treefrog *Boana albomarginata*. Consistent with the pattern observed in the geographic variation of their advertisement call frequencies (Rebouças et al. 2020), we also document substantial geographic variation in advertisement call temporal properties and body color traits across populations (Fig. 4). Geographic variation in call and color traits is widely reported for anurans (Summers et al. 2003; Heyer and Barrio-Amorós 2009; Zornosa-Torres and Toledo 2019; Augusto-Alves et al. 2020; de Souza et al. 2021). For instance, color pattern polymorphisms were observed in two Central American species, *Dendropsophus ebraccatus* and *Agalychnis callidryas*, demonstrating significant geographic variation across populations throughout the regions of Costa Rica and Panama (Robertson et al. 2009). In another example, *Dendropsophus cruzi* exhibits call variation in spectral and temporal properties across populations, with dominant frequency, call duration, and rate showing a negative correlation with latitude (Tessarolo et al. 2016). Several reasons have been suggested for the inter-population variation, including influence of climate, morphology, geographic and genetic isolation, population density, vegetation and community structure (Pröhl et al. 2007; Rudh et al. 2007; Robertson et al. 2009; Forti et al. 2016; Köhler et al. 2017; Serrano et al. 2020; Fernandes et al. 2021; da Rosa et al. 2023).

The pigment categories of carotenoids and pteridines are responsible for producing yellow-orange-red coloration in vertebrates (Mills and Patterson 2009; Weiss et al. 2012; Umbers et al. 2016; Merklings et al. 2018), including frogs (Suga and Munesada 1988; Umbers et al. 2016; Brenes-Soto et al. 2017). Unlike pteridines, that are synthesized during purine production (Ziegler 2003; Le Guyader et al. 2005), carotenoids have to be acquired from the

diet (Feltl et al. 2005; Umbers et al. 2016). Carotenoid-supplementation studies with captive frogs support this link between diet and color (Brenes-Soto and Dierenfeld 2014; Umbers et al. 2016). For instance, *Pseudophryne corroboree* individuals fed with carotenoid-poor diet still developed the species yellow-black patched pattern, but individuals fed with a diet supplemented with carotenoids presented patches more orange (yellow in the poor dietary), and significant difference in chroma (higher saturation) (Umbers et al. 2016). In this manner, both the observed variation in orange patch among populations (Fig. 4) and the differences in how these patches are associated with body condition (Fig. 6, Table 4) may be outcomes related to dietary quality and availability in the different studied sites.

Acoustic allometry describes how information about body size is conveyed by acoustic signals (Ryan 1988; Fletcher 2004), and the pattern where larger body size is associated with lower-frequency calls is widely present across anuran taxa (Gingras et al. 2013; Tonini et al. 2020; Augusto-Alves et al. 2021). Yet, exceptions from this rule have been documented (Tonini et al. 2020), including some populations of *B. albomarginata* (Rebouças et al. 2020), and a study involving a range of acoustically communicating taxa concluded that most acoustic signals do not appear to have been selected to function as indicators of body size (Rodríguez et al. 2015). Our study corroborates the previously described frequency escape of the acoustic allometry in *B. albomarginata* and extends it to include temporal and orange patch traits which were also not predictors of male size in all populations.

On the other hand, we did find that calls (both spectral and temporal properties) and sometimes also color traits were associated with SMI. Therefore, both color and calls potentially serve as indicators of male body condition for conspecifics. Aggressive interactions between males of *B. albomarginata* involve aggressive calls combined with visual signals (leg kicking, limb lifting and body raising; Giasson and Haddad 2006), some of which increase visibility of their orange color patches. We do not have information about the use of visual signaling/exposure of orange patches during courtship, but the elevated stance of calling males exposes those patches and allows for inspection by mate-searching females. Evaluating the traits of orange patches between unmated and mated males could provide new evidence of the use of color traits in the mate choice process, if the traits between groups differ. However, to examine the relative importance of call and color signals during male competition, courtship, and mate choice in *B. albomarginata*, behavioral experiments will be necessary. Furthermore, based on these behavioral experiments, future studies could address how these different communication modes are related in a multimodal context or if they are completely independent within the behavioral repertoire.

Conclusion

Based on a multi-population survey, we demonstrated that the Brazilian treefrog *Boana albomarginata* exhibits geographic variation in both color and call traits. Additionally, we found that information about body condition may potentially be transmitted through different components within the same communication modality, the spectral and temporal properties of the calls. Furthermore, we observed an association between body color traits and body condition in some populations, indicating that orange patches may also convey information about males' body condition. Behavioral experiments will be necessary to test whether males and/or females attend to variations in orange patch color to mediate different social contexts.

Acknowledgments

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SUPPLEMENTARY MATERIAL

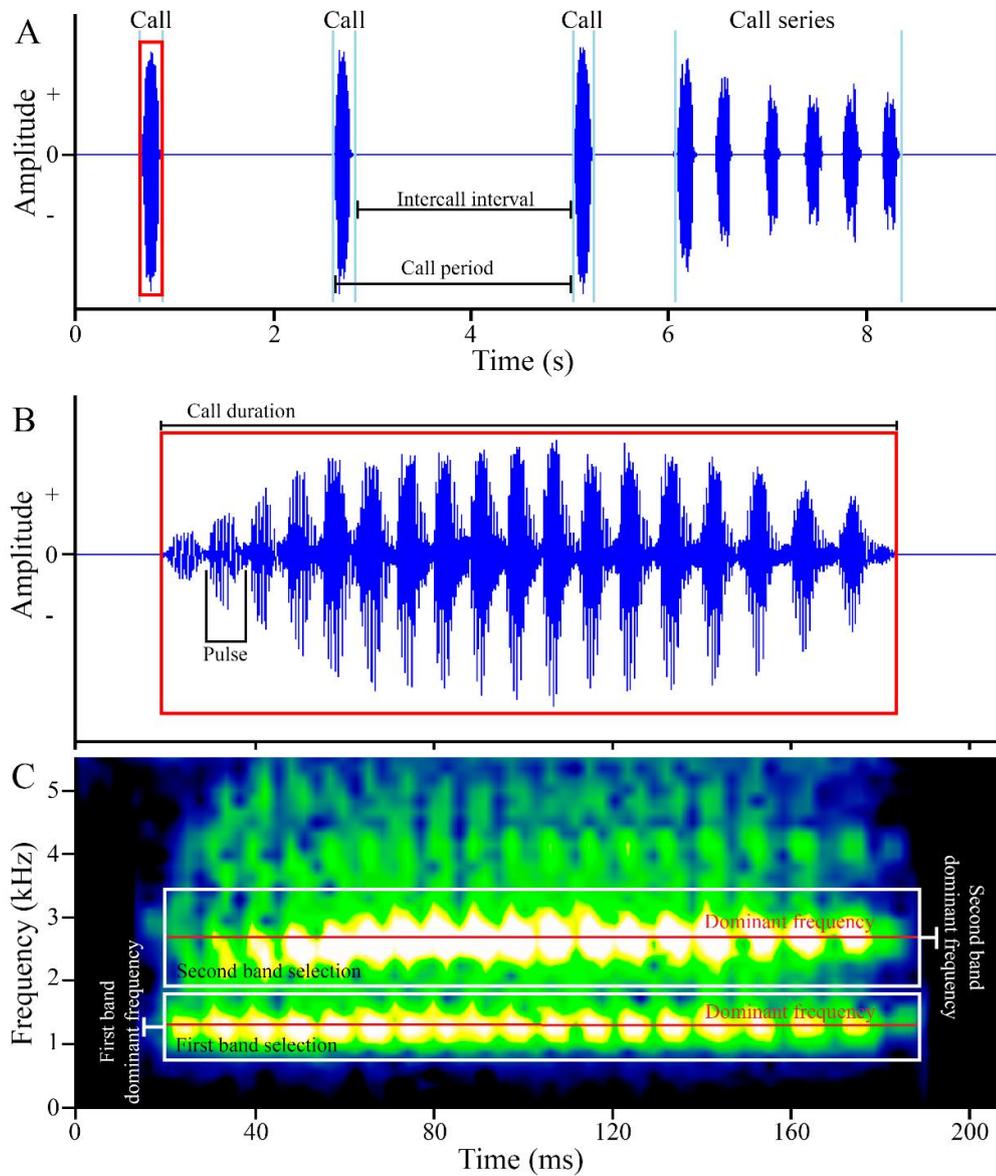


Fig. S1 Demonstration of how we selected each property during the audio analyses. Frequency properties were measured in the spectrogram, while the temporal properties were measured in the waveform. a) *Boana albomarginata* individuals emit advertisement calls as simple units or in call series of two or more calls rhythmically emitted, in other to standard we only included single calls in this study. We measured call period selecting from the beginning of the call to the end of the silence period subsequently; intercall interval represents the measure between two calls. b) we measured call duration selecting from the beginning of the first pulse to the end of the last one. Pulses per call was the number of the pulse peaks in each call (18 pulses in this example). c) The call harmonic that contains the dominant frequency (frequency with the highest sound energy) changes according with the analyzed call (see main text for details). For this reason, we select separately the two harmonics that contain most energy (white boxes), instead of one selection for the entire call. From each selection, using the ‘peak frequency’ function in Raven PRO 1.6.1 (Center for Conservation Bioacoustics 2019), we extracted the dominant frequency automatically.

Appendix I. Color data for *Boana albomarginata* females.

Our study was focused on males' body color, thus we did not sample females. Despite it, we sampled data for two females from two different populations. They presented relative size of the orange patches smaller than males from same population. The female from Bertioga presented orange patch relative size of 24.3 %, while the males from Bertioga presented mean of 46.3 %, ranging from 31.6 to 73.4 %; another female from Itacuruçá Island presented orange patch relative size of 18.18 %, while males from same population presented mean of 50.3 %, ranging from 25.0 to 71.5 %. Although the sample size is limited for females, it might be an indication that there is color sexual dimorphism in *Boana albomarginata*.

Table S1. Catalogue numbers of audio analyzed and spectral and temporal properties of each *Boana albomarginata* male. Audios are deposited at Fonoteca Neotropical Jacques Vielliard (FNJV), Museu de Diversidade Biológica (MDBio), Universidade Estadual de Campinas (Unicamp), Campinas, SP, Brazil. Values are presented as mean \pm standard deviation (range; number of calls).

Male ID	Voucher number	Call duration (s)	Pulses per call	Intercall interval (s)	Call period (s)	Fist band dominant frequency (kHz)	First band minimum frequency (kHz)	First band maximum frequency (kHz)	Second band dominant frequency (kHz)	Second band minimum frequency (kHz)	Second band maximum frequency (kHz)	dB difference (dB FS)
001	43831	0.17 \pm 0.01 (0.16 - 0.19; 10)	19.1 \pm 1.64 (17 - 22; 10)	1.75 \pm 0.76 (0.94 - 3.49; 10)	1.86 \pm 0.75 (1.07 - 3.58; 10)	1.21 \pm 0.01 (1.19 - 1.23; 10)	1 \pm 0.11 (0.84 - 1.14; 10)	1.43 \pm 0.033 (1.39 - 1.5; 10)	2.57 \pm 0.059 (2.44 - 2.65; 10)	2.35 \pm 0.057 (2.23 - 2.44; 10)	2.75 \pm 0.018 (2.71 - 2.78; 10)	5.41 \pm 2.32 (2.79 - 10.72; 10)
002	43832	0.17 \pm 0.01 (0.16 - 0.18; 8)	18.63 \pm 0.86 (17 - 20; 8)	2.68 \pm 1.6 (0.68 - 6.09; 8)	2.85 \pm 1.6 (0.84 - 6.27; 8)	1.16 \pm 0.047 (1.12 - 1.28; 8)	1.04 \pm 0.012 (1.03 - 1.07; 8)	1.37 \pm 0.03 (1.3 - 1.4; 8)	2.53 \pm 0.06 (2.4 - 2.59; 8)	2.26 \pm 0.04 (2.2 - 2.31; 8)	2.67 \pm 0.048 (2.57 - 2.72; 8)	5.69 \pm 1.52 (2.99 - 8.39; 8)
003	43833	0.17 \pm 0.02 (0.16 - 0.21; 7)	19.71 \pm 1.48 (18 - 23; 7)	3.56 \pm 2.72 (1.57 - 9.53; 6)	3.74 \pm 2.72 (1.73 - 9.69; 6)	1.22 \pm 0.011 (1.21 - 1.24; 7)	1.12 \pm 0.004 (1.11 - 1.12; 7)	1.44 \pm 0.004 (1.43 - 1.44; 7)	2.58 \pm 0.008 (2.56 - 2.58; 7)	2.4 \pm 0.016 (2.38 - 2.42; 7)	2.77 \pm 0.017 (2.76 - 2.8; 7)	9.64 \pm 1.01 (7.92 - 11.03; 7)
004	43834	0.16 \pm 0.01 (0.13 - 0.17; 10)	18.9 \pm 1.76 (15 - 20; 10)	2.19 \pm 0.86 (0.82 - 3.73; 8)	2.39 \pm 0.88 (0.99 - 3.9; 8)	1.22 \pm 0.016 (1.19 - 1.25; 10)	1.12 \pm 0.012 (1.11 - 1.14; 10)	1.4 \pm 0.018 (1.38 - 1.42; 10)	2.59 \pm 0.07 (2.49 - 2.71; 10)	2.34 \pm 0.029 (2.29 - 2.39; 10)	3.49 \pm 0.232 (3.05 - 3.76; 10)	2.12 \pm 1.26 (0.01 - 3.95; 10)
005	43835	0.15 \pm 0.01 (0.14 - 0.15; 10)	15.7 \pm 0.9 (14 - 17; 10)	1.96 \pm 0.72 (0.93 - 3.43; 10)	2.11 \pm 0.72 (1.09 - 3.58; 10)	1.18 \pm 0.01 (1.16 - 1.19; 10)	1.09 \pm 0.008 (1.08 - 1.1; 10)	1.4 \pm 0.013 (1.38 - 1.41; 10)	2.51 \pm 0.033 (2.48 - 2.57; 10)	2.32 \pm 0.06 (2.17 - 2.39; 10)	2.75 \pm 0.029 (2.7 - 2.8; 10)	5.08 \pm 2 (0.13 - 7.52; 10)
006	43836	0.18 \pm 0.01 (0.17 - 0.18; 10)	19 \pm 0.45 (18 - 20; 10)	1.84 \pm 0.71 (0.97 - 2.9; 10)	2.01 \pm 0.71 (1.14 - 3.09; 10)	1.23 \pm 0.015 (1.21 - 1.26; 10)	1.13 \pm 0.005 (1.12 - 1.13; 10)	1.45 \pm 0.01 (1.43 - 1.46; 10)	2.55 \pm 0.016 (2.53 - 2.57; 10)	2.39 \pm 0.013 (2.37 - 2.41; 10)	2.76 \pm 0.014 (2.72 - 2.77; 10)	11.39 \pm 0.4 (10.71 - 12.12; 10)
007	43837	0.15 \pm 0.01 (0.15 - 0.16; 6)	17.33 \pm 0.47 (17 - 18; 6)	4.4 \pm 1.31 (3.25 - 6.63; 5)	4.55 \pm 1.31 (3.4 - 6.79; 5)	1.17 \pm 0.009 (1.16 - 1.18; 6)	1.09 \pm 0.007 (1.08 - 1.1; 6)	1.35 \pm 0.015 (1.34 - 1.38; 6)	2.46 \pm 0.011 (2.44 - 2.48; 6)	2.34 \pm 0.011 (2.31 - 2.35; 6)	2.62 \pm 0.014 (2.59 - 2.64; 6)	7.21 \pm 0.71 (6.3 - 8.46; 6)
008	43838	0.15 \pm 0.01 (0.13 - 0.16; 10)	15.9 \pm 0.94 (14 - 17; 10)	2.08 \pm 0.44 (1.07 - 2.61; 10)	2.22 \pm 0.44 (1.22 - 2.74; 10)	1.24 \pm 0.012 (1.26 - 1.26; 10)	1.14 \pm 0.007 (1.12 - 1.14; 10)	1.45 \pm 0.031 (1.4 - 1.49; 10)	2.53 \pm 0.051 (2.45 - 2.63; 10)	2.32 \pm 0.058 (2.22 - 2.38; 10)	2.81 \pm 0.05 (2.72 - 2.87; 10)	3.65 \pm 1.88 (0.52 - 5.56; 10)
009	43839	0.17 \pm 0.01 (0.16 - 0.18; 10)	19.7 \pm 1.27 (18 - 22; 10)	2.93 \pm 1.09 (1.43 - 4.93; 10)	3.1 \pm 1.09 (1.6 - 5.11; 10)	1.13 \pm 0.015 (1.1 - 1.14; 10)	1.04 \pm 0.012 (1.01 - 1.04; 10)	1.32 \pm 0.027 (1.27 - 1.37; 10)	2.41 \pm 0.067 (2.26 - 2.48; 10)	2.18 \pm 0.045 (2.1 - 2.24; 10)	2.57 \pm 0.039 (2.5 - 2.62; 10)	12.34 \pm 2.73 (7.61 - 15.54; 10)
010	43840	0.11 \pm 0.03 (0.09 - 0.18; 10)	12.5 \pm 3.5 (9 - 21; 10)	3.06 \pm 1.81 (1.11 - 7.43; 9)	3.17 \pm 1.82 (1.24 - 7.62; 9)	1.24 \pm 0.015 (1.23 - 1.28; 10)	1.15 \pm 0.014 (1.12 - 1.16; 10)	1.45 \pm 0.036 (1.39 - 1.51; 10)	2.63 \pm 0.051 (2.53 - 2.71; 10)	2.42 \pm 0.056 (2.31 - 2.52; 10)	2.77 \pm 0.046 (2.69 - 2.84; 10)	9.59 \pm 2.34 (4.81 - 12.65; 10)
011	43841	0.12 \pm 0.01 (0.11 - 0.14; 10)	13.7 \pm 1.19 (12 - 16; 10)	2.66 \pm 0.57 (1.73 - 3.35; 9)	2.78 \pm 0.56 (1.86 - 3.47; 9)	1.24 \pm 0.019 (1.22 - 1.28; 10)	1.11 \pm 0.008 (1.1 - 1.12; 10)	1.42 \pm 0.024 (1.39 - 1.48; 10)	2.51 \pm 0.046 (2.41 - 2.61; 10)	2.18 \pm 0.033 (2.12 - 2.24; 10)	2.69 \pm 0.042 (2.62 - 2.78; 10)	1.03 \pm 0.85 (-0.79 - 2.01; 10)
012	43842	0.17 \pm 0.02 (0.13 - 0.19; 4)	20.25 \pm 2.68 (16 - 23; 4)	7.22 \pm 1.24 (5.98 - 8.46; 2)	7.39 \pm 1.23 (6.16 - 8.62; 2)	1.32 \pm 0.041 (1.29 - 1.39; 4)	1.21 \pm 0.016 (1.2 - 1.24; 4)	1.61 \pm 0.036 (1.57 - 1.67; 4)	2.79 \pm 0.075 (2.71 - 2.9; 4)	2.54 \pm 0.032 (2.5 - 2.57; 4)	3.03 \pm 0.025 (3 - 3.07; 4)	6.57 \pm 1.22 (5.11 - 7.97; 4)
013	43843	0.16 \pm 0.02 (0.12 - 0.18; 10)	18.9 \pm 1.92 (14 - 21; 10)	10.83 \pm 4.77 (4.72 - 20.91; 9)	10.99 \pm 4.76 (4.88 - 21.03; 9)	1.22 \pm 0.037 (1.17 - 1.27; 10)	1.1 \pm 0.006 (1.09 - 1.11; 10)	1.37 \pm 0.013 (1.34 - 1.38; 10)	2.47 \pm 0.021 (2.42 - 2.5; 10)	2.3 \pm 0.029 (2.26 - 2.34; 10)	2.64 \pm 0.022 (2.62 - 2.69; 10)	5.33 \pm 1.62 (1.15 - 7.05; 10)
014	43844	0.16 \pm 0.01 (0.15 - 0.18; 10)	17.9 \pm 0.7 (17 - 19; 10)	3.14 \pm 0.56 (2.23 - 3.8; 9)	3.3 \pm 0.55 (2.4 - 3.95; 9)	1.16 \pm 0.012 (1.14 - 1.17; 10)	1.07 \pm 0.011 (1.04 - 1.08; 10)	1.35 \pm 0.032 (1.3 - 1.4; 10)	2.47 \pm 0.056 (2.36 - 2.58; 10)	2.29 \pm 0.038 (2.19 - 2.33; 10)	2.64 \pm 0.055 (2.53 - 2.71; 10)	5.34 \pm 1.86 (2.39 - 8.57; 10)
015	43845	0.15 \pm 0.01 (0.13 - 0.17; 10)	17.4 \pm 1.1 (15 - 19; 10)	2.69 \pm 0.86 (2.03 - 4.51; 7)	2.85 \pm 0.86 (2.19 - 4.66; 7)	1.16 \pm 0.011 (1.14 - 1.17; 10)	1.07 \pm 0.014 (1.06 - 1.09; 10)	1.3 \pm 0.013 (1.28 - 1.31; 10)	2.43 \pm 0.036 (2.38 - 2.49; 10)	2.22 \pm 0.056 (2.13 - 2.29; 10)	2.56 \pm 0.038 (2.52 - 2.63; 10)	3.17 \pm 0.95 (1.62 - 4.92; 10)
016	43846	0.16 \pm 0.01 (0.14 - 0.17; 4)	19.25 \pm 1.48 (17 - 21; 4)	15.12 \pm 18.36 (1.23 - 41.07; 3)	15.29 \pm 18.37 (1.37 - 41.24; 3)	1.19 \pm 0.005 (1.18 - 1.19; 4)	1.09 \pm 0.013 (1.08 - 1.11; 4)	1.43 \pm 0.012 (1.42 - 1.45; 4)	2.49 \pm 0.021 (2.46 - 2.52; 4)	2.29 \pm 0.03 (2.25 - 2.34; 4)	2.68 \pm 0.03 (2.65 - 2.72; 4)	9.52 \pm 0.57 (8.76 - 10.16; 4)
017	43847	0.19 \pm 0.01 (0.17 - 0.2; 10)	19.44 \pm 0.96 (18 - 21; 10)	2.51 \pm 1.07 (0.96 - 4.14; 10)	2.6 \pm 1.08 (1.14 - 4.34; 10)	1.17 \pm 0.011 (1.15 - 1.18; 10)	1.07 \pm 0.01 (1.06 - 1.09; 10)	1.33 \pm 0.014 (1.31 - 1.36; 10)	2.49 \pm 0.047 (2.42 - 2.57; 10)	2.29 \pm 0.027 (2.24 - 2.35; 10)	2.64 \pm 0.023 (2.59 - 2.67; 10)	6.84 \pm 1.61 (3.26 - 9.19; 10)
018	43848	0.18 \pm 0.01 (0.17 - 0.2; 10)	20.7 \pm 1.1 (18 - 22; 10)	2.47 \pm 0.86 (0.94 - 3.62; 9)	2.65 \pm 0.87 (1.12 - 3.82; 9)	1.25 \pm 0.039 (1.19 - 1.32; 10)	1.13 \pm 0.013 (1.11 - 1.14; 10)	1.41 \pm 0.014 (1.39 - 1.43; 10)	2.6 \pm 0.019 (2.57 - 2.64; 10)	2.36 \pm 0.044 (2.29 - 2.44; 10)	2.74 \pm 0.038 (2.69 - 2.8; 10)	2.42 \pm 1.14 (0.66 - 4.31; 10)
019	43849	0.12 \pm 0 (0.11 - 0.12; 9)	14.7 \pm 0.46 (14 - 15; 9)	1.37 \pm 0.44 (0.12 - 1.93; 9)	1.62 \pm 0.16 (1.46 - 2.05; 9)	1.17 \pm 0.014 (1.15 - 1.19; 9)	1.07 \pm 0.003 (1.07 - 1.08; 9)	1.38 \pm 0.008 (1.37 - 1.39; 9)	2.6 \pm 0.015 (2.58 - 2.63; 9)	2.4 \pm 0.024 (2.36 - 2.44; 9)	2.72 \pm 0.011 (2.7 - 2.73; 9)	11.09 \pm 2.14 (8.21 - 14.07; 9)

020	43850	0.14 ± 0.02 (0.11 - 0.16; 7)	17.14 ± 1.73 (15 - 20; 7)	17.35 ± 15.17 (2.08 - 37.23; 4)	17.48 ± 15.18 (2.21 - 37.39; 4)	1.23 ± 0.057 (1.18 - 1.36; 7)	1.12 ± 0.031 (1.08 - 1.16; 7)	1.43 ± 0.085 (1.31 - 1.53; 7)	2.54 ± 0.077 (2.41 - 2.69; 7)	2.26 ± 0.089 (2.14 - 2.42; 7)	2.76 ± 0.064 (2.68 - 2.83; 7)	2.64 ± 2.81 (-2.02 - 6.93; 7)
021	43851	0.13 ± 0.01 (0.11 - 0.15; 7)	16 ± 1.6 (14 - 18; 7)	2.05 ± 0.65 (1.27 - 3.27; 6)	2.3 ± 0.65 (1.42 - 3.39; 6)	1.15 ± 0.012 (1.13 - 1.16; 7)	1.06 ± 0.012 (1.03 - 1.07; 7)	1.4 ± 0.025 (1.35 - 1.42; 7)	2.46 ± 0.027 (2.41 - 2.51; 7)	2.18 ± 0.074 (2.07 - 2.27; 7)	2.65 ± 0.05 (2.57 - 2.76; 7)	6.35 ± 1.5 (4.32 - 8.76; 7)
022	43852	0.17 ± 0.01 (0.16 - 0.18; 10)	20.1 ± 0.54 (19 - 21; 10)	2.49 ± 0.58 (1.75 - 3.96; 9)	2.66 ± 0.58 (1.91 - 4.13; 9)	1.23 ± 0.078 (0.1.11 - 1.38; 10)	0.89 ± 0.257 (0.42 - 1.18; 10)	1.48 ± 0.053 (1.36 - 1.55; 10)	2.83 ± 0.332 (2.55 - 3.56; 10)	2.49 ± 0.241 (2.19 - 3.08; 10)	3.15 ± 0.265 (2.82 - 3.56; 10)	8.37 ± 8.85 (-1.98 - 28.24; 10)
023	43853	0.18 ± 0.01 (0.16 - 0.18; 10)	21.4 ± 0.8 (20 - 23; 10)	4.21 ± 0.92 (2.84 - 6.17; 9)	4.39 ± 0.92 (3.03 - 6.34; 9)	1.24 ± 0.005 (1.24 - 1.25; 10)	1.14 ± 0.006 (1.13 - 1.15; 10)	1.48 ± 0.011 (1.46 - 1.51; 10)	2.77 ± 0.027 (2.71 - 2.8; 10)	2.5 ± 0.019 (2.48 - 2.54; 10)	2.9 ± 0.02 (2.86 - 2.93; 10)	10.49 ± 1.5 (8.22 - 13.31; 10)
024	43854	0.09 ± 0.01 (0.08 - 0.12; 10)	10.6 ± 1.62 (9 - 15; 10)	5.75 ± 4.23 (2.6 - 17.11; 9)	5.83 ± 4.24 (2.69 - 17.21; 9)	1.19 ± 0.013 (1.17 - 1.22; 10)	1.1 ± 0.012 (1.08 - 1.12; 10)	1.37 ± 0.019 (1.34 - 1.4; 10)	2.49 ± 0.031 (2.42 - 2.53; 10)	2.26 ± 0.041 (2.19 - 2.33; 10)	2.65 ± 0.045 (2.55 - 2.71; 10)	3.69 ± 1.32 (1.02 - 5.81; 10)
025	43855	0.11 ± 0.02 (0.06 - 0.13; 7)	12.71 ± 2.66 (7 - 16; 7)	6.29 ± 2.33 (3.83 - 10.04; 6)	6.4 ± 2.33 (3.93 - 10.15; 6)	1.27 ± 0.025 (1.25 - 1.31; 7)	1.15 ± 0.012 (1.13 - 1.16; 7)	1.47 ± 0.024 (1.43 - 1.51; 7)	2.65 ± 0.094 (2.47 - 2.75; 7)	2.49 ± 0.069 (2.33 - 2.54; 7)	2.83 ± 0.055 (2.71 - 2.87; 7)	5.77 ± 2.8 (-0.31 - 9.71; 7)
026	43856	0.15 ± 0.01 (0.14 - 0.16; 9)	17 ± 0.67 (16 - 18; 9)	2.41 ± 1.78 (0.15 - 4.89; 8)	3.12 ± 1.54 (1.08 - 5.04; 8)	1.13 ± 0.015 (1.11 - 1.16; 9)	1.03 ± 0.01 (1.01 - 1.04; 9)	1.33 ± 0.018 (1.3 - 1.36; 9)	2.43 ± 0.066 (2.25 - 2.48; 9)	2.2 ± 0.043 (2.12 - 2.26; 9)	2.6 ± 0.043 (2.52 - 2.65; 9)	4.15 ± 1.07 (2.44 - 5.86; 9)
027	43857	0.14 ± 0.01 (0.13 - 0.15; 8)	17.13 ± 1.17 (15 - 19; 8)	6.03 ± 3.01 (2.59 - 11.42; 7)	6.17 ± 3.01 (2.74 - 11.56; 7)	1.13 ± 0.017 (1.11 - 1.15; 8)	1.03 ± 0.022 (0.99 - 1.06; 8)	1.35 ± 0.038 (1.29 - 1.4; 8)	2.46 ± 0.088 (2.34 - 2.57; 8)	2.17 ± 0.14 (1.95 - 2.33; 8)	2.64 ± 0.062 (2.53 - 2.71; 8)	4.83 ± 2.86 (-0.72 - 9; 8)
028	43858	0.12 ± 0.01 (0.1 - 0.13; 10)	14.9 ± 1.22 (13 - 16; 10)	6.59 ± 5.28 (3.73 - 21.75; 9)	7.43 ± 5.29 (3.85 - 21.87; 9)	1.2 ± 0.004 (1.19 - 1.21; 10)	1.11 ± 0.008 (1.1 - 1.12; 10)	1.38 ± 0.037 (1.35 - 1.48; 10)	2.56 ± 0.027 (2.53 - 2.63; 10)	2.36 ± 0.065 (2.21 - 2.44; 10)	2.72 ± 0.023 (2.68 - 2.76; 10)	7.36 ± 2.97 (3.9 - 13.45; 10)
029	43860	0.17 ± 0.02 (0.14 - 0.19; 10)	18.8 ± 2.09 (16 - 21; 10)	1.71 ± 0.3 (0.96 - 2.01; 9)	1.88 ± 0.3 (1.14 - 2.2; 9)	1.2 ± 0.007 (1.18 - 1.21; 10)	1.1 ± 0.009 (1.09 - 1.12; 10)	1.33 ± 0.008 (1.32 - 1.35; 10)	2.44 ± 0.018 (2.41 - 2.47; 10)	2.2 ± 0.035 (2.12 - 2.25; 10)	2.58 ± 0.014 (2.55 - 2.59; 10)	4.68 ± 0.94 (2.94 - 6.1; 10)
030	43861	0.19 ± 0.01 (0.18 - 0.2; 9)	21.43 ± 1.4 (20 - 24; 9)	3.56 ± 1.07 (2.62 - 5.69; 10)	3.75 ± 1.07 (2.8 - 5.87; 10)	1.13 ± 0.005 (1.12 - 1.14; 9)	1.03 ± 0.008 (1.02 - 1.04; 9)	1.48 ± 0.011 (1.46 - 1.5; 9)	2.59 ± 0.013 (2.58 - 2.62; 9)	2.35 ± 0.019 (2.33 - 2.38; 9)	2.71 ± 0.006 (2.7 - 2.72; 9)	12.91 ± 1.77 (9.75 - 15.85; 9)
031	43859	0.17 ± 0.01 (0.14 - 0.19; 10)	18.8 ± 1.08 (17 - 20; 10)	4.02 ± 1.91 (1.12 - 7.98; 10)	4.17 ± 1.81 (1.28 - 8.13; 10)	1.18 ± 0.007 (1.16 - 1.18; 10)	1.09 ± 0.008 (1.08 - 1.1; 10)	1.34 ± 0.011 (1.31 - 1.35; 10)	2.36 ± 0.107 (2.19 - 2.48; 10)	2.07 ± 0.053 (1.98 - 2.15; 10)	2.57 ± 0.063 (2.48 - 2.71; 10)	-7.52 ± 1.88 (-10.3 - -5.1; 10)
032	43862	0.15 ± 0.01 (0.13 - 0.16; 10)	18.11 ± 1.1 (16 - 19; 10)	4.93 ± 1.46 (3.1 - 6.91; 9)	5.07 ± 1.46 (3.23 - 7.04; 9)	1.12 ± 0.012 (1.1 - 1.14; 10)	1.03 ± 0.014 (1.01 - 1.06; 10)	1.33 ± 0.028 (1.28 - 1.38; 10)	2.44 ± 0.026 (2.4 - 2.5; 10)	2.13 ± 0.035 (2.08 - 2.19; 10)	2.55 ± 0.019 (2.53 - 2.61; 10)	0.73 ± 3.02 (-3.8 - 4.5; 10)
033	43863	0.18 ± 0.01 (0.17 - 0.2; 10)	21.4 ± 1.02 (20 - 23; 10)	5.3 ± 1.81 (3.32 - 8.32; 10)	5.48 ± 1.8 (3.49 - 8.48; 10)	1.09 ± 0.016 (1.07 - 1.12; 10)	0.99 ± 0.018 (0.97 - 1.02; 10)	1.24 ± 0.011 (1.23 - 1.26; 10)	2.25 ± 0.064 (2.14 - 2.35; 10)	2 ± 0.031 (1.93 - 2.05; 10)	2.51 ± 0.088 (2.39 - 2.66; 10)	-2.21 ± 0.76 (-3.52 - -0.97; 10)
034	43864	0.12 ± 0.03 (0.08 - 0.14; 5)	15 ± 2.9 (11 - 18; 5)	28.7 (1)	28.87 (1)	1.39 ± 0.015 (1.38 - 1.42; 5)	1.27 ± 0.02 (1.25 - 1.3; 5)	1.58 ± 0.008 (1.57 - 1.59; 5)	2.7 ± 0.094 (2.56 - 2.81; 5)	2.1 ± 0.094 (1.96 - 2.2; 5)	2.98 ± 0.019 (2.96 - 3; 5)	-11.18 ± 1.63 (-12.88 - -8.44; 5)
035	43865	0.13 ± 0.01 (0.12 - 0.15; 10)	16.7 ± 1.62 (13 - 19; 10)	4.86 ± 2.24 (2.76 - 10.2; 9)	5 ± 2.24 (2.88 - 10.33; 9)	1.23 ± 0.012 (1.21 - 1.25; 10)	1.15 ± 0.014 (1.11 - 1.16; 10)	1.41 ± 0.021 (1.36 - 1.43; 10)	2.53 ± 0.13 (2.15 - 2.64; 10)	2.26 ± 0.148 (1.85 - 2.39; 10)	2.71 ± 0.04 (2.62 - 2.78; 10)	-5.18 ± 3.45 (-14.67 - -2.24; 10)
036	43866	0.16 ± 0 (0.15 - 0.17; 9)	19.56 ± 0.68 (18 - 20; 9)	4.74 ± 2.42 (2.1 - 8.73; 7)	4.9 ± 2.42 (2.27 - 8.89; 7)	1.22 ± 0.009 (1.21 - 1.24; 9)	1.13 ± 0.012 (1.11 - 1.15; 9)	1.4 ± 0.031 (1.35 - 1.43; 9)	2.58 ± 0.047 (2.5 - 2.63; 9)	2.29 ± 0.098 (2.08 - 2.42; 9)	2.75 ± 0.04 (2.68 - 2.8; 9)	3.71 ± 3.7 (-2.06 - 10.28; 9)
037	43867	0.18 ± 0.01 (0.17 - 0.19; 10)	22.1 ± 0.94 (21 - 24; 10)	3.6 ± 3.16 (1.29 - 12.35; 9)	3.77 ± 3.16 (1.46 - 12.53; 9)	1.25 ± 0.008 (1.24 - 1.26; 10)	1.16 ± 0.005 (1.15 - 1.16; 10)	1.48 ± 0.017 (1.44 - 1.5; 10)	2.63 ± 0.015 (2.62 - 2.66; 10)	2.47 ± 0.035 (2.4 - 2.52; 10)	2.77 ± 0.009 (2.76 - 2.78; 10)	9.51 ± 1.86 (5.97 - 11.54; 10)
038	43868	0.1 ± 0.01 (0.08 - 0.11; 10)	12.2 ± 1.25 (10 - 14; 10)	7.03 ± 3.09 (4.57 - 13.81; 9)	7.13 ± 3.09 (4.67 - 13.92; 9)	1.22 ± 0.016 (1.18 - 1.24; 10)	1.12 ± 0.012 (1.1 - 1.14; 10)	1.41 ± 0.04 (1.36 - 1.45; 10)	2.56 ± 0.03 (2.5 - 2.61; 10)	2.33 ± 0.058 (2.24 - 2.42; 10)	2.71 ± 0.037 (2.65 - 2.77; 10)	3.66 ± 3.26 (-0.47 - 8.49; 10)
039	43869	0.11 ± 0.01 (0.09 - 0.13; 10)	13.5 ± 1.02 (11 - 15; 10)	4.66 ± 1.68 (2.97 - 7.9; 9)	4.77 ± 1.68 (3.07 - 7.99; 9)	1.18 ± 0.058 (1.12 - 1.32; 10)	1.03 ± 0.017 (1 - 1.06; 10)	1.45 ± 0.018 (1.43 - 1.49; 10)	2.38 ± 0.019 (2.35 - 2.41; 10)	2.18 ± 0.091 (2 - 2.27; 10)	2.6 ± 0.022 (2.56 - 2.64; 10)	5.92 ± 2.23 (2.36 - 8.64; 10)
040	43870	0.11 ± 0.01 (0.09 - 0.12; 10)	13 ± 1.34 (11 - 15; 10)	3.49 ± 2.16 (0.12 - 8.46; 9)	3.97 ± 1.94 (2.13 - 8.55; 9)	1.2 ± 0.012 (1.18 - 1.22; 10)	1.11 ± 0.009 (1.1 - 1.12; 10)	1.39 ± 0.014 (1.37 - 1.42; 10)	2.51 ± 0.035 (2.47 - 2.59; 10)	2.2 ± 0.063 (2.09 - 2.33; 10)	2.69 ± 0.032 (2.64 - 2.76; 10)	-2.16 ± 2.12 (-6.4 - 1.5; 10)
041	43871	0.13 ± 0.02 (0.11 - 0.17; 10)	15.33 ± 2.16 (13 - 21; 10)	3.25 ± 1.96 (1.28 - 6.51; 9)	3.39 ± 1.96 (1.41 - 6.64; 9)	1.16 ± 0.03 (1.14 - 1.25; 10)	1.08 ± 0.029 (1.06 - 1.16; 10)	1.3 ± 0.025 (1.29 - 1.38; 10)	2.44 ± 0.053 (2.39 - 2.56; 10)	2.21 ± 0.093 (2.1 - 2.39; 10)	2.57 ± 0.094 (2.51 - 2.83; 10)	2.04 ± 2.75 (-2.57 - 5.71; 10)
042	43872	0.09 ± 0.02 (0.07 - 0.11; 4)	10.5 ± 2.5 (8 - 13; 4)	10.57 ± 8.92 (3.58 - 23.16; 3)	10.67 ± 8.94 (3.69 - 23.28; 3)	1.23 ± 0.019 (1.21 - 1.26; 4)	1.15 ± 0.019 (1.12 - 1.17; 4)	1.43 ± 0.051 (1.35 - 1.48; 4)	2.62 ± 0.053 (2.53 - 2.67; 4)	2.29 ± 0.093 (2.15 - 2.38; 4)	2.77 ± 0.06 (2.67 - 2.82; 4)	-3.12 ± 4.93 (-9.61 - 2.08; 4)
043	43873	0.17 ± 0.01	20.4 ± 0.49	5.71 ± 4.85	5.88 ± 4.85	1.23 ± 0.006	1.13 ± 0.005	1.45 ± 0.016	2.66 ± 0.031	2.48 ± 0.052	2.83 ± 0.013	10.3 ± 2.79

044	43874	(0.16 - 0.18; 10) 0.14 ± 0.02 (0.11 - 0.15; 5)	(20 - 21; 10) 17.6 ± 1.85 (14 - 19; 5)	(2.24 - 19.14; 9) 14.89 ± 13.57 (3.89 - 38.12; 4)	(2.41 - 19.32; 9) 15.02 ± 13.57 (4.04 - 38.27; 4)	(1.22 - 1.24; 10) 1.31 ± 0.018 (1.3 - 1.34; 5)	(1.13 - 1.14; 10) 1.18 ± 0.014 (1.15 - 1.2; 5)	(1.43 - 1.48; 10) 1.48 ± 0.011 (1.46 - 1.5; 5)	(2.61 - 2.71; 10) 2.53 ± 0.022 (2.51 - 2.56; 5)	(2.4 - 2.55; 10) 2.28 ± 0.038 (2.24 - 2.33; 5)	(2.81 - 2.85; 10) 2.73 ± 0.071 (2.62 - 2.82; 5)	(6.24 - 14.21; 10) 1.93 ± 1.03 (0.68 - 3.21; 5)
045	43875	0.13 ± 0.01 (0.13 - 0.15; 6)	17.5 ± 0.76 (17 - 19; 6)	5.2 ± 1.82 (2.64 - 8.27; 5)	5.33 ± 1.83 (2.77 - 8.41; 5)	1.33 ± 0.01 (1.31 - 1.34; 6)	1.22 ± 0.005 (1.22 - 1.23; 6)	1.52 ± 0.004 (1.51 - 1.52; 6)	2.81 ± 0.023 (2.77 - 2.83; 6)	2.47 ± 0.02 (2.44 - 2.51; 6)	3 ± 0.025 (2.95 - 3.03; 6)	1.47 ± 0.72 (0.09 - 2.34; 6)
046	43876	0.16 ± 0.01 (0.14 - 0.17; 5)	19.4 ± 1.36 (17 - 21; 5)	4.79 ± 1.98 (3.23 - 7.59; 3)	5 ± 2.06 (3.39 - 7.9; 3)	1.34 ± 0.011 (1.32 - 1.36; 5)	1.23 ± 0.008 (1.22 - 1.24; 5)	1.49 ± 0.012 (1.46 - 1.5; 5)	2.63 ± 0.017 (2.59 - 2.64; 5)	2.34 ± 0.045 (2.28 - 2.41; 5)	2.8 ± 0.009 (2.79 - 2.81; 5)	3.22 ± 2.53 (-0.16 - 6.24; 5)
047	43877	0.11 ± 0.01 (0.1 - 0.12; 2)	13.5 ± 1.5 (12 - 15; 2)	6.7 (1)	6.8 (1)	1.36 ± 0.016 (1.35 - 1.38; 2)	1.23 ± 0.005 (1.23 - 1.24; 2)	1.58 ± 0.016 (1.56 - 1.59; 2)	2.78 ± 0 (2.78 - 2.78; 2)	2.44 ± 0.011 (2.43 - 2.45; 2)	2.96 ± 0.011 (2.95 - 2.97; 2)	2.12 ± 0.57 (1.55 - 2.68)
048	43878	0.09 ± 0.02 (0.07 - 0.12; 9)	10.33 ± 2.21 (8 - 13; 9)	7.53 ± 6.81 (0.81 - 21.97; 8)	7.62 ± 6.81 (0.93 - 22.04; 8)	1.12 ± 0.009 (1.11 - 1.13; 9)	1.02 ± 0.01 (1 - 1.03; 9)	1.28 ± 0.023 (1.25 - 1.32; 9)	2.26 ± 0.107 (2.08 - 2.4; 9)	1.96 ± 0.088 (1.83 - 2.13; 9)	2.46 ± 0.044 (2.39 - 2.55; 9)	-3.55 ± 3.57 (-7.85 - 3.64; 9)
049	43879	0.17 ± 0.01 (0.16 - 0.18; 7)	21.43 ± 1.05 (20 - 23; 7)	11.09 ± 7.4 (6.75 - 19.63; 3)	11.25 ± 7.4 (6.92 - 19.79; 3)	1.3 ± 0.005 (1.3 - 1.3; 7)	1.2 ± 0.004 (1.2 - 1.21; 7)	1.47 ± 0.004 (1.46 - 1.48; 7)	2.59 ± 0.008 (2.58 - 2.61; 7)	2.41 ± 0.022 (2.37 - 2.43; 7)	2.82 ± 0.012 (2.8 - 2.84; 7)	-1.07 ± 1.22 (-3.34 - 0.34; 7)
050	43880	0.13 ± 0.02 (0.08 - 0.16; 10)	14.3 ± 2.72 (8 - 18; 10)	5.6 ± 6.04 (1.29 - 22.24; 9)	5.74 ± 6.0 (1.44 - 22.38; 9)	1.18 ± 0.015 (1.14 - 1.19; 10)	1.06 ± 0.01 (1.06 - 1.09; 10)	1.3 ± 0.008 (1.29 - 1.31; 10)	2.33 ± 0.052 (2.28 - 2.45; 10)	2.08 ± 0.054 (1.99 - 2.15; 10)	2.5 ± 0.039 (2.45 - 2.58; 10)	-1.35 ± 1.88 (-6.56 - 0.29; 10)
051	43881	0.11 ± 0.01 (0.1 - 0.13; 10)	13.1 ± 1.04 (11 - 15; 10)	16.87 ± 13.34 (5.11 - 43.75; 9)	16.98 ± 13.34 (5.23 - 43.87; 9)	1.27 ± 0.015 (1.25 - 1.29; 10)	1.17 ± 0.01 (1.16 - 1.2; 10)	1.45 ± 0.018 (1.42 - 1.48; 10)	2.67 ± 0.049 (2.59 - 2.76; 10)	2.36 ± 0.105 (2.14 - 2.5; 10)	2.83 ± 0.041 (2.75 - 2.9; 10)	0.75 ± 2.7 (-4.56 - 4.39; 10)
052	43882	0.1 ± 0.01 (0.09 - 0.12; 9)	13.33 ± 1.41 (11 - 15; 9)	8.23 ± 2.88 (4.74 - 13.26; 6)	8.33 ± 2.88 (4.84 - 13.35; 6)	1.38 ± 0.044 (1.35 - 1.49; 9)	1.26 ± 0.03 (1.23 - 1.31; 9)	1.55 ± 0.072 (1.48 - 1.65; 9)	2.71 ± 0.053 (2.59 - 2.77; 9)	2.41 ± 0.102 (2.26 - 2.56; 9)	2.9 ± 0.074 (2.79 - 3.05; 9)	-0.11 ± 2.66 (-5.02 - 4.66; 9)
053	43883	0.15 ± 0.02 (0.12 - 0.17; 6)	17.67 ± 2.13 (14 - 20; 6)	3.17 ± 0.34 (2.69 - 3.44; 3)	3.32 ± 0.32 (2.86 - 3.59; 3)	1.26 ± 0.041 (1.22 - 1.32; 6)	1.14 ± 0.013 (1.12 - 1.15; 6)	1.4 ± 0.012 (1.38 - 1.41; 6)	2.53 ± 0.011 (2.52 - 2.55; 6)	2.36 ± 0.023 (2.33 - 2.4; 6)	2.64 ± 0.019 (2.62 - 2.67; 6)	8.35 ± 0.97 (7.05 - 10; 6)
054	43884	0.19 ± 0.01 (0.18 - 0.21; 8)	22.63 ± 1.32 (21 - 25; 8)	9.17 ± 3.97 (3.69 - 14.88; 4)	9.36 ± 3.97 (3.88 - 15.08; 4)	1.29 ± 0.017 (1.26 - 1.31; 8)	1.17 ± 0.016 (1.16 - 1.21; 8)	1.47 ± 0.013 (1.45 - 1.5; 8)	2.58 ± 0.035 (2.51 - 2.63; 8)	2.33 ± 0.039 (2.26 - 2.38; 8)	2.78 ± 0.043 (2.69 - 2.82; 8)	5.12 ± 1.8 (3.1 - 8.35; 8)
055	43885	0.15 ± 0.01 (0.14 - 0.16; 5)	17.2 ± 0.4 (17 - 18; 5)	6.04 ± 2.12 (3.83 - 9.22; 4)	6.19 ± 2.13 (3.98 - 9.37; 4)	1.38 ± 0.011 (1.37 - 1.4; 5)	1.21 ± 0.01 (1.2 - 1.22; 5)	1.5 ± 0.005 (1.5 - 1.51; 5)	2.64 ± 0.008 (2.63 - 2.65; 5)	2.42 ± 0.028 (2.38 - 2.45; 5)	2.82 ± 0.05 (2.77 - 2.91; 5)	3.88 ± 1.02 (2.22 - 5.34; 5)
056	43886	0.15 ± 0.01 (0.14 - 0.16; 3)	16.8 ± 0.75 (15 - 18; 3)	9.74 ± 2.55 (3.55 - 11.96; 2)	9.89 ± 2.55 (3.69 - 12.11; 2)	1.13 ± 0.009 (1.19 - 1.14; 3)	1.03 ± 0.01 (1.01 - 1.04; 3)	1.3 ± 0.019 (1.27 - 1.34; 3)	2.36 ± 0.056 (2.28 - 2.47; 3)	2 ± 0.09 (1.84 - 2.14; 3)	2.51 ± 0.043 (2.42 - 2.56; 3)	-1.36 ± 2.57 (-7.66 - 1.41; 3)
057	43887	0.07 ± 0.01 (0.05 - 0.09; 10)	8.5 ± 1.36 (6 - 11; 10)	7.3 ± 2.22 (5.24 - 12.3; 8)	7.36 ± 2.32 (5.59 - 12.39; 8)	1.19 ± 0.017 (1.15 - 1.21; 10)	1.09 ± 0.022 (1.03 - 1.11; 10)	1.35 ± 0.021 (1.32 - 1.38; 10)	2.38 ± 0.152 (2.14 - 2.55; 10)	1.99 ± 0.083 (1.85 - 2.13; 10)	2.7 ± 0.095 (2.5 - 2.79; 10)	-6.8 ± 1.78 (-8.59 - -2.55; 10)
058	43888	0.15 ± 0.01 (0.13 - 0.16; 8)	17.38 ± 1.11 (15 - 19; 8)	7.04 ± 2.18 (4.69 - 10.77; 5)	7.18 ± 2.18 (4.84 - 10.91; 5)	1.21 ± 0.005 (1.21 - 1.22; 8)	1.12 ± 0.005 (1.11 - 1.12; 8)	1.4 ± 0.012 (1.38 - 1.41; 8)	2.6 ± 0.125 (2.44 - 2.73; 8)	2.27 ± 0.05 (2.21 - 2.37; 8)	2.8 ± 0.026 (2.5 - 2.79; 10)	2.87 ± 1.86 (-0.07 - 6.67; 8)
059	43889	0.15 ± 0.01 (0.12 - 0.17; 10)	19.4 ± 1.85 (15 - 21; 10)	5.81 ± 8.98 (0.98 - 30.8; 9)	5.96 ± 8.98 (1.13 - 30.95; 9)	1.26 ± 0.063 (1.22 - 1.4; 10)	1.14 ± 0.011 (1.13 - 1.16; 10)	1.43 ± 0.03 (1.4 - 1.49; 10)	2.56 ± 0.024 (2.52 - 2.59; 10)	2.19 ± 0.083 (2.06 - 2.35; 10)	2.7 ± 0.023 (2.67 - 2.73; 10)	-0.03 ± 4.09 (-4.63 - 10.15; 10)
060	43890	0.13 ± 0.02 (0.1 - 0.15; 5)	16.6 ± 2.65 (13 - 20; 5)	6.44 ± 7 (0.79 - 18.24; 4)	6.57 ± 6.98 (0.93 - 18.34; 4)	1.21 ± 0.061 (1.15 - 1.31; 5)	1.1 ± 0.029 (1.07 - 1.14; 5)	1.41 ± 0.027 (1.37 - 1.44; 5)	2.45 ± 0.035 (2.41 - 2.5; 5)	2.19 ± 0.04 (2.13 - 2.24; 5)	2.67 ± 0.062 (2.56 - 2.75; 5)	-1.95 ± 1.24 (-3.84 - -0.59; 5)
061	43891	0.14 ± 0.01 (0.13 - 0.16; 10)	18 ± 0.82 (17 - 19; 10)	3.37 ± 0.49 (2.61 - 4.08; 9)	3.51 ± 0.49 (2.74 - 4.22; 9)	1.56 ± 0.139 (1.46 - 1.8; 10)	1.37 ± 0.015 (1.35 - 1.39; 10)	1.73 ± 0.152 (1.63 - 1.99; 10)	3.01 ± 0.041 (2.94 - 3.05; 10)	2.73 ± 0.071 (2.63 - 2.8; 10)	3.17 ± 0.027 (3.12 - 3.19; 10)	9.48 ± 2.84 (4.65 - 13.37; 10)
062	43892	0.12 ± 0.01 (0.12 - 0.13; 5)	14.75 ± 0.43 (14 - 15; 5)	2.54 ± 0.42 (1.88 - 2.96; 4)	2.66 ± 0.42 (2 - 3.08; 4)	1.44 ± 0.056 (1.32 - 1.47; 5)	1.22 ± 0.004 (1.22 - 1.23; 5)	1.8 ± 0.086 (1.68 - 1.92; 5)	2.86 ± 0.007 (2.85 - 2.87; 5)	2.62 ± 0.036 (2.57 - 2.67; 5)	3.12 ± 0.144 (2.98 - 3.32; 5)	4.09 ± 1.27 (2.18 - 5.57; 5)
063	43893	0.13 ± 0.01 (0.12 - 0.13; 7)	15 ± 0 (15 - 15; 7)	9.61 ± 14.28 (1.68 - 41.44; 6)	9.77 ± 14.26 (1.84 - 41.56; 6)	1.56 ± 0.048 (1.51 - 1.6; 7)	1.37 ± 0.005 (1.37 - 1.38; 7)	1.78 ± 0.097 (1.68 - 1.87; 7)	3.02 ± 0 (3.02 - 3.02; 7)	2.69 ± 0.032 (2.66 - 2.72; 7)	3.37 ± 0.14 (3.23 - 3.51; 7)	5.26 ± 2.06 (3.2 - 7.31; 7)
064	43894	0.12 ± 0.01 (0.1 - 0.13; 10)	14.8 ± 1.08 (13 - 16; 10)	5.07 ± 1.84 (1.59 - 8.35; 9)	5.19 ± 1.84 (1.69 - 8.47; 9)	1.43 ± 0.132 (1.31 - 1.81; 10)	1.25 ± 0.029 (1.2 - 1.31; 10)	1.67 ± 0.126 (1.56 - 2; 10)	2.81 ± 0.042 (2.72 - 2.87; 10)	2.46 ± 0.082 (2.27 - 2.61; 10)	3 ± 0.097 (2.85 - 3.23; 10)	5.76 ± 1.07 (3.88 - 7.93; 10)
065	43899	0.15 ± 0.02 (0.13 - 0.18; 6)	18.83 ± 1.95 (16 - 21; 6)	4.77 ± 2.93 (2.34 - 10.28; 5)	4.92 ± 2.92 (2.49 - 10.42; 5)	1.42 ± 0.011 (1.41 - 1.44; 6)	1.16 ± 0.135 (0.93 - 1.27; 6)	1.62 ± 0.03 (1.6 - 1.68; 6)	2.92 ± 0.044 (2.87 - 2.97; 6)	2.7 ± 0.033 (2.65 - 2.73; 6)	3.08 ± 0.025 (3.05 - 3.12; 6)	8.21 ± 1.75 (5.46 - 10.32; 6)
066	43900	0.12 ± 0.02 (0.1 - 0.15; 10)	15.1 ± 2.17 (12 - 19; 10)	6.79 ± 6.5 (1.51 - 19.84; 8)	6.91 ± 6.49 (1.66 - 19.96; 8)	1.42 ± 0.029 (1.37 - 1.47; 10)	1.1 ± 0.111 (0.94 - 1.23; 10)	1.67 ± 0.119 (1.57 - 1.94; 10)	2.78 ± 0.024 (2.73 - 2.81; 10)	2.53 ± 0.042 (2.47 - 2.59; 10)	3.02 ± 0.128 (2.9 - 3.29; 10)	6.18 ± 5.74 (-9.75 - 11.15; 10)

067	43901	0.16 ± 0.01 (0.15 - 0.17; 7)	19.29 ± 1.48 (16 - 21; 7)	4.35 ± 2.82 (1.26 - 9.63; 6)	4.52 ± 2.82 (1.43 - 9.8; 6)	1.37 ± 0.017 (1.35 - 1.4; 7)	1.2 ± 0.082 (1.07 - 1.27; 7)	1.6 ± 0.049 (1.55 - 1.69; 7)	2.74 ± 0.081 (2.57 - 2.85; 7)	2.44 ± 0.074 (2.33 - 2.53; 7)	3.04 ± 0.059 (2.98 - 3.17; 7)	2.94 ± 1.45 (0.8 - 5.11; 7)
068	43902	0.17 ± 0.02 (0.13 - 0.21; 9)	21.56 ± 2.71 (17 - 27; 9)	3.21 ± 1.19 (1.84 - 5.92; 9)	3.38 ± 1.2 (1.98 - 6.08; 9)	1.29 ± 0.009 (1.27 - 1.3; 9)	1.18 ± 0.007 (1.16 - 1.18; 9)	1.54 ± 0.068 (1.46 - 1.71; 9)	2.74 ± 0.02 (2.69 - 2.78; 9)	2.52 ± 0.03 (2.48 - 2.55; 9)	2.97 ± 0.068 (2.89 - 3.13; 9)	3.63 ± 1.3 (0.53 - 4.96; 9)
069	43903	0.13 ± 0.01 (0.11 - 0.14; 10)	16.3 ± 1.19 (14 - 18; 10)	2.47 ± 0.8 (0.88 - 4.19; 10)	2.6 ± 0.8 (0.99 - 4.3; 10)	1.31 ± 0.012 (1.29 - 1.33; 10)	1.19 ± 0.018 (1.15 - 1.21; 10)	1.53 ± 0.017 (1.5 - 1.56; 10)	2.8 ± 0.021 (2.77 - 2.84; 10)	2.65 ± 0.025 (2.61 - 2.68; 10)	2.97 ± 0.013 (2.95 - 2.99; 10)	8.89 ± 1.12 (6.89 - 10.97; 10)
070	43904	0.14 ± 0.01 (0.13 - 0.15; 10)	17.2 ± 0.87 (16 - 19; 10)	2.43 ± 1.32 (1.46 - 5.94; 10)	2.49 ± 1.27 (1.46 - 5.94; 10)	1.34 ± 0.012 (1.32 - 1.36; 10)	1.23 ± 0.011 (1.22 - 1.25; 10)	1.53 ± 0.021 (1.49 - 1.56; 10)	2.81 ± 0.046 (2.75 - 2.9; 10)	2.58 ± 0.048 (2.47 - 2.65; 10)	3.03 ± 0.081 (2.96 - 3.26; 10)	3.31 ± 1.45 (0.91 - 6.07; 10)
071	43905	0.12 ± 0.01 (0.1 - 0.15; 10)	15.3 ± 2.05 (12 - 19; 10)	5.34 ± 4.02 (2.57 - 16.2; 10)	5.47 ± 4.02 (2.69 - 16.31; 10)	1.23 ± 0.015 (1.21 - 1.25; 10)	1.1 ± 0.032 (1.03 - 1.13; 10)	1.48 ± 0.043 (1.41 - 1.55; 10)	2.55 ± 0.089 (2.41 - 2.69; 10)	2.33 ± 0.082 (2.11 - 2.4; 10)	2.82 ± 0.044 (2.77 - 2.89; 10)	2.07 ± 2.03 (-0.76 - 6.23; 10)
072	43906	0.15 ± 0.01 (0.13 - 0.16; 9)	17.89 ± 0.99 (16 - 19; 9)	5.23 ± 3.03 (1.09 - 9.6; 4)	5.38 ± 3.04 (1.22 - 9.76; 4)	1.41 ± 0.011 (1.39 - 1.42; 9)	1.29 ± 0.015 (1.26 - 1.31; 9)	1.62 ± 0.025 (1.57 - 1.67; 9)	2.92 ± 0.036 (2.84 - 2.96; 9)	2.56 ± 0.078 (2.47 - 2.73; 9)	3.09 ± 0.039 (3 - 3.14; 9)	3.24 ± 1.18 (1.44 - 5.6; 9)
073	43907	0.18 ± 0.01 (0.16 - 0.2; 10)	23.5 ± 1.28 (21 - 26; 10)	7.02 ± 1.4 (4.81 - 8.75; 9)	7.2 ± 1.4 (4.99 - 8.94; 9)	1.32 ± 0.01 (1.3 - 1.34; 10)	1.21 ± 0.011 (1.2 - 1.23; 10)	1.5 ± 0.021 (1.46 - 1.53; 10)	2.77 ± 0.042 (2.7 - 2.83; 10)	2.61 ± 0.035 (2.56 - 2.66; 10)	2.94 ± 0.046 (2.87 - 3; 10)	5.81 ± 0.74 (4.93 - 7.03; 10)
074	43908	0.13 ± 0.01 (0.12 - 0.14; 10)	16.5 ± 1.36 (14 - 18; 10)	1.37 ± 0.18 (1.07 - 1.72; 9)	1.5 ± 0.18 (1.22 - 1.86; 9)	1.29 ± 0.015 (1.27 - 1.31; 10)	1.18 ± 0.007 (1.16 - 1.18; 10)	1.66 ± 0.06 (1.54 - 1.78; 10)	2.76 ± 0.028 (2.72 - 2.8; 10)	2.56 ± 0.029 (2.5 - 2.58; 10)	2.95 ± 0.037 (2.9 - 3; 10)	8.55 ± 4.94 (-1.73 - 12.43; 10)
075	43909	0.14 ± 0.01 (0.14 - 0.15; 10)	19 ± 0.63 (18 - 20; 10)	2.24 ± 0.2 (1.94 - 2.54; 10)	2.45 ± 0.17 (2.16 - 2.69; 10)	1.32 ± 0.04 (1.3 - 1.43; 10)	1.21 ± 0.014 (1.18 - 1.23; 10)	1.55 ± 0.012 (1.53 - 1.57; 10)	2.85 ± 0.048 (2.77 - 2.93; 10)	2.59 ± 0.029 (2.53 - 2.64; 10)	3 ± 0.014 (2.97 - 3.03; 10)	9.96 ± 1.04 (8.53 - 11.31; 10)
076	43910	0.1 ± 0.01 (0.08 - 0.11; 10)	12.3 ± 1 (10 - 14; 10)	2.82 ± 1 (1.2 - 4.65; 8)	2.92 ± 1 (1.28 - 4.74; 8)	1.33 ± 0.012 (1.3 - 1.35; 10)	1.23 ± 0.01 (1.22 - 1.25; 10)	1.62 ± 0.09 (1.57 - 1.88; 10)	2.87 ± 0.073 (2.68 - 2.95; 10)	2.53 ± 0.054 (2.39 - 2.61; 10)	3.08 ± 0.076 (2.86 - 3.14; 10)	-1.81 ± 2.07 (-6.29 - 1.9; 10)
077	43911	0.11 ± 0.01 (0.09 - 0.12; 10)	13.8 ± 0.87 (12 - 15; 10)	3.72 ± 1.65 (2.03 - 7.75; 9)	3.82 ± 1.65 (2.13 - 7.85; 9)	1.25 ± 0.007 (1.24 - 1.26; 10)	1.16 ± 0.007 (1.15 - 1.17; 10)	1.63 ± 0.089 (1.57 - 1.83; 10)	2.88 ± 0.048 (2.77 - 2.95; 10)	2.58 ± 0.023 (2.53 - 2.61; 10)	3.04 ± 0.022 (3 - 3.08; 10)	8.57 ± 1.2 (6.4 - 10.33; 10)
078	43912	0.12 ± 0.01 (0.11 - 0.14; 10)	15.9 ± 0.7 (15 - 17; 10)	4.05 ± 0.88 (2.95 - 5.97; 9)	4.17 ± 0.88 (3.06 - 6.1; 9)	1.48 ± 0.011 (1.46 - 1.51; 10)	1.37 ± 0.014 (1.35 - 1.39; 10)	1.65 ± 0.016 (1.64 - 1.68; 10)	2.99 ± 0.059 (2.85 - 3.11; 10)	2.62 ± 0.077 (2.49 - 2.71; 10)	3.2 ± 0.049 (3.07 - 3.25; 10)	0.88 ± 3.45 (-4.76 - 6.1; 10)
079	43917	0.12 ± 0.01 (0.1 - 0.13; 10)	16.2 ± 1.33 (13 - 17; 10)	4.54 ± 2.49 (2.59 - 11.16; 9)	4.66 ± 2.49 (2.69 - 11.29; 9)	1.34 ± 0.005 (1.33 - 1.34; 10)	1.24 ± 0.007 (1.23 - 1.25; 10)	1.6 ± 0.014 (1.57 - 1.63; 10)	2.86 ± 0.022 (2.82 - 2.9; 10)	2.64 ± 0.025 (2.61 - 2.68; 10)	3.07 ± 0.025 (3.01 - 3.1; 10)	7.95 ± 1.29 (6.02 - 10.34; 10)
080	43918	0.16 ± 0 (0.15 - 0.17; 10)	19.1 ± 0.54 (18 - 20; 10)	3.05 ± 1.91 (1.31 - 7.94; 9)	3.21 ± 1.91 (1.47 - 8.11; 9)	1.26 ± 0.013 (1.24 - 1.27; 10)	1.16 ± 0.01 (1.15 - 1.17; 10)	1.48 ± 0.023 (1.44 - 1.51; 10)	2.74 ± 0.063 (2.64 - 2.83; 10)	2.51 ± 0.048 (2.45 - 2.58; 10)	2.89 ± 0.035 (2.83 - 2.93; 10)	9.5 ± 2.42 (6.8 - 13.45; 10)
081	43919	0.15 ± 0 (0.14 - 0.15; 8)	19.13 ± 0.6 (18 - 20; 8)	3.69 ± 1.65 (0.98 - 6.65; 8)	3.83 ± 1.65 (1.12 - 6.8; 8)	1.36 ± 0.013 (1.34 - 1.38; 8)	1.26 ± 0.01 (1.24 - 1.27; 8)	1.64 ± 0.014 (1.61 - 1.66; 8)	2.89 ± 0.059 (2.8 - 2.97; 8)	2.66 ± 0.076 (2.47 - 2.72; 8)	3.11 ± 0.037 (3.03 - 3.14; 8)	4.8 ± 1.78 (0.54 - 7.1; 8)
082	43920	0.1 ± 0.01 (0.08 - 0.11; 10)	12.8 ± 1.47 (10 - 15; 10)	3.34 ± 1.54 (1.05 - 6.29; 9)	3.43 ± 1.54 (1.14 - 6.39; 9)	1.41 ± 0.011 (1.39 - 1.43; 10)	1.32 ± 0.012 (1.3 - 1.34; 10)	1.67 ± 0.02 (1.63 - 1.69; 10)	2.94 ± 0.02 (2.91 - 2.97; 10)	2.67 ± 0.044 (2.58 - 2.72; 10)	3.16 ± 0.028 (3.1 - 3.19; 10)	6.37 ± 0.96 (4.67 - 8.05; 10)
083	43921	0.11 ± 0.01 (0.1 - 0.12; 4)	14 ± 1 (13 - 15; 4)	1.55 ± 1.42 (0.69 - 4.01; 4)	1.66 ± 1.42 (0.79 - 4.11; 4)	1.29 ± 0.021 (1.27 - 1.32; 4)	1.19 ± 0.014 (1.18 - 1.22; 4)	1.58 ± 0.02 (1.55 - 1.6; 4)	2.84 ± 0.012 (2.82 - 2.85; 4)	2.44 ± 0.011 (2.43 - 2.45; 4)	3.01 ± 0.028 (2.97 - 3.05; 4)	2.42 ± 0.4 (1.93 - 2.99; 4)
084	43922	0.17 ± 0.02 (0.13 - 0.19; 10)	20.5 ± 1.57 (17 - 23; 10)	4.21 ± 1.48 (2.52 - 7.33; 9)	4.38 ± 1.47 (2.7 - 7.46; 9)	1.3 ± 0.013 (1.28 - 1.32; 10)	1.18 ± 0.018 (1.16 - 1.23; 10)	1.54 ± 0.102 (1.49 - 1.84; 10)	2.58 ± 0.118 (2.42 - 2.8; 10)	2.28 ± 0.103 (2.21 - 2.56; 10)	2.84 ± 0.072 (2.72 - 3; 10)	3.87 ± 1.98 (1.8 - 8.63; 10)
085	43923	0.1 ± 0.01 (0.08 - 0.12; 10)	13.3 ± 1.95 (9 - 16; 10)	3.55 ± 0.98 (2.65 - 5.17; 4)	3.66 ± 0.99 (2.76 - 5.28; 4)	1.34 ± 0.018 (1.3 - 1.36; 10)	1.23 ± 0.015 (1.21 - 1.25; 10)	1.53 ± 0.028 (1.49 - 1.57; 10)	2.8 ± 0.045 (2.71 - 2.84; 10)	2.54 ± 0.05 (2.44 - 2.61; 10)	2.96 ± 0.041 (2.87 - 3; 10)	4.79 ± 1.69 (2.73 - 8.59; 10)
086	43924	0.11 ± 0.01 (0.1 - 0.13; 3)	14.33 ± 1.89 (13 - 17; 3)	2.5 (1)	2.63 (1)	1.34 ± 0.013 (1.32 - 1.36; 3)	1.25 ± 0.018 (1.23 - 1.27; 3)	1.57 ± 0.027 (1.54 - 1.6; 3)	2.94 ± 0.047 (2.9 - 3; 3)	2.6 ± 0.057 (2.53 - 2.67; 3)	3.06 ± 0.044 (3 - 3.11; 3)	5.12 ± 1.07 (4.27 - 6.62; 3)
087	43925	0.14 ± 0.02 (0.09 - 0.18; 10)	17.6 ± 2.91 (12 - 22; 10)	6.79 ± 11.76 (1.44 - 40.03; 9)	6.92 ± 11.78 (1.56 - 40.21; 9)	1.29 ± 0.009 (1.27 - 1.3; 10)	1.19 ± 0.013 (1.17 - 1.22; 10)	1.5 ± 0.009 (1.48 - 1.51; 10)	2.68 ± 0.044 (2.62 - 2.73; 10)	2.43 ± 0.058 (2.34 - 2.52; 10)	2.88 ± 0.028 (2.84 - 2.93; 10)	5.1 ± 2.47 (-0.86 - 7.38; 10)
088	43926	0.15 ± 0.02 (0.12 - 0.18; 10)	18.4 ± 2.15 (15 - 22; 10)	4.39 ± 1.36 (1.89 - 6.73; 9)	4.54 ± 1.36 (2.02 - 6.87; 9)	1.32 ± 0.032 (1.29 - 1.41; 10)	1.19 ± 0.03 (1.17 - 1.28; 10)	1.53 ± 0.062 (1.5 - 1.71; 10)	2.76 ± 0.06 (2.68 - 2.92; 10)	2.59 ± 0.029 (2.56 - 2.66; 10)	3 ± 0.069 (2.91 - 3.18; 10)	7.53 ± 0.64 (6.81 - 8.96; 10)
089	43927	0.14 ± 0.01 (0.13 - 0.16; 10)	17.7 ± 1.1 (16 - 19; 10)	3.41 ± 0.85 (2.36 - 4.89; 9)	3.55 ± 0.85 (2.51 - 5.03; 9)	1.25 ± 0.013 (1.22 - 1.26; 10)	1.14 ± 0.008 (1.12 - 1.15; 10)	1.63 ± 0.021 (1.58 - 1.66; 10)	2.78 ± 0.016 (2.76 - 2.82; 10)	2.62 ± 0.014 (2.59 - 2.64; 10)	2.93 ± 0.013 (2.91 - 2.95; 10)	12.65 ± 0.47 (11.99 - 13.49; 10)
090	43928	0.15 ± 0	18.6 ± 0.8	2.39 ± 0.28	2.54 ± 0.28	1.37 ± 0.012	1.27 ± 0.014	1.65 ± 0.016	2.79 ± 0.029	2.63 ± 0.025	2.98 ± 0.039	9.23 ± 0.76

		(0.14 - 0.15; 10)	(17 - 20; 10)	(1.84 - 2.8; 9)	(1.98 - 2.94; 9)	(1.36 - 1.4; 10)	(1.24 - 1.29; 10)	(1.61 - 1.66; 10)	(2.73 - 2.82; 10)	(2.57 - 2.66; 10)	(2.9 - 3.03; 10)	(8.21 - 11.07; 10)
091	43932	0.11 ± 0.01	12.9 ± 1.51	2.31 ± 0.74	2.42 ± 0.73	1.34 ± 0.014	1.23 ± 0.007	1.63 ± 0.073	2.9 ± 0.046	2.65 ± 0.032	3.1 ± 0.044	5.37 ± 0.94
		(0.09 - 0.13; 10)	(10 - 15; 10)	(1.34 - 3.7; 9)	(1.46 - 3.79; 9)	(1.32 - 1.37; 10)	(1.22 - 1.24; 10)	(1.55 - 1.77; 10)	(2.81 - 2.96; 10)	(2.58 - 2.69; 10)	(3.01 - 3.15; 10)	(4.24 - 6.79; 10)
092	43895	0.14 ± 0.01	18.3 ± 1.1	6.91 ± 2.36	7.06 ± 2.36	1.28 ± 0.015	1.2 ± 0.01	1.56 ± 0.029	2.66 ± 0.039	2.32 ± 0.081	2.87 ± 0.024	4.33 ± 2.12
		(0.13 - 0.15; 10)	(16 - 20; 10)	(4.42 - 11.51; 8)	(4.55 - 11.65; 8)	(1.27 - 1.32; 10)	(1.17 - 1.21; 10)	(1.52 - 1.61; 10)	(2.56 - 2.71; 10)	(2.12 - 2.44; 10)	(2.84 - 2.91; 10)	(-0.84 - 7.67; 10)
093	43896	0.13 ± 0	16 ± 1	13.53 ± 6.16	13.66 ± 6.16	1.32 ± 0.009	1.2 ± 0.02	1.48 ± 0.022	2.62 ± 0.188	2.06 ± 0.133	2.9 ± 0.046	-6.38 ± 2.46
		(0.12 - 0.13; 3)	(15 - 17; 3)	(7.37 - 19.69; 2)	(7.5 - 19.82; 2)	(1.31 - 1.33; 3)	(1.17 - 1.22; 3)	(1.45 - 1.51; 3)	(2.36 - 2.77; 3)	(1.92 - 2.24; 3)	(2.86 - 2.96; 3)	(-8.99 - -3.08; 3)
094	43897	0.1 ± 0.01	13.33 ± 1.49	18.05 ± 3.98	18.15 ± 3.97	1.35 ± 0.058	1.2 ± 0.014	1.63 ± 0.032	2.66 ± 0.092	2.27 ± 0.105	2.89 ± 0.037	2.61 ± 2.15
		(0.08 - 0.11; 6)	(11 - 15; 6)	(13.53 - 24.05; 5)	(13.64 - 24.15; 4)	(1.3 - 1.46; 6)	(1.18 - 1.23; 6)	(1.59 - 1.68; 6)	(2.53 - 2.75; 6)	(2.12 - 2.37; 6)	(2.84 - 2.94; 6)	(-0.65 - 5.57; 6)
095	43898	0.15 ± 0.01	19.2 ± 1.17	19.56 ± 26.22	19.71 ± 26.23	1.22 ± 0.007	1.11 ± 0.011	1.38 ± 0.011	2.5 ± 0.022	2.17 ± 0.046	2.68 ± 0.038	-0.57 ± 1.05
		(0.14 - 0.17; 5)	(18 - 21; 5)	(2.55 - 64.91; 4)	(2.71 - 65.06; 4)	(1.21 - 1.23; 5)	(1.1 - 1.13; 5)	(1.37 - 1.4; 5)	(2.47 - 2.53; 5)	(2.13 - 2.26; 5)	(2.64 - 2.73; 5)	(-1.6 - 1.43; 5)
096	43913	0.12 ± 0.01	16.4 ± 1.28	5.93 ± 2.38	6.04 ± 2.38	1.3 ± 0.016	1.21 ± 0.014	1.59 ± 0.017	2.71 ± 0.07	2.4 ± 0.011	2.92 ± 0.017	4.91 ± 0.98
		(0.1 - 0.13; 10)	(14 - 19; 10)	(3.3 - 10.75; 9)	(3.41 - 10.88; 9)	(1.28 - 1.32; 10)	(1.2 - 1.23; 10)	(1.57 - 1.63; 10)	(2.58 - 2.78; 10)	(2.38 - 2.41; 10)	(2.9 - 2.95; 10)	(2.93 - 6.49; 10)
097	43914	0.13 ± 0.02	17 ± 2.16	13.03 ± 21.82	13.16 ± 21.81	1.31 ± 0.01	1.18 ± 0.026	1.45 ± 0.066	2.73 ± 0.019	2.38 ± 0.149	2.87 ± 0.05	4.87 ± 3.8
		(0.09 - 0.18; 9)	(14 - 21; 5)	(1.08 - 61.73; 6)	(1.08 - 61.84; 6)	(1.3 - 1.32; 9)	(1.14 - 1.22; 9)	(1.39 - 1.58; 9)	(2.69 - 2.76; 9)	(2.12 - 2.57; 9)	(2.82 - 2.98; 9)	(-0.06 - 11.55; 9)
098	43915	0.12 ± 0.02	13.3 ± 2.53	17.56 ± 18.91	17.68 ± 18.91	1.34 ± 0.031	1.15 ± 0.017	1.54 ± 0.037	2.52 ± 0.087	2.3 ± 0.07	2.85 ± 0.053	-0.05 ± 2.69
		(0.08 - 0.16; 9)	(10 - 18; 9)	(3.7 - 68.79; 9)	(3.81 - 68.9; 9)	(1.25 - 1.36; 9)	(1.12 - 1.17; 9)	1.49 - 1.61; 9)	(2.4 - 2.71; 9)	(2.15 - 2.45; 9)	(2.77 - 2.96; 9)	(-3.05 - 6.45; 9)
099	43916	0.13 ± 0.01	13 ± 0	1.33 ± 0.14	1.45 ± 0.14	1.26 ± 0.016	1.16 ± 0.027	1.43 ± 0.059	2.61 ± 0.022	2.27 ± 0.081	2.78 ± 0.065	-0.42 ± 0.84
		(0.12 - 0.13; 2)	(13 - 13; 2)	(1.19 - 1.46; 2)	(1.31 - 1.59; 2)	(1.25 - 1.28; 2)	(1.13 - 1.18)	(1.37 - 1.49)	(2.58 - 2.63; 2)	(2.19 - 2.35; 2)	(2.71 - 2.84; 2)	(-1.26 - 0.42; 2)
100	43929	0.13 ± 0.01	16.2 ± 1.25	4.76 ± 5.55	4.89 ± 5.55	1.27 ± 0.021	1.17 ± 0.017	1.5 ± 0.037	2.71 ± 0.059	2.52 ± 0.065	2.87 ± 0.048	9.09 ± 2.2
		(0.11 - 0.14; 10)	(13 - 18; 10)	(1.65 - 20.35; 9)	(1.77 - 20.49; 9)	(1.24 - 1.31; 10)	(1.15 - 1.21; 10)	(1.45 - 1.55; 10)	(2.63 - 2.79; 10)	(2.41 - 2.59; 10)	(2.8 - 2.95; 10)	(6 - 11.75; 10)
101	43930	0.15 ± 0.02	17.67 ± 2.49	2.27 ± 0.53	2.42 ± 0.56	1.2 ± 0.018	1.09 ± 0.013	1.36 ± 0.018	2.34 ± 0.009	2.14 ± 0.04	2.66 ± 0.058	-2.25 ± 0.67
		(0.12 - 0.18; 9)	(15 - 21; 9)	(1.73 - 2.8; 2)	(1.86 - 2.98; 2)	(1.17 - 1.22; 9)	(1.08 - 1.11; 9)	(1.35 - 1.39; 9)	(2.33 - 2.35; 9)	(2.09 - 2.19; 9)	(2.59 - 2.73; 9)	(-3.19 - -1.76; 9)
102	43931	0.17 ± 0.01	19.67 ± 1.25	18.51 ± 16.01	18.68 ± 16.01	1.36 ± 0.026	1.19 ± 0.018	1.52 ± 0.013	2.47 ± 0.121	2.28 ± 0.03	2.8 ± 0.071	1.62 ± 0.96
		(0.15 - 0.18; 6)	(18 - 21; 6)	(3.64 - 48.93; 5)	(3.82 - 49.11; 5)	(1.31 - 1.4; 6)	(1.16 - 1.22; 6)	(1.51 - 1.54; 6)	(2.41 - 2.75; 6)	(2.25 - 2.34; 6)	(2.66 - 2.89; 6)	(-0.05 - 2.73; 6)
103	43933	0.17 ± 0.01	19.67 ± 0.94	5.87 ± 2.76	6.03 ± 2.77	1.27 ± 0.011	1.16 ± 0.005	1.5 ± 0.014	2.74 ± 0.05	2.51 ± 0.048	2.92 ± 0.048	8.1 ± 1.11
		(0.15 - 0.18; 7)	(18 - 21; 7)	(2.27 - 10.99; 6)	(2.43 - 11.17; 6)	(1.26 - 1.29; 7)	(1.15 - 1.16; 7)	(1.48 - 1.52; 7)	(2.64 - 2.79; 7)	(2.42 - 2.57; 7)	(2.82 - 2.98; 7)	(6.21 - 9.41; 7)
104	43934	0.24 ± 0.01	26.2 ± 0.98	1.47 ± 0.54	1.71 ± 0.54	1.19 ± 0.008	1.09 ± 0.004	1.45 ± 0.015	2.56 ± 0.039	2.27 ± 0.019	2.76 ± 0.039	4.13 ± 0.77
		(0.24 - 0.25; 5)	(25 - 28; 5)	(0.97 - 2.48; 5)	(1.21 - 2.73; 5)	(1.18 - 1.21; 5)	(1.08 - 1.09; 5)	(1.43 - 1.48; 5)	(2.49 - 2.59; 5)	(2.23 - 2.28; 5)	(2.69 - 2.81; 5)	(3.23 - 5; 5)
105	43935	0.17 ± 0.01	17 ± 1.1	2.94 ± 1.46	3.11 ± 1.46	1.25 ± 0.041	1.1 ± 0.019	1.4 ± 0.022	2.56 ± 0.053	2.37 ± 0.049	2.7 ± 0.054	9.34 ± 1.2
		(0.15 - 0.19; 10)	(15 - 19; 10)	(1.03 - 5.8; 9)	(1.2 - 5.98; 9)	(1.16 - 1.29; 10)	(1.07 - 1.13; 10)	(1.36 - 1.42; 10)	(2.46 - 2.62; 10)	(2.25 - 2.42; 10)	(2.59 - 2.76; 10)	(6.13 - 10.55; 10)
106	43936	0.18 ± 0.02	18.5 ± 1.5	2.88 ± 1.07	3.06 ± 1.08	1.34 ± 0.021	1.15 ± 0.018	1.49 ± 0.018	2.78 ± 0.023	2.53 ± 0.039	2.91 ± 0.027	11.5 ± 0.87
		(0.16 - 0.2; 6)	(16 - 20; 6)	(1.85 - 5.18; 6)	(2.03 - 5.37; 6)	(1.3 - 1.37; 6)	(1.12 - 1.17; 6)	(1.46 - 1.52; 6)	(2.75 - 2.8; 6)	(2.47 - 2.58; 6)	(2.86 - 2.94; 6)	(10.14 - 12.7; 6)
107	43937	0.14 ± 0.01	15.22 ± 1.31	2.47 ± 0.48	2.61 ± 0.47	1.27 ± 0.009	1.17 ± 0.008	1.43 ± 0.012	2.69 ± 0.032	2.51 ± 0.018	2.85 ± 0.037	6.08 ± 1.05
		(0.13 - 0.16; 9)	(13 - 17; 9)	(1.68 - 2.98; 8)	(1.83 - 3.12; 8)	(1.26 - 1.29; 9)	(1.16 - 1.18; 9)	(1.41 - 1.45; 9)	(2.67 - 2.78; 9)	(2.49 - 2.55; 9)	(2.81 - 2.94; 9)	(4.66 - 8.4; 9)
108	43938	0.19 ± 0.01	21.1 ± 0.83	7.12 ± 1.11	7.31 ± 1.11	1.54 ± 0.006	1.36 ± 0.011	1.67 ± 0.013	2.92 ± 0.018	2.65 ± 0.009	3.15 ± 0.039	3.11 ± 1.35
		(0.18 - 0.2; 10)	(20 - 22; 10)	(5.55 - 8.8; 9)	(5.74 - 9; 9)	(1.54 - 1.55; 10)	(1.34 - 1.38; 10)	(1.65 - 1.69; 10)	(2.9 - 2.96; 10)	(2.64 - 2.66; 10)	(3.07 - 3.19; 10)	(1.39 - 5.52; 10)
109	43939	0.17 ± 0.01	18 ± 1.18	4 ± 0.89	4.17 ± 0.9	1.29 ± 0.034	1.13 ± 0.011	1.44 ± 0.009	2.63 ± 0.061	2.37 ± 0.026	2.82 ± 0.02	5.18 ± 1.24
		(0.15 - 0.2; 10)	(16 - 20; 10)	(2.79 - 5.4; 9)	(2.96 - 5.57; 9)	(1.23 - 1.33; 10)	(1.11 - 1.15; 10)	(1.42 - 1.45; 10)	(2.54 - 2.72; 10)	(2.31 - 2.4; 10)	(2.78 - 2.84; 10)	(3.41 - 8.16; 10)
110	43941	0.16 ± 0.02	16 ± 1.41	1.37 ± 0.77	1.53 ± 0.77	1.33 ± 0.005	1.22 ± 0.01	1.48 ± 0.009	2.77 ± 0.036	2.36 ± 0.032	2.89 ± 0.037	-0.83 ± 0.93
		(0.15 - 0.18; 3)	(15 - 18; 3)	(0.78 - 2.46; 3)	(0.93 - 2.61; 3)	(1.32 - 1.33; 3)	(1.21 - 1.23; 3)	(1.46 - 1.49; 3)	(2.72 - 2.81; 3)	(2.31 - 2.39; 3)	(2.84 - 2.93; 3)	(-1.82 - 0.41; 3)
111	43942	0.19 ± 0.01	19.8 ± 0.87	2.49 ± 0.9	2.67 ± 0.9	1.36 ± 0.009	1.25 ± 0.006	1.51 ± 0.01	2.77 ± 0.024	2.57 ± 0.036	2.93 ± 0.022	1.81 ± 0.53
		(0.18 - 0.2; 10)	(19 - 21; 10)	(1.38 - 4.42; 10)	(1.58 - 4.62; 10)	(1.35 - 1.38; 10)	(1.24 - 1.26; 10)	(1.5 - 1.53; 10)	(2.73 - 2.82; 10)	(2.51 - 2.62; 10)	(2.91 - 2.97; 10)	(0.81 - 2.72; 10)
112	43943	0.14 ± 0.01	15.67 ± 0.47	na	na	1.29 ± 0.013	1.13 ± 0.009	1.46 ± 0.01	2.58 ± 0.018	2.33 ± 0.048	2.82 ± 0.064	3.5 ± 1.02
		(0.14 - 0.15; 9)	(15 - 16; 9)			(1.28 - 1.31; 9)	(1.12 - 1.14; 9)	(1.44 - 1.46; 9)	(2.55 - 2.59; 9)	(2.27 - 2.39; 9)	(2.73 - 2.87; 9)	(2.2 - 4.7; 9)
113	43944	0.16 ± 0.01	17.7 ± 0.78	6.9 ± 2.05	7.06 ± 2.04	1.38 ± 0.011	1.25 ± 0.009	1.52 ± 0.008	2.72 ± 0.017	2.45 ± 0.02	2.9 ± 0.02	1.49 ± 1.39
		(0.15 - 0.18; 10)	(16 - 19; 10)	(4.22 - 10.43; 9)	(4.39 - 10.58; 9)	(1.36 - 1.4; 10)	(1.24 - 1.26; 10)	(1.51 - 1.53; 10)	(2.7 - 2.76; 10)	(2.43 - 2.49; 10)	(2.89 - 2.94; 10)	(-0.81 - 4.56; 10)

114	43945	0.14 ± 0.01 (0.1 - 0.16; 10)	15.6 ± 1.69 (11 - 17; 10)	4.56 ± 1.28 (2.6 - 6.54; 7)	4.7 ± 1.28 (2.74 - 6.69; 7)	1.34 ± 0.017 (1.3 - 1.36; 10)	1.22 ± 0.008 (1.21 - 1.23; 10)	1.49 ± 0.016 (1.46 - 1.52; 10)	2.7 ± 0.032 (2.63 - 2.73; 10)	2.31 ± 0.068 (2.14 - 2.38; 10)	2.85 ± 0.042 (2.76 - 2.9; 10)	-1.31 ± 2.25 (-5.56 - 2.65; 10)
115	43946	0.16 ± 0.01 (0.15 - 0.17; 10)	16.7 ± 0.64 (16 - 18; 10)	3.22 ± 1.33 (1.11 - 6.28; 9)	3.39 ± 1.33 (1.28 - 6.45; 9)	1.27 ± 0.015 (1.25 - 1.3; 10)	1.17 ± 0.013 (1.15 - 1.2; 10)	1.46 ± 0.025 (1.41 - 1.5; 10)	2.74 ± 0.027 (2.7 - 2.79; 10)	2.5 ± 0.046 (2.42 - 2.57; 10)	2.88 ± 0.03 (2.83 - 2.93; 10)	5.51 ± 1.81 (2.14 - 7.84; 10)
116	43947	0.18 ± 0.01 (0.18 - 0.19; 10)	19.8 ± 0.75 (18 - 21; 10)	11.41 ± 9.1 (3.51 - 31.92; 9)	11.59 ± 9.1 (3.7 - 32.09; 9)	1.31 ± 0.015 (1.28 - 1.33; 10)	1.18 ± 0.015 (1.14 - 1.2; 10)	1.46 ± 0.023 (1.42 - 1.51; 10)	2.61 ± 0.104 (2.47 - 2.78; 10)	2.35 ± 0.052 (2.29 - 2.49; 10)	2.8 ± 0.108 (2.61 - 2.96; 10)	3.22 ± 2.46 (0.37 - 7.6; 10)
117	43948	0.15 ± 0.01 (0.13 - 0.16; 10)	17 ± 0.89 (15 - 18; 10)	8.01 ± 1.11 (6.22 - 9.86; 8)	8.16 ± 1.11 (6.38 - 9.99; 8)	1.36 ± 0.024 (1.33 - 1.41; 10)	1.25 ± 0.017 (1.22 - 1.27; 10)	1.55 ± 0.016 (1.53 - 1.58; 10)	2.82 ± 0.034 (2.76 - 2.87; 10)	2.56 ± 0.082 (2.43 - 2.67; 10)	2.97 ± 0.049 (2.9 - 3.06; 10)	4.01 ± 2.69 (0.3 - 8.69; 10)
118	43949	0.15 ± 0.01 (0.13 - 0.18; 10)	16.2 ± 1.47 (14 - 19; 10)	6.41 ± 2.35 (4.04 - 10.57; 7)	6.56 ± 2.35 (4.2 - 10.7; 7)	1.29 ± 0.01 (1.27 - 1.3; 10)	1.18 ± 0.008 (1.17 - 1.2; 10)	1.43 ± 0.014 (1.4 - 1.44; 10)	2.71 ± 0.138 (2.55 - 2.89; 10)	2.26 ± 0.054 (2.19 - 2.37; 10)	2.9 ± 0.08 (2.77 - 2.99; 10)	-5.44 ± 1.54 (-7.14 - -1.43; 10)
119	43950	0.16 ± 0.01 (0.15 - 0.18; 6)	18.33 ± 0.94 (17 - 20; 6)	12.99 ± 6.46 (7.71 - 25.25; 5)	13.16 ± 6.46 (7.89 - 25.41; 5)	1.29 ± 0.012 (1.27 - 1.3; 6)	1.19 ± 0.013 (1.17 - 1.21; 6)	1.45 ± 0.026 (1.41 - 1.48; 6)	2.7 ± 0.034 (2.65 - 2.73; 6)	2.52 ± 0.084 (2.35 - 2.58; 6)	2.88 ± 0.046 (2.81 - 2.96; 6)	6.31 ± 2.94 (0.42 - 9.72; 6)
120	43951	0.16 ± 0.01 (0.15 - 0.18; 5)	18 ± 1.1 (17 - 20; 5)	12.1 ± 10.71 (2.35 - 32.18; 5)	12.26 ± 10.71 (2.51 - 32.32; 5)	1.33 ± 0.104 (1.21 - 1.46; 5)	1.15 ± 0.025 (1.11 - 1.17; 5)	1.51 ± 0.066 (1.41 - 1.58; 5)	2.69 ± 0.044 (2.63 - 2.75; 5)	2.39 ± 0.063 (2.29 - 2.47; 5)	2.85 ± 0.044 (2.79 - 2.9; 5)	5.47 ± 4.23 (-0.92 - 10.31; 5)
121	43952	0.16 ± 0.02 (0.14 - 0.19; 8)	18.13 ± 1.62 (16 - 21; 8)	24.53 ± 24.44 (2.36 - 77.67; 7)	24.69 ± 24.44 (2.53 - 77.85; 7)	1.35 ± 0.009 (1.33 - 1.37; 8)	1.24 ± 0.008 (1.23 - 1.25; 8)	1.51 ± 0.013 (1.49 - 1.53; 8)	2.8 ± 0.037 (2.73 - 2.86; 8)	2.61 ± 0.028 (2.57 - 2.66; 8)	2.94 ± 0.024 (2.9 - 2.98; 8)	7.75 ± 1.11 (5.75 - 9.6; 8)
122	43953	0.15 ± 0.01 (0.14 - 0.17; 10)	16.6 ± 1.5 (15 - 19; 10)	6.16 ± 2.19 (2.35 - 8.31; 5)	6.31 ± 2.19 (2.51 - 8.45; 5)	1.34 ± 0.026 (1.32 - 1.41; 10)	1.22 ± 0.023 (1.15 - 1.24; 10)	1.49 ± 0.023 (1.46 - 1.53; 10)	2.71 ± 0.046 (2.64 - 2.8; 10)	2.44 ± 0.091 (2.17 - 2.49; 10)	2.88 ± 0.043 (2.78 - 2.93; 10)	1.45 ± 2.73 (-2.41 - 5.78; 10)
123	43955	0.18 ± 0.01 (0.17 - 0.2; 10)	20.7 ± 1 (20 - 23; 10)	9.56 ± 9.36 (2.2 - 34.7; 9)	9.72 ± 9.37 (2.2 - 34.87; 9)	1.3 ± 0.013 (1.27 - 1.31; 10)	1.18 ± 0.009 (1.16 - 1.2; 10)	1.49 ± 0.031 (1.43 - 1.54; 10)	2.62 ± 0.036 (2.55 - 2.67; 10)	2.38 ± 0.025 (2.33 - 2.41; 10)	2.81 ± 0.045 (2.72 - 2.89; 10)	5.68 ± 1.22 (4.51 - 7.96; 10)
124	43956	0.14 ± 0.01 (0.12 - 0.16; 10)	17 ± 1.55 (14 - 19; 10)	3.76 ± 1.79 (1.52 - 8.4; 9)	3.9 ± 1.78 (1.66 - 8.52; 9)	1.38 ± 0.014 (1.36 - 1.4; 10)	1.21 ± 0.031 (1.16 - 1.26; 10)	1.55 ± 0.023 (1.5 - 1.58; 10)	2.72 ± 0.053 (2.63 - 2.78; 10)	2.56 ± 0.043 (2.47 - 2.62; 10)	2.87 ± 0.05 (2.79 - 2.94; 10)	11.24 ± 0.97 (9.5 - 13.1; 10)
125	43957	0.17 ± 0.01 (0.16 - 0.18; 10)	21.8 ± 0.87 (20 - 23; 10)	4.02 ± 2.65 (1.06 - 9.1; 10)	4.19 ± 2.65 (1.24 - 9.28; 10)	1.44 ± 0.019 (1.42 - 1.49; 10)	1.27 ± 0.034 (1.23 - 1.32; 10)	1.61 ± 0.019 (1.57 - 1.64; 10)	2.71 ± 0.058 (2.61 - 2.8; 10)	2.43 ± 0.17 (2.16 - 2.63; 10)	2.89 ± 0.058 (2.83 - 3.01; 10)	6.23 ± 3.14 (-0.23 - 10.58; 10)
126	43958	0.13 ± 0.01 (0.12 - 0.14; 8)	16.5 ± 0.87 (15 - 18; 8)	6.92 ± 2.72 (3.36 - 11.72; 6)	7.05 ± 2.73 (3.48 - 11.86; 6)	1.48 ± 0.026 (1.42 - 1.51; 8)	1.31 ± 0.012 (1.29 - 1.32; 8)	1.63 ± 0.01 (1.63 - 1.66; 8)	2.87 ± 0.029 (2.82 - 2.93; 8)	2.59 ± 0.046 (2.52 - 2.67; 8)	3.04 ± 0.025 (2.99 - 3.08; 8)	8.08 ± 0.89 (6.99 - 9.9; 8)
127	43959	0.16 ± 0.01 (0.15 - 0.17; 10)	18.8 ± 0.6 (18 - 20; 10)	4.44 ± 1.29 (3.08 - 6.73; 9)	4.6 ± 1.29 (3.23 - 6.89; 9)	1.37 ± 0.034 (1.3 - 1.39; 10)	1.22 ± 0.014 (1.18 - 1.24; 10)	1.5 ± 0.019 (1.45 - 1.52; 10)	2.76 ± 0.035 (2.66 - 2.79; 10)	2.58 ± 0.031 (2.49 - 2.61; 10)	2.9 ± 0.033 (2.81 - 2.93; 10)	6.34 ± 0.45 (5.37 - 6.93; 10)
128	43960	0.19 ± 0.01 (0.17 - 0.2; 10)	21.5 ± 0.81 (20 - 23; 10)	3.48 ± 0.79 (2.6 - 4.99; 8)	3.66 ± 0.79 (2.78 - 5.18; 8)	1.31 ± 0.011 (1.29 - 1.33; 10)	1.2 ± 0.014 (1.17 - 1.22; 10)	1.49 ± 0.018 (1.46 - 1.52; 10)	2.7 ± 0.054 (2.63 - 2.78; 10)	2.5 ± 0.096 (2.29 - 2.62; 10)	2.86 ± 0.051 (2.78 - 2.95; 10)	3.71 ± 1.8 (1.42 - 6.83; 10)
129	43961	0.17 ± 0.01 (0.16 - 0.19; 10)	19 ± 1.18 (17 - 21; 10)	1.76 ± 0.6 (1.06 - 3.09; 9)	1.94 ± 0.61 (1.22 - 3.28; 9)	1.4 ± 0.028 (1.37 - 1.45; 10)	1.28 ± 0.017 (1.25 - 1.3; 10)	1.65 ± 0.029 (1.61 - 1.7; 10)	2.93 ± 0.039 (2.9 - 3.01; 10)	2.78 ± 0.024 (2.75 - 2.83; 10)	3.1 ± 0.041 (3.05 - 3.15; 10)	11.06 ± 1.23 (8.87 - 13.72; 10)
130	43962	0.17 ± 0.01 (0.14 - 0.2; 10)	19.8 ± 1.25 (17 - 21; 10)	1.84 ± 0.49 (1.22 - 2.44; 9)	2.01 ± 0.5 (1.39 - 2.61; 9)	1.43 ± 0.013 (1.42 - 1.46; 10)	1.3 ± 0.027 (1.23 - 1.32; 10)	1.6 ± 0.017 (1.57 - 1.63; 10)	2.99 ± 0.066 (2.87 - 3.07; 10)	2.69 ± 0.031 (2.65 - 2.76; 10)	3.14 ± 0.044 (3.07 - 3.19; 10)	5.54 ± 2 (3.87 - 11; 10)
131	43963	0.16 ± 0.01 (0.14 - 0.17; 7)	17.43 ± 1.4 (15 - 19; 7)	1.96 ± 0.84 (1.06 - 3.2; 6)	2.12 ± 0.83 (1.22 - 3.35; 6)	1.34 ± 0.016 (1.3 - 1.36; 7)	1.22 ± 0.017 (1.184 - 1.24; 7)	1.48 ± 0.011 (1.464 - 1.5; 7)	2.74 ± 0.033 (2.69 - 2.78; 7)	2.5 ± 0.067 (2.41 - 2.59; 7)	2.88 ± 0.03 (2.84 - 2.92; 7)	6.42 ± 2.67 (2.22 - 10.26; 7)
132	43964	0.14 ± 0.02 (0.12 - 0.16; 6)	17.83 ± 1.21 (16 - 19; 6)	15.86 ± 9.31 (4.75 - 27.04; 4)	16 ± 9.3 (4.9 - 27.17; 4)	1.19 ± 0.012 (1.17 - 1.21; 6)	1.09 ± 0.017 (1.06 - 1.11; 6)	1.37 ± 0.028 (1.31 - 1.4; 6)	2.51 ± 0.04 (2.48 - 2.59; 6)	2.2 ± 0.077 (2.12 - 2.36; 6)	2.64 ± 0.034 (2.59 - 2.7; 6)	5.11 ± 2.55 (2.29 - 8.65; 6)
133	43965	0.15 ± 0.02 (0.11 - 0.17; 10)	18.7 ± 2.1 (14 - 21; 10)	10.9 ± 8.59 (1.98 - 27.87; 9)	11.06 ± 8.59 (2.16 - 28.04; 9)	1.29 ± 0.021 (1.26 - 1.31; 10)	1.16 ± 0.016 (1.14 - 1.2; 10)	1.46 ± 0.013 (1.43 - 1.48; 10)	2.59 ± 0.048 (2.51 - 2.65; 10)	2.31 ± 0.044 (2.26 - 2.39; 10)	2.78 ± 0.035 (2.7 - 2.81; 10)	1.79 ± 1.7 (-1.13 - 5.29; 10)
134	43967	0.2 ± 0.01 (0.18 - 0.22; 10)	22.8 ± 0.98 (21 - 24; 10)	2.32 ± 0.56 (1.82 - 3.48; 10)	2.52 ± 0.56 (2.04 - 3.68; 10)	1.19 ± 0.068 (1.1 - 1.25; 10)	1.04 ± 0.014 (1.01 - 1.06; 10)	1.37 ± 0.015 (1.32 - 1.38; 10)	2.34 ± 0.016 (2.33 - 2.38; 10)	2.06 ± 0.035 (2 - 2.11; 10)	2.54 ± 0.036 (2.47 - 2.57; 10)	5.65 ± 1.69 (1.37 - 6.99; 10)
135	43968	0.12 ± 0.02 (0.09 - 0.14; 10)	14.8 ± 2.09 (12 - 18; 10)	6.55 ± 2.89 (3.18 - 13.3; 9)	6.67 ± 2.89 (3.32 - 13.43; 9)	1.21 ± 0.019 (1.16 - 1.23; 10)	1.11 ± 0.022 (1.08 - 1.14; 10)	1.37 ± 0.02 (1.34 - 1.4; 10)	2.46 ± 0.063 (2.35 - 2.6; 10)	2.14 ± 0.1 (1.97 - 2.26; 10)	2.65 ± 0.077 (2.54 - 2.76; 10)	-1.5 ± 1.79 (-4.78 - 1.7; 10)
136	43969	0.1 ± 0.02 (0.08 - 0.14; 10)	14.13 ± 2.76 (10 - 18; 10)	3.26 ± 1.07 (0.99 - 4.34; 6)	3.37 ± 1.07 (1.08 - 4.45; 6)	1.17 ± 0.032 (1.12 - 1.21; 10)	1.07 ± 0.027 (1.03 - 1.11; 10)	1.33 ± 0.027 (1.28 - 1.36; 10)	2.4 ± 0.034 (2.35 - 2.45; 10)	2.1 ± 0.076 (2.01 - 2.22; 10)	2.61 ± 0.048 (2.51 - 2.68; 10)	-2.75 ± 3.52 (-7.61 - 3.2; 10)
137	43970	0.08 ± 0.01	11.38 ± 1.49	11.37 ± 4.87	11.46 ± 4.87	1.16 ± 0.007	1.08 ± 0.007	1.34 ± 0.019	2.42 ± 0.057	2.19 ± 0.057	2.55 ± 0.031	4.75 ± 2.05

138	43971	(0.07 - 0.1; 8)	(9 - 14; 8)	(4.17 - 20.07; 7)	(4.25 - 20.15; 7)	(1.15 - 1.17; 8)	(1.07 - 1.09; 8)	(1.31 - 1.36; 8)	(2.31 - 2.48; 8)	(2.08 - 2.26; 8)	(2.51 - 2.61; 8)	(2.03 - 7.33; 8)
		0.09 ± 0.01	10.5 ± 1.5	19.78 ± 19.33	19.87 ± 19.32	1.21 ± 0.061	1.06 ± 0.023	1.36 ± 0.024	2.39 ± 0.108	2.05 ± 0.121	2.55 ± 0.059	0.38 ± 5.14
139	43972	(0.08 - 0.12; 7)	(9 - 12; 7)	(1.24 - 46.24; 4)	(1.36 - 46.33; 4)	(1.14 - 1.29; 7)	(1.03 - 1.1; 7)	(1.32 - 1.4; 7)	(2.14 - 2.49; 7)	(1.88 - 2.2; 7)	(2.44 - 2.66; 7)	(-9.28 - 5.45; 7)
		0.14 ± 0.02	19.6 ± 2.37	2.79 ± 1.2	2.93 ± 1.21	1.15 ± 0.024	1.04 ± 0.016	1.35 ± 0.03	2.38 ± 0.061	2.12 ± 0.034	2.55 ± 0.07	4.12 ± 1.44
140	43973	(0.1 - 0.18; 10)	(14 - 23; 10)	(1.07 - 5.26; 8)	(1.2 - 5.41; 8)	(1.12 - 1.21; 10)	(1.02 - 1.08; 10)	(1.3 - 1.4; 10)	(2.28 - 2.48; 10)	(2.06 - 2.17; 10)	(2.44 - 2.66; 10)	(2.23 - 6.31; 10)
		0.08 ± 0	11.33 ± 0.47	na	na	1.19 ± 0.018	1.11 ± 0.013	1.36 ± 0.022	2.53 ± 0.033	2.23 ± 0.097	2.7 ± 0.018	-1.6 ± 1.13
141	43974	(0.08 - 0.09; 3)	(11 - 12; 3)			(1.16 - 1.21; 3)	(1.09 - 1.12; 3)	(1.34 - 1.39; 3)	(2.48 - 2.56; 3)	(2.09 - 2.3; 3)	(2.67 - 2.71; 3)	(-3.19 - -0.72; 3)
		0.08 ± 0.01	11.25 ± 0.83	18.25 ± 17.5	18.33 ± 17.5	1.15 ± 0.016	1.06 ± 0.013	1.36 ± 0.025	2.43 ± 0.038	2.24 ± 0.048	2.6 ± 0.04	1.84 ± 3.34
142	43966	(0.07 - 0.09; 8)	(10 - 12; 8)	(2.82 - 43.9; 6)	(2.89 - 43.96; 6)	(1.13 - 1.18; 8)	(1.04 - 1.09; 8)	(1.34 - 1.41; 8)	(2.4 - 2.48; 8)	(2.17 - 2.33; 8)	(2.54 - 2.68; 8)	(-3.51 - 6.98; 8)
		0.16 ± 0.01	19.1 ± 0.7	1.42 ± 0.28	1.58 ± 0.28	1.39 ± 0.028	1.22 ± 0.009	1.55 ± 0.011	2.78 ± 0.024	2.61 ± 0.03	3 ± 0.013	7.73 ± 0.81
143	43975	(0.14 - 0.17; 10)	(18 - 20; 10)	(0.96 - 1.87; 10)	(1.13 - 2.04; 10)	(1.35 - 1.45; 10)	(1.21 - 1.24; 10)	(1.53 - 1.56; 10)	(2.75 - 2.82; 10)	(2.53 - 2.64; 10)	(2.97 - 3.01; 10)	(6.32 - 9.27; 10)
		0.17 ± 0.01	24.5 ± 1.63	2.05 ± 0.71	2.23 ± 0.71	1.41 ± 0.017	1.32 ± 0.014	1.62 ± 0.023	2.99 ± 0.028	2.64 ± 0.085	3.14 ± 0.023	1.73 ± 2.71
144	43976	(0.15 - 0.2; 10)	(22 - 28; 10)	(0.82 - 3.21; 9)	(0.99 - 3.39; 9)	(1.39 - 1.44; 10)	(1.3 - 1.35; 10)	(1.59 - 1.67; 10)	(2.95 - 3.05; 10)	(2.52 - 2.77; 10)	(3.11 - 3.19; 10)	(-0.85 - 7.97; 10)
		0.13 ± 0	18 ± 0.63	8.59 ± 12.29	8.71 ± 12.29	1.34 ± 0.038	1.15 ± 0.017	1.49 ± 0.019	2.44 ± 0.098	2.19 ± 0.037	2.78 ± 0.031	1.89 ± 1.78
145	43977	(0.13 - 0.14; 6)	(17 - 19; 6)	(1.16 - 29.86; 4)	(1.29 - 29.99; 4)	(1.27 - 1.38; 6)	(1.12 - 1.17; 6)	(1.45 - 1.51; 6)	(2.34 - 2.64; 6)	(2.13 - 2.25; 6)	(2.72 - 2.81; 6)	(-1.14 - 4.08; 6)
		0.1 ± 0.02	13.5 ± 2.5	7.73 ± 7.55	7.83 ± 7.54	1.23 ± 0.027	1.14 ± 0.023	1.46 ± 0.058	2.55 ± 0.211	2.25 ± 0.179	2.79 ± 0.077	1.14 ± 6.19
146	43978	(0.06 - 0.11; 6)	(8 - 15; 6)	(1.25 - 22.49; 6)	(1.36 - 22.6; 6)	(1.17 - 1.26; 6)	(1.09 - 1.15; 6)	(1.35 - 1.53; 6)	(2.09 - 2.68; 6)	(1.87 - 2.38; 6)	(2.64 - 2.89; 6)	(-11.53 - 6.64; 6)
		0.1 ± 0.01	13 ± 2.37	2.66 ± 2.28	2.76 ± 2.29	1.26 ± 0.01	1.17 ± 0.01	1.43 ± 0.033	2.64 ± 0.072	2.21 ± 0.072	2.81 ± 0.044	-6.22 ± 2.46
147	43979	(0.07 - 0.12; 10)	(9 - 17; 10)	(0.96 - 8.84; 9)	(1.05 - 8.97; 9)	(1.25 - 1.28; 10)	(1.15 - 1.18; 10)	(1.37 - 1.49; 10)	(2.53 - 2.71; 10)	(2.09 - 2.34; 10)	(2.72 - 2.87; 10)	(-11.01 - -3.06; 10)
		0.12 ± 0.01	15.9 ± 0.83	3.46 ± 1.11	3.58 ± 1.06	1.42 ± 0.017	1.28 ± 0.026	1.72 ± 0.016	2.88 ± 0.047	2.67 ± 0.054	3.04 ± 0.047	10.92 ± 1.43
148	43980	(0.11 - 0.13; 10)	(15 - 17; 10)	(1.8 - 5.31; 9)	(1.92 - 5.43; 9)	(1.4 - 1.45; 10)	(1.23 - 1.31; 10)	(1.69 - 1.74; 10)	(2.79 - 2.98; 10)	(2.52 - 2.71; 10)	(2.95 - 3.12; 10)	(7.74 - 13.32; 10)
		0.19 ± 0.02	24.75 ± 2.49	2.27 ± 1.01	2.45 ± 1	1.34 ± 0.008	1.24 ± 0.005	1.67 ± 0.084	2.71 ± 0.024	2.44 ± 0.067	2.91 ± 0.027	4.87 ± 1.46
149	43981	(0.16 - 0.21; 4)	(21 - 28; 4)	(1.17 - 3.86; 4)	(1.35 - 4.02; 4)	(1.34 - 1.36; 4)	(1.24 - 1.25; 4)	(1.58 - 1.81; 4)	(2.68 - 2.74; 4)	(2.34 - 2.52; 4)	(2.89 - 2.95; 4)	(2.78 - 6.53; 4)
		0.17 ± 0.01	22.2 ± 1.54	2.02 ± 0.52	2.18 ± 0.52	1.39 ± 0.02	1.27 ± 0.028	1.64 ± 0.012	2.82 ± 0.081	2.54 ± 0.052	3 ± 0.024	-0.04 ± 2.55
150	43982	(0.15 - 0.19; 10)	(20 - 26; 10)	(1.11 - 2.83; 8)	(1.28 - 3; 8)	(1.37 - 1.43; 10)	(1.22 - 1.3; 10)	(1.61 - 1.66; 10)	(2.69 - 2.9; 10)	(2.42 - 2.62; 10)	(2.96 - 3.04; 10)	(-3.99 - 3.72; 10)
		0.14 ± 0.01	18.17 ± 1.07	2.58 ± 0.41	2.73 ± 0.41	1.33 ± 0.019	1.2 ± 0.012	1.47 ± 0.016	2.67 ± 0.064	2.43 ± 0.033	2.81 ± 0.041	8.99 ± 1.21
151	43983	(0.13 - 0.15; 10)	(17 - 20; 10)	(2.09 - 3.45; 9)	(2.22 - 3.59; 9)	(1.3 - 1.37; 10)	(1.17 - 1.22; 10)	(1.44 - 1.49; 10)	(2.49 - 2.72; 10)	(2.36 - 2.48; 10)	(2.76 - 2.87; 10)	(6.64 - 10.67; 10)
		0.15 ± 0	na	2.29	2.44	1.31 ± 0.011	1.21 ± 0	1.49 ± 0	2.72 ± 0.011	2.44 ± 0	2.85 ± 0.022	6.77 ± 0.6
152	43985	(0.15 - 0.15; 2)		(1)	(1)	(1.31 - 1.32; 2)	(1.21 - 1.21; 2)	(1.49 - 1.49; 2)	(2.71 - 2.73; 2)	(2.44 - 2.44; 2)	(2.83 - 2.87; 2)	(6.16 - 7.37; 2)
		0.17 ± 0.01	20.13 ± 1.69	3.08 ± 0.53	3.25 ± 0.52	1.28 ± 0.066	1.14 ± 0.013	1.48 ± 0.061	2.64 ± 0.031	2.48 ± 0.026	2.83 ± 0.025	10.27 ± 4.48
153	43986	(0.14 - 0.19; 10)	(18 - 22; 10)	(2.06 - 3.51; 5)	(2.24 - 3.69; 5)	(1.23 - 1.42; 10)	(1.12 - 1.15; 10)	(1.4 - 1.56; 10)	(2.59 - 2.69; 10)	(2.44 - 2.52; 10)	(2.79 - 2.87; 10)	(3.73 - 15.84; 10)
		0.14 ± 0.01	19.33 ± 1.7	3.16 ± 1.95	3.31 ± 1.96	1.33 ± 0.018	1.22 ± 0.018	1.52 ± 0.026	2.78 ± 0.05	2.6 ± 0.037	2.93 ± 0.048	10.15 ± 0.97
154	43987	(0.13 - 0.15; 3)	(17 - 21; 3)	(0.95 - 5.69; 3)	(1.08 - 5.85; 3)	(1.3 - 1.35; 3)	(1.2 - 1.24; 3)	(1.49 - 1.55; 3)	(2.71 - 2.83; 3)	(2.55 - 2.64; 3)	(2.86 - 2.97; 3)	(8.83 - 11.14; 3)
		0.12 ± 0.01	15.7 ± 1.68	2.65 ± 0.81	2.77 ± 0.81	1.23 ± 0.013	1.12 ± 0.012	1.49 ± 0.05	2.72 ± 0.028	2.5 ± 0.06	2.87 ± 0.034	7.27 ± 2.81
155	43988	(0.09 - 0.13; 10)	(13 - 18; 10)	(1.78 - 4.52; 9)	(1.9 - 4.65; 9)	(1.21 - 1.25; 10)	(1.1 - 1.14; 10)	(1.36 - 1.53; 10)	(2.68 - 2.79; 10)	(2.35 - 2.58; 10)	(2.8 - 2.94; 10)	(-0.53 - 9.62; 10)
		0.15 ± 0.01	19.9 ± 0.83	2.66 ± 0.71	2.81 ± 0.71	1.33 ± 0.003	1.22 ± 0.007	1.56 ± 0.014	2.85 ± 0.058	2.65 ± 0.017	3.02 ± 0.025	9.12 ± 0.94
156	43989	(0.15 - 0.16; 10)	(19 - 21; 10)	(1.7 - 4.36; 9)	(1.86 - 4.52; 9)	(1.31 - 1.32; 10)	(1.21 - 1.23; 10)	(1.55 - 1.59; 10)	(2.76 - 2.93; 10)	(2.63 - 2.68; 10)	(2.98 - 3.06; 10)	(7.34 - 11.06; 10)
		0.15 ± 0.02	18.4 ± 2.11	2.48 ± 1.31	2.63 ± 1.32	1.25 ± 0.013	1.16 ± 0.013	1.42 ± 0.034	2.63 ± 0.031	2.45 ± 0.055	2.79 ± 0.046	6.21 ± 2.19
157	43990	(0.11 - 0.16; 10)	(14 - 21; 10)	(1.03 - 5.63; 9)	(1.15 - 5.79; 9)	(1.24 - 1.27; 10)	(1.14 - 1.18; 10)	(1.37 - 1.49; 10)	(2.58 - 2.68; 10)	(2.36 - 2.53; 10)	(2.73 - 2.87; 10)	(2.77 - 10.52; 10)
		0.13 ± 0.02	16.2 ± 2.71	1.93 ± 0.64	2.05 ± 0.63	1.3 ± 0.012	1.2 ± 0.008	1.42 ± 0.017	2.73 ± 0.121	2.45 ± 0.042	2.93 ± 0.113	-1.21 ± 1.4
158	43991	(0.09 - 0.15; 10)	(11 - 20; 10)	(1.39 - 3.2; 8)	(1.51 - 3.33; 8)	(1.27 - 1.31; 10)	(1.18 - 1.21; 10)	(1.38 - 1.44; 10)	(2.55 - 2.94; 10)	(2.34 - 2.49; 10)	(2.75 - 3.06; 10)	(-4.2 - 1.32; 10)
		0.15 ± 0.01	20.83 ± 2.19	1.81 ± 0.32	1.97 ± 0.31	1.3 ± 0.027	1.2 ± 0.029	1.54 ± 0.098	2.64 ± 0.125	2.3 ± 0.136	2.84 ± 0.148	-2.49 ± 5.66
159	43992	(0.14 - 0.17; 6)	(18 - 24; 6)	(1.24 - 2.12; 5)	(1.4 - 2.28; 5)	(1.27 - 1.34; 6)	(1.16 - 1.25; 6)	(1.42 - 1.7; 6)	(2.55 - 2.92; 6)	(2.05 - 2.45; 6)	(2.71 - 3.06; 6)	(-12.82 - 2.28; 6)
		0.13 ± 0.01	18.5 ± 0.5	3.03 ± 1.27	3.16 ± 1.27	1.34 ± 0.063	1.18 ± 0.014	1.55 ± 0.07	2.53 ± 0.017	2.3 ± 0.035	2.85 ± 0.027	-0.14 ± 0.42
160	43993	(0.11 - 0.14; 4)	(18 - 19; 4)	(0.97 - 4.23; 4)	(1.1 - 4.35; 4)	(1.26 - 1.41; 4)	(1.16 - 1.2; 4)	(1.51 - 1.67; 4)	(2.51 - 2.55; 4)	(2.25 - 2.35; 4)	(2.82 - 2.9; 4)	(-0.63 - 0.54; 4)
		0.15 ± 0.01	20.67 ± 1.25	2.14 ± 0.82	2.29 ± 0.81	1.38 ± 0.063	1.23 ± 0.05	1.66 ± 0.05	2.64 ± 0.207	2.26 ± 0.214	3.07 ± 0.128	5.31 ± 0.69
		(0.13 - 0.16; 4)	(19 - 22; 4)	(1.57 - 3.56; 4)	(1.73 - 3.69; 4)	(1.29 - 1.46; 4)	(1.16 - 1.29; 4)	(1.61 - 1.73; 4)	(2.31 - 2.89; 4)	(2.09 - 2.63; 4)	(2.96 - 3.28; 4)	(4.61 - 6.42; 4)

161	43994	0.18 ± 0 (0.17 - 0.18; 8)	22.88 ± 0.33 (22 - 23; 8)	4.79 ± 2.44 (2.43 - 9.59; 7)	4.96 ± 2.44 (2.61 - 9.76; 7)	1.36 ± 0.013 (1.33 - 1.38; 8)	1.22 ± 0.013 (1.21 - 1.25; 8)	1.54 ± 0.012 (1.52 - 1.55; 8)	2.76 ± 0.06 (2.66 - 2.84; 8)	2.56 ± 0.017 (2.53 - 2.57; 8)	2.96 ± 0.027 (2.92 - 2.99; 8)	7.35 ± 0.88 (5.92 - 8.88; 8)
162	43995	0.17 ± 0.01 (0.15 - 0.19; 8)	24.75 ± 1.79 (22 - 28; 8)	3.46 ± 1.28 (1.96 - 5.56; 8)	3.63 ± 1.27 (2.14 - 5.73; 8)	1.3 ± 0.006 (1.29 - 1.31; 8)	1.21 ± 0.01 (1.2 - 1.23; 8)	1.6 ± 0.027 (1.56 - 1.64; 8)	2.78 ± 0.0128 (2.55 - 2.93; 8)	2.49 ± 0.058 (2.37 - 2.54; 8)	2.99 ± 0.044 (2.91 - 3.04; 8)	5.05 ± 2.29 (2.22 - 9.66; 8)
163	43996	0.18 ± 0.01 (0.17 - 0.2; 7)	23.71 ± 1.39 (21 - 26; 7)	7.36 ± 7.58 (2.25 - 23.14; 6)	7.54 ± 7.58 (2.43 - 23.32; 6)	1.47 ± 0.065 (1.37 - 1.55; 7)	1.32 ± 0.027 (1.27 - 1.36; 7)	1.67 ± 0.032 (1.63 - 1.72; 7)	2.96 ± 0.064 (2.86 - 3.07; 7)	2.64 ± 0.049 (2.54 - 2.71; 7)	3.17 ± 0.047 (3.11 - 3.23; 7)	7.45 ± 2.7 (3.92 - 12.57; 7)
164	43997	0.19 ± 0.02 (0.17 - 0.23; 8)	24.25 ± 3.11 (19 - 30; 8)	7.89 ± 4.17 (2.04 - 14.13; 6)	8.09 ± 4.17 (2.28 - 14.33; 6)	1.51 ± 0.048 (1.41 - 1.56; 8)	1.33 ± 0.009 (1.31 - 1.35; 8)	1.7 ± 0.026 (1.67 - 1.75; 8)	2.9 ± 0.044 (2.82 - 2.96; 8)	2.64 ± 0.036 (2.57 - 2.7; 8)	3.2 ± 0.048 (3.13 - 3.31; 8)	7.85 ± 2.52 (3.92 - 11.1)
165	43998	0.17 ± 0.01 (0.16 - 0.18; 10)	20.3 ± 0.9 (19 - 22; 10)	3.01 ± 0.68 (2.04 - 4.37; 8)	3.18 ± 0.68 (2.2 - 4.53; 8)	1.32 ± 0.033 (1.28 - 1.38; 10)	1.2 ± 0.007 (1.2 - 1.22; 10)	1.49 ± 0.017 (1.46 - 1.53; 10)	2.69 ± 0.02 (2.65 - 2.72; 10)	2.54 ± 0.039 (2.43 - 2.57; 10)	2.88 ± 0.036 (2.83 - 2.97; 10)	11.57 ± 1.72 (8.31 - 14.47; 10)
166	43999	0.15 ± 0.01 (0.15 - 0.17; 7)	19.29 ± 0.88 (18 - 21; 7)	4.21 ± 2.23 (1.93 - 6.95; 5)	4.36 ± 2.23 (2.1 - 7.1; 5)	1.42 ± 0.097 (1.27 - 1.52; 7)	1.17 ± 0.026 (1.14 - 1.23; 7)	1.61 ± 0.071 (1.44 - 1.66; 7)	2.67 ± 0.044 (2.61 - 2.75; 7)	2.49 ± 0.049 (2.43 - 2.61; 7)	2.87 ± 0.058 (2.79 - 2.96; 7)	12.1 ± 2.2 (8.13 - 15.4; 7)
167	44000	0.19 ± 0.02 (0.16 - 0.22; 10)	21.6 ± 2.87 (16 - 25; 10)	2.74 ± 0.91 (1.04 - 3.75; 9)	2.94 ± 0.91 (1.22 - 3.91; 9)	1.29 ± 0.009 (1.28 - 1.3; 10)	1.18 ± 0.007 (1.17 - 1.2; 10)	1.42 ± 0.011 (1.41 - 1.44; 10)	2.69 ± 0.046 (2.62 - 2.75; 10)	2.31 ± 0.077 (2.15 - 2.4; 10)	2.85 ± 0.043 (2.78 - 2.93; 10)	0.41 ± 1.89 (-2.7 - 3.52; 10)
168	44001	0.18 ± 0.01 (0.16 - 0.19; 5)	21.2 ± 1.17 (20 - 23; 5)	4.19 ± 2.35 (1.63 - 7.3; 3)	4.38 ± 2.35 (1.81 - 7.49; 3)	1.31 ± 0.042 (1.27 - 1.39; 5)	1.18 ± 0.008 (1.16 - 1.18; 5)	1.52 ± 0.036 (1.45 - 1.55; 5)	2.74 ± 0.014 (2.71 - 2.76; 5)	2.58 ± 0.029 (2.52 - 2.59; 5)	2.88 ± 0.017 (2.85 - 2.91; 5)	13.3 ± 3.18 (7.61 - 16.48; 5)
169	44002	0.16 ± 0 (0.16 - 0.16; 2)	22 ± 0 (22 - 22; 2)	10.96 ± 1.09 (9.87 - 12.06; 2)	11.13 ± 1.1 (10.03 - 12.22; 2)	1.31 ± 0.005 (1.3 - 1.31; 2)	1.21 ± 0.005 (1.21 - 1.22; 2)	1.48 ± 0.027 (1.45 - 1.51; 2)	2.82 ± 0.022 (2.8 - 2.84; 2)	2.57 ± 0.027 (2.54 - 2.59; 2)	3.06 ± 0.022 (3.04 - 3.08; 2)	2.13 ± 1.07 (1.06 - 3.2; 2)
170	44003	0.12 ± 0.02 (0.08 - 0.15; 10)	14.2 ± 3.43 (10 - 19; 10)	1.75 ± 0.76 (0.94 - 3.49; 9)	1.86 ± 0.75 (1.07 - 3.58; 9)	1.31 ± 0.02 (1.27 - 1.34; 10)	1.2 ± 0.016 (1.17 - 1.23; 10)	1.47 ± 0.047 (1.39 - 1.55; 10)	2.7 ± 0.06 (2.63 - 2.81; 10)	2.38 ± 0.145 (2.16 - 2.59; 10)	2.83 ± 0.054 (2.77 - 2.92; 10)	-1.09 ± 5.31 (-7.75 - 7.56; 10)

Table S2. Air temperature information for each studied population during the sampling days. We encountered a problem with our data logger during the Picinguaba expedition, resulting in the loss of temperature data for this population. Since the temporal properties of the advertisement call were temperature-dependent, we utilized the mean temperature of all populations (25.4 °C) to correct these properties.

Population	Mean (°C)	SD	Minimum (°C)	Maximum (°C)
Angra dos Reis	24.4	1.6	22.5	27.9
Bertioga	23.9	1.0	21.8	25.9
Gipóia island	29.4	1.4	27.6	31.5
Itacuruçá island	27.7	2.7	24.4	32.2
Marambaia island	22.8	4.0	18.0	28.1
Picinguaba	na	na	na	na
Seropédica	28.0	2.5	24.0	34.0

Table S3. Mean of color, call, and body condition measurements from evaluated populations. Abbreviations: SVL – Snout-Vent Length; SMI – Scaled Mass Index. Values are presented as mean \pm standard deviation (range; number of calls).

	Angra dos Reis	Bertioga	Gipóia island	Itacuruçá island	Marambaia island	Picinguaba	Seropédica
<i>Color traits</i>							
Patch relative size	58.36 \pm 8.03 (39.88 - 73.18; 29)	46.34 \pm 9.85 (31.62 - 73.41; 30)	57.62 \pm 11.13 (42.65 - 83.44; 10)	50.26 \pm 10.84 (24.95 - 71.45; 13)	55.68 \pm 6.45 (40.04 - 67.57; 27)	42.35 \pm 8.21 (20.6 - 59.07; 30)	42.64 \pm 18.46 (0 - 65.99; 31)
Orange hue value	0.31 \pm 0.03 (0.22 - 0.36; 29)	0.29 \pm 0.03 (0.25 - 0.34; 19)	0.25 \pm 0.03 (0.21 - 0.3; 10)	0.27 \pm 0.04 (0.21 - 0.32; 10)	0.3 \pm 0.03 (0.25 - 0.36; 27)	0.3 \pm 0.04 (0.2 - 0.39; 28)	0.37 \pm 0.09 (0.25 - 0.62; 31)
Green hue value	0.63 \pm 0.05 (0.54 - 0.74; 29)	0.63 \pm 0.06 (0.54 - 0.78; 19)	0.71 \pm 0.1 (0.55 - 0.89; 10)	0.62 \pm 0.06 (0.56 - 0.75; 10)	0.6 \pm 0.07 (0.43 - 0.73; 27)	0.64 \pm 0.05 (0.54 - 0.77; 28)	0.68 \pm 0.06 (0.56 - 0.77; 31)
Contrast hue value	-0.35 \pm 0.05 (-0.53 - -0.24; 29)	-0.37 \pm 0.05 (-0.49 - -0.26; 19)	-0.48 \pm 0.04 (-0.58 - -0.43; 10)	-0.39 \pm 0.06 (-0.51 - -0.3; 10)	-0.33 \pm 0.06 (-0.45 - -0.17; 27)	-0.36 \pm 0.07 (-0.57 - -0.18; 28)	-0.3 \pm 0.11 (-0.44 - -0.01; 31)
<i>Call properties</i>							
Call duration (ms)	148.78 \pm 24.52 (101 - 190.22; 29)	148.09 \pm 25.51 (83.02 - 187.55; 30)	130.96 \pm 40.31 (84.93 - 213.47; 10)	149.82 \pm 33.32 (93.31 - 240.65; 13)	155.72 \pm 17.57 (124.99 - 194.46; 27)	135.65 \pm 29.68 (69.76 - 194.8; 30)	140.53 \pm 21.9 (99.16 - 180.13; 31)
Pulses per call	19.75 \pm 3.31 (13 - 24.75; 29)	17.53 \pm 2.69 (10.6 - 21.43; 30)	15.23 \pm 4.06 (10.5 - 22.8; 10)	17.38 \pm 3.39 (13 - 26.2; 13)	18.07 \pm 1.83 (15.22 - 21.8; 27)	16.44 \pm 3.6 (8.5 - 22.63; 30)	16.76 \pm 2.67 (12.3 - 23.5; 31)
Intercall interval (s)	3.73 \pm 2.36 (1.43 - 10.94; 29)	4.42 \pm 3.77 (1.36 - 17.34; 30)	10.14 \pm 6.4 (2.35 - 19.8; 10)	9.92 \pm 6.71 (1.35 - 19.55; 13)	6.03 \pm 4.9 (1.33 - 24.49; 27)	7.55 \pm 5.05 (3.17 - 28.73; 30)	3.95 \pm 1.77 (1.37 - 9.66; 31)
Call period (s)	3.88 \pm 2.36 (1.6 - 11.1; 29)	4.61 \pm 3.77 (1.6 - 17.47; 30)	9.26 \pm 6.79 (0.04 - 19.9; 10)	10.07 \pm 6.7 (1.48 - 19.7; 13)	5.95 \pm 4.94 (0.02 - 24.62; 27)	7.7 \pm 5.04 (3.32 - 28.87; 30)	4.1 \pm 1.78 (1.5 - 9.86; 31)
First band dominant frequency (kHz)	1.35 \pm 0.05 (1.26 - 1.51; 29)	1.2 \pm 0.05 (1.1 - 1.29; 30)	1.28 \pm 0.05 (1.22 - 1.4; 10)	1.28 \pm 0.06 (1.17 - 1.42; 13)	1.34 \pm 0.06 (1.22 - 1.5; 27)	1.25 \pm 0.07 (1.12 - 1.41; 30)	1.31 \pm 0.07 (1.19 - 1.48; 31)
First band minimum frequency (kHz)	1.21 \pm 0.05 (1.12 - 1.33; 29)	1.09 \pm 0.06 (0.89 - 1.21; 30)	1.08 \pm 0.04 (1.04 - 1.16; 10)	1.16 \pm 0.04 (1.09 - 1.21; 13)	1.21 \pm 0.06 (1.1 - 1.36; 27)	1.13 \pm 0.07 (0.99 - 1.27; 30)	1.22 \pm 0.07 (1.1 - 1.37; 31)
First band maximum frequency (kHz)	1.54 \pm 0.09 (1.42 - 1.72; 29)	1.41 \pm 0.06 (1.3 - 1.61; 30)	1.37 \pm 0.03 (1.33 - 1.46; 10)	1.49 \pm 0.07 (1.36 - 1.63; 13)	1.51 \pm 0.07 (1.4 - 1.67; 27)	1.42 \pm 0.08 (1.24 - 1.58; 30)	1.6 \pm 0.08 (1.48 - 1.8; 31)
Second band dominant frequency (kHz)	2.75 \pm 0.1 (2.55 - 2.9; 29)	2.55 \pm 0.11 (2.37 - 2.83; 30)	2.62 \pm 0.08 (2.5 - 2.79; 10)	2.6 \pm 0.12 (2.28 - 2.74; 13)	2.72 \pm 0.09 (2.55 - 2.93; 27)	2.55 \pm 0.13 (2.26 - 2.77; 30)	2.74 \pm 0.11 (2.49 - 2.98; 31)
Second band minimum frequency (kHz)	2.47 \pm 0.14 (2.19 - 2.67; 29)	2.32 \pm 0.1 (2.17 - 2.54; 30)	2.16 \pm 0.08 (2.05 - 2.31; 10)	2.3 \pm 0.13 (2.06 - 2.52; 13)	2.49 \pm 0.12 (2.26 - 2.78; 27)	2.25 \pm 0.15 (1.96 - 2.48; 30)	2.57 \pm 0.1 (2.28 - 2.73; 31)
Second band maximum frequency (kHz)	2.93 \pm 0.12 (2.78 - 3.2; 29)	2.75 \pm 0.19 (2.56 - 3.49; 30)	2.62 \pm 0.07 (2.54 - 2.78; 10)	2.83 \pm 0.08 (2.66 - 2.92; 13)	2.9 \pm 0.1 (2.7 - 3.15; 27)	2.72 \pm 0.14 (2.46 - 3; 30)	3.03 \pm 0.11 (2.82 - 3.37; 31)
dB difference (dB FS)	5.12 \pm 4.9 (-6.22 - 13.3; 29)	6.49 \pm 3.14 (1.03 - 12.91; 30)	1.78 \pm 2.92 (-2.75 - 5.65; 10)	2.31 \pm 4.1 (-6.38 - 9.09; 13)	4.91 \pm 3.81 (-5.44 - 11.5; 27)	0.6 \pm 4.79 (-11.18 - 10.3; 30)	5.8 \pm 3.03 (-1.81 - 12.65; 31)
<i>Body condition traits</i>							
SVL (mm)	47.28 \pm 2.84 (42.2 - 51.8; 29)	46.34 \pm 2.46 (42.1 - 52; 30)	52.29 \pm 1.24 (50.1 - 54.3; 10)	46.18 \pm 2.23 (41.8 - 50.3; 13)	45.94 \pm 1.79 (41.3 - 48.9; 27)	46.9 \pm 2.36 (42.9 - 52.3; 28)	43.62 \pm 2.65 (37.6 - 49.1; 31)
SMI	4.38 \pm 0.63 (3.33 - 5.9; 29)	5.26 \pm 0.56 (4.37 - 6.77; 30)	6.69 \pm 0.81 (5.66 - 8.57; 10)	4.97 \pm 0.47 (4.28 - 5.7; 13)	4.52 \pm 0.56 (3.47 - 5.79; 27)	5.07 \pm 0.74 (3.99 - 7.04; 28)	4.11 \pm 0.54 (2.73 - 5.17; 31)

Table S4. Slopes of the regressions between orange color patch and advertisement call properties indicating how much variation of the dependent variable (PC1c of the color PCA) is explained by the independent variables [spectral (PC1_A of the call PCA) or temporal (PC2_A of the call PCA) properties] in the regression model. Bold font indicates significant p-values (see main text for details).

Population	Spectral properties (PC1_A)	Temporal properties (PC2_A)
Angra dos Reis	0.09	-0.07
Bertioga	-0.22	0.13
Gipóia island	0.02	-0.16
Itacuruçá island	-0.4	0.2
Marambaia island	0.13	0.09
Picinguaba	0.33	0.07
Seropédica	0.76	0.96

Table S5 Results of the standard least squares models showing the relation of orange patch traits (loaded in the PC1_C of the PCA with color traits - patch relative size, orange hue, and contrast hue) with acoustic properties excluding three outliers males. The models returned very similar results when compared with the models based entire data (table 3 in the main text), for this reason we presented in the manuscript the models based on the entire dataset. PC1_A of the PCA loaded with call traits was loaded by spectral properties (first harmonic dominant frequency, first harmonic minimum frequency, first harmonic maximum frequency, second harmonic dominant frequency, second harmonic minimum frequency, and second harmonic maximum frequency) and PC2_A with temporal properties (call duration; pulses per call; intercall interval; call period), and dB difference. Bold font indicates significant p-values.

Spectral traits (PC1 _A)				
factor	DF	Sum of squares	F	P
PC1 _C	1,6	4.695	2.383	0.125
population	1,6	24.792	2.097	0.057
PC1 _C *population	1,6	40.627	3.436	0.003
Temporal traits (PC2 _A)				
factor	DF	Sum of squares	F	P
PC2 _C	1,6	9.343	4.278	0.04
population	1,6	115.578	8.820	<0.001
PC2 _C *population	1,6	31.551	2.408	0.03

Table S6 Results of the standard least squares models showing the relation of each orange patch trait with body condition measurements, and population. Abbreviations: SVL – Snout-Vent Length, SMI – Scaled Mass Index. Bold font indicates significant p-values.

Stain relative size				
factor	DF	Sum of squares	F	P
SVL	1,6	13.297	0.124	0.726
SMI	1,6	419.735	3.902	0.05
population	1,6	3993.205	6.188	<0.001
SVL*population	1,6	782.605	1.213	0.303
SMI*population	1,6	2480.531	3.844	0.001
Orange hue				
factor	DF	Sum of squares	F	P
SVL	1,6	0.006	2.908	0.091
SMI	1,6	0.009	4.586	0.034
population	1,6	0.014	1.163	0.330
SVL*population	1,6	0.010	0.853	0.531
SMI*population	1,6	0.024	1.927	0.081
Contrast hue				
factor	DF	Sum of squares	F	P
SVL	1,6	0.002	0.567	0.453
SMI	1,6	0.039	9.482	0.002
population	1,6	0.039	1.586	0.156
SVL*population	1,6	0.013	0.514	0.797
SMI*population	1,6	0.065	2.652	0.018

Table S7. Slopes of the regressions between orange color patch and body condition (SMI: Scaled Mass Index) indicating how much variation of the dependent variable (PC1c of the color PCA) is explained by the independent variable (SMI) in the regression model. Bold font indicates significant p-values (see main text for details).

Population	SMI
Angra dos Reis	-0.29
Bertioga	0.52
Gipóia island	0.1
Itacuruçá island	-1.82
Marambaia island	-0.55
Picinguaba	-0.85
Seropédica	-2.64

CHAPTER IV

VISUAL CUES DO NOT FUNCTION IN A MULTIMODAL SIGNALING CONTEXT FOR MATE ATTRACTION IN EASTERN GRAY TREEFROGS

**Pistas visuais não funcionam em um contexto de sinalização multimodal para atração de
parceiros em *Dryophytes versicolor***

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**Visual cues do not function in a multimodal signaling context for mate attraction in
Eastern Gray Treefrogs**

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Abstract

Anurans use different sensory modalities to communicate and interact socially, including acoustic, chemical, tactile, and visual signals. In a multimodal context, these sensory modes can transmit different information to the receiver, or even reinforce the sending of the same message. In this study, we hypothesized that body color traits and vocal sac movements of male Eastern Gray Treefrogs [*Dryophytes versicolor* (= *Hyla versicolor*)] serve as secondary visual cues that increase their attractiveness while calling to attract mates. We tested this hypothesis using playback trials combining synthetic advertisement calls with a variety of visual cues, including differently colored resin frog models, simulated vocal sacs and live frogs. None of the visual stimuli tested impacted female choice, and there was no difference in response rate, response time, choice angle, or distance covered in the arena between multimodal and unimodal stimuli. However, females showed a strong preference for longer calls even if the less attractive calls were paired with a visual stimulus. The study provides comprehensive insights into the role of visual stimuli in the mate attraction of Eastern Gray Treefrogs and suggests that call traits play a dominant role in the male selection process of this species, contrary to what was observed for visual stimuli.

Keywords: body coloration; *Hyla versicolor*; multicomponent signal; sexual selection; two-alternative trials; vocal sac.

Introduction

In the last few decades, multimodal communication, also termed multisensory communication/signaling, has received significant attention from behavioral ecologists (Candolin, 2003; Coss et al., 2022; Møller & Pomiankowski, 1993), in no small part because of the intriguing potential of providing receivers with either redundant or different information in different sensory modalities (e.g. ‘backup signals’ vs. ‘multiple messages’; Johnstone, 1996). Animals may use a range of sensory modalities to mediate social interactions (i.e., acoustic, chemical, electric, tactile, seismic/vibrational, and visual), but the combination of acoustic and visual cues is particularly common. It involves the emission of vocalizations coupled or alternated with stereotyped gestures/movements, as well as the transmission of visual information through body color or postures (Ferrer et al., 2021; Grafe & Wanger, 2007; Ligon et al., 2018).

Anurans are a useful model system for conducting research on multimodal communication. Frogs communicate mainly by sounds, using a vast vocal repertoire to attract female and repel rival males (Köhler et al., 2017; Toledo et al., 2015), but many species also display visual signals (Giasson & Haddad, 2006; Hartmann et al., 2005; Hirschmann & Hödl, 2006). The emission of calls coupled or alternated with visual displays is commonly associated with diurnal species (Furtado et al., 2019; Haddad & Giaretta, 1999; Narins et al., 2005). However, frogs possess well-adapted vision for low-light conditions, including the ability for color discrimination (Kelber et al., 2017; Robertson et al., 2022; Yovanovich et al., 2017), and visual displays are also well documented for nocturnal species (Augusto-Alves et al., 2018; Barros & Feio, 2011; Toledo et al., 2007). For example, females of *Scaphiopus couchii* can assess males' size and body condition based on dorsal color traits, and they tend to prefer brighter males (Vásquez & Pfennig, 2007). Furthermore, most frogs inflate and deflate their vocal sac during call emission, de facto generating a ‘fixed’ multimodal (audiovisual) signal that may provide additional visual and even olfactory stimulation (Augusto-Alves & Toledo, 2022; Starnberger et al., 2014; Smith, 1977).

Dryophytes versicolor, the Eastern Gray Treefrog, is a common nocturnal species widely distributed in the eastern United States and Southern Canada (Frost 2023). During the breeding season, males aggregate and form choruses around small ponds, where females search for mates (Reichert et al., 2014; Schwartz et al., 2001, 2002). Females attend more to longer calls, i.e., calls with more pulses (Feagles & Höbel, 2022; Gerhardt et al., 2000; Reichert & Höbel, 2015). In this species, males have darker throats than females (sexually dimorphic), the color of the vocal sac is related to male body condition, and the vocal sac color of mated males

is darker when compared with unmated males (Höbel et al., 2022). Furthermore, males emit more courtship calls when they are able to see an approaching female, indicating that they attend to visual cues (Reichert, 2013). On the other hand, both males and females exhibit sexually monomorphic color traits, such as yellow coloration in the femoral and flank regions. Additionally, males and females have a high capacity for color change (Edgren, 1954), and individuals can change their dorsal color between gray, green, or brown (G. Höbel pers. observ.).

In the present study, we investigate the role of various visual cues in the mate choice process of *D. versicolor*. We used frog-like models with various color traits (dorsal color, throat color, thigh color) and synthetic advertisement calls in two-choice trials with females. We predicted that *D. versicolor* females prefer multimodal signals (average call + visual signals) over unimodal signal (average call alone). We also tested if females show a preference for dorsal color (gray, green, and brown), vocal sac coloration (black and white patterns), and thigh coloration (presence of yellow patches). Lastly, we tested whether visual stimulation (presence of model) would increase females' acceptance of a shorter, less attractive calls.

Methods

Study site and data sampling

We conducted the study at a pond located at the University of Wisconsin-Milwaukee's (UWM) field station in Saukville, WI, USA (43°23'10"N, 88°01'57"W). Sampling was performed from May 10 to June 9, 2022. We collected 72 *Dryophytes versicolor* females, all of which were in amplexus to ensure that they were sexually receptive. The collected females were transported to the laboratory and individually kept in boxes (~10 x 10 x 7 cm), which were placed inside coolers with melting ice to decrease females' metabolism and postpone oviposition. Females were tested within three days after capture and then released in the collection site.

Stimuli preparation

We used frog-like models (except for experiment 4) and synthetic calls to standardize the traits of the stimuli, including body size and color, and spectral and temporal properties of calls. Using live individuals for visual stimulation might introduce confounding factors, such as chemical cues and uncontrolled movements, potentially biasing the results.

Synthetic calls – Male *D. versicolor* produce pulsed advertisement calls comprising a series of short pulses with a duration of approximately 25 ms (at 20 °C) and that are repeated

after a pause of 25 ms. At a given temperature, the duration of the call can be expressed in ms or number of pulses (used here). Females prefer longer duration calls (i.e., Feagles & Höbel, 2022; Reichert & Höbel, 2015). To create synthetic *D. versicolor* advertisement calls, we used the *seewave* package (Sueur et al. 2008) in R version 3.1.0 (R Core Team 2014). We created four calls varying in duration and hence, attractiveness (6, 15, 18, and 21 pulses, respectively). These values cover much of the range of call durations observed in our study population (Reichert & Höbel, 2015). All other call characteristics were maintained at the population average; length of pulse = 25 ms, pulse period = 25 ms, call period = 7750 ms, low frequency peak (first visible harmonic band) = 1071 Hz, and high frequency peak (second visible harmonic band) = 2142 Hz (which is 10 dB louder in low frequency) (Reichert & Höbel, 2015).

Frog-like Model – Using living and preserved frogs for reference, we constructed a clay model (Sculpey Oven-Bake Clay) of a calling male *D. versicolor*. Subsequently, we used the clay model, to create a silicone mold, from which we produced all resin frog models used in the experiments. Since Eastern Gray Treefrogs exhibit substantial color change ability, we selected numerous paint colors reflecting the most common dorsal displays. Dorsal coloration can vary from light gray to different shades of brown and green, with brown being the most common and gray the last common nocturnal color (G. Höbel, pers. obs). Both sexes have yellow groins and thighs (sexually monomorphic), and the vocal sac of mature males darkens and can range from light gray to almost black (Höbel et al. 2022). To select the paint colors for the frog models, we used an Ocean Optics USB 2000+ spectrometer to measure reflectance spectra of the dorsal surface of live male frogs. We measured 10 males each that were gray, brown or green, respectively, as well as thigh and throat coloration of 10 of these frogs. Then we sampled a large number of color swatches from a Sherwin Williams Paint booklet until we found paint colors that matched the frog colors: Green (Sherwin Williams #146-C3), Brown (#209-C5), Gray (#239-C2), Yellow (#131-C4) and Black (#237-C7) (Figure S1). To avoid model biases, we produced two models for each stimulus tested and randomly exchanged them during the trials.

Vocal sac device – To test whether vocal sac movement is used as a visual cue, we built a device that simulated male vocal sac inflation and deflation during calling activity. The device consists in a square wooden base painted with black paint. A small inflatable white balloon was attached to this base, and this balloon was linked in a clear silicone tube. In the other side of this tube, we coupled one plastic syringe. During the trials (experiment 12, see below), we manually pressed and depressed the syringe, inflating and deflating the balloon

whenever the speaker broadcast a call. To isolate the response that only the vocal sac, as a visual cue, would provoke in female preference, no frog model was associated with the balloon.

Live males - We ran one experiment using live males (experiment 4) to observe whether the females responded similarly to experiments with resin models and real males. To prevent these males from emitting calls, we used cold males (from the same ice-filled coolers we used to store females awaiting testing). We took the males out of the cooler three minutes before the trials, so they could assume a natural sitting posture but not yet emit calls. In the arena, the males were placed inside a transparent container (~ 5 x 5 x 5 cm). An equal but empty container was placed on the opposite side of the test arena to prevent biases. These transparent containers were used to prevent movement of the live frogs; consequently, they were not employed in trials that used resin models.

Experiment design

Experiments took place in a semi-anechoic room containing a 2 m diameter circular arena (Figure S2). This arena was wire-fenced covered by a black fabric with dark gray foam exercise mats on the floor. A circular small wire container was placed in the center of the arena to be the release point, where females were placed at the beginning of each trial. Temperature in the arena was maintained at $20 \pm 1^\circ\text{C}$. The arena was dimly illuminated with a dimmed incandescent light bulb dimmed to a light intensity of 1 lux; light level was monitored using a Extech EasyView EA31 Digital Light Meter. Due to variations in moon phase, cloud cover, vegetation around calling perches, etc., nocturnal light levels are characterized by significant fluctuations (Li et al., 2022). This makes it challenging to pinpoint the "most appropriate" light level for experimental trials. Previously studies with *Dryophytes (=Hyla) versicolor* (i.e., same study species and same population), have shown call preferences, including preference functions and choosiness for longer calls, are unaffected by variation in nocturnal light levels (Underhill & Höbel, 2017) or even anthropogenic light pollution (Underhill & Höbel, 2018). In light of the robustness of acoustic preferences to variation in light levels, we opted to supply maybe too much light rather than risk the elimination of potential effects of visual cues due to insufficient light.

The two alternative stimuli were presented at an angle of 90° between them. For acoustic stimulation, we placed two speakers outside the arena wall, and for visual stimulation the models, live males, or the vocal sac device, respectively, were placed inside the arena in front of the speaker (Figure S2). In all trials, we offered two different stimuli for females (Table 1). All acoustic stimuli had the same spectral properties and were presented at an amplitude of

85 dB SPL, measured from the females' release point at the center of the arena (verified using a Sound Level Meter; Extech Instruments; C-weighting, fast RMS). The call duration (= number of pulses) was mostly set to 18 pulses (average of the study population), except for trials that tested for an interaction between call attractiveness and presence of a multimodal cue (experiments 5 and 6) (Table 1).

Before the experiments, females were removed from the coolers with ice for acclimation to the test temperature of 20 °C. Before each trial, the female was placed at the release point and stimulated by playing the calls three times in each speaker, and then released. The same female was tested in all trials with five minutes of resting period between them. Rest periods of two to five minutes duration between trials “erases” memory of the prior trial in treefrogs (Boyd & Gordon 2021). The order of the trials (experiments 1 – 12), as well as the position of the stimuli, were randomly established and changed with each female tested. In all trials, we scored a choice when the female arrived in a demarcated area in front of the model/speaker (Figure S2). Each trial had a maximum duration of five minutes. Females that did not respond in a trial were tested again after five minutes resting period. Females that did not respond in a second attempt were considered unreceptive, and we stopped testing them in other trials. On average (\pm SD) we tested 43.3 ± 2.7 (range 35 – 45) females per experiment.

Our study included 12 different experiments (Table 1), in which we explored the function of visual stimuli (presence/absence of males/frog-like models; dorsal, vocal sac, and thigh color; and vocal sac movement) and acoustic stimuli (advertisement call with different numbers of pulses) in a unimodal or multimodal context. We did this by using frog-like models with different color traits in two-choice trials with females, exploring differences on dorsal colors (gray, green, and brown), vocal sac coloration (black and white), and the presence of yellow patches on the thigh (yellow and white). The experiments were organized into five sets (Table 1):

Set 1: Influence of the frog-like model/male presence as visual cue

In this set of experiments, we explored whether a multimodal signal elicits a stronger response than a unimodal one. For this, we paired frog-like models with different body colors (green, gray, or brown models; Figure 1ab) with an average (18 pulses) call (multimodal stimulus) and presented it against an average (18 pulses) call (unimodal stimulus) (experiments 1 – 3). We also executed a trial where the visual stimulus in the multimodal condition was a live male (experiment 4).

Set 2: Attractive calls vs. unattractive calls in multimodal context

We tested whether visual stimulation (i.e., presence of the model) would increase the females' attraction to a less attractive (shorter) call. We tested this at two different levels of variation: i) unimodal 18 pulses vs. multimodal 6 pulses + model (experiment 5: 67 % of variation between the acoustic stimuli); ii) unimodal 21 pulses vs. multimodal 15 pulses + model (experiment 6: 29 % of variation between the acoustic stimuli). Here, we only used brown models, since brown males represent 60 – 80 % of the males found chorusing at the pond (G. Höbel pers. observ.).

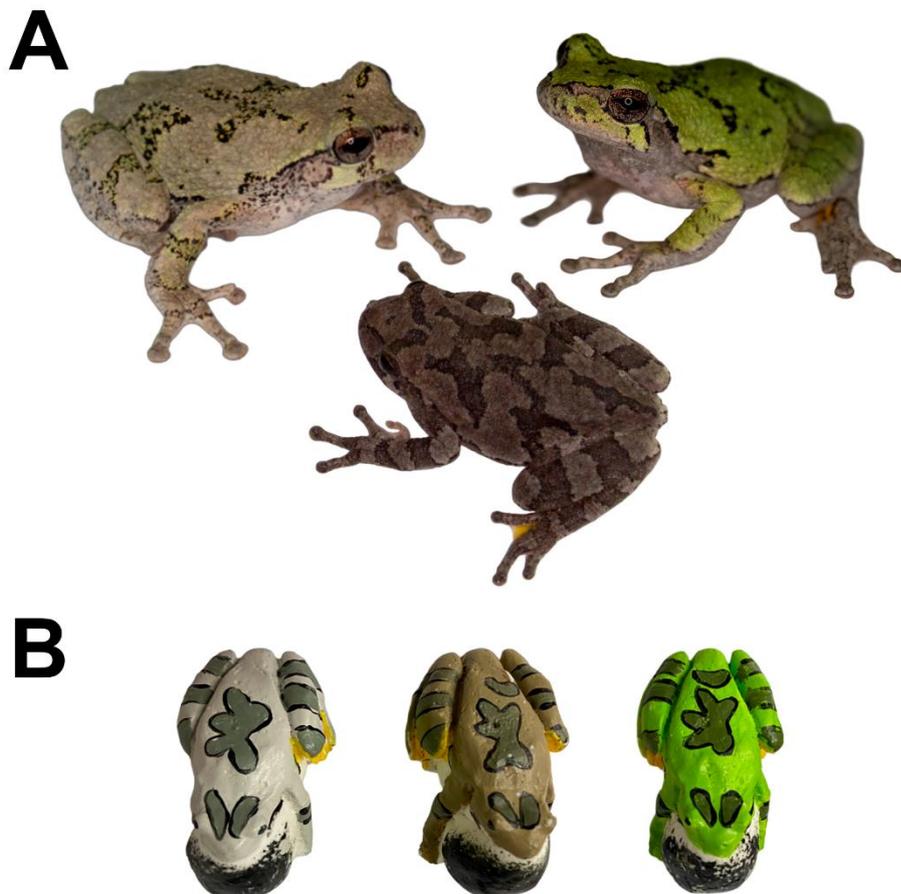


Figure 1. A) *Dryophytes versicolor* body color patterns. All individuals were collected at same pond at the University of Wisconsin-Milwaukee's (UWM) field station in Saukville, WI, USA; B) models painted based on the males' color traits.

Set 3: Female preference for dorsal color pattern

In this set of experiments, we tested whether the females show preferences for any specific dorsal color among those observed in nature (green, gray, and brown; Figure 1a), by offering the different color patterns combinations in the two-choice trials (experiments 7 – 9: Table 1). Here, models were always presented in multimodal condition, i.e., paired with a speaker broadcasting an 18 pulses call).

Table 1. Description of the stimuli offered in each experiment, which were organized into sets according to the tested aims. As described in the main text, brown models were used for the experiments 5 – 6 and 10 – 12, as males with this color are more common when they are in calling activity. Despite the stimuli being identified as "a" and "b", the sides were randomized during the trials to avoid any biases. Number of females that responded to each stimulus in all experiments. Bold value represents significant difference in the two-tailed binomial test between offered stimuli.

Experiment	Stimulus (context)	Female response (n)	<i>P</i>
1	a - green frog-like model + call 18 pulses (multimodal)	25	0.451
	b - call 18 pulses (unimodal)	19	
2	a - gray frog-like model + call 18 pulses (multimodal)	27	0.174
	b - call 18 pulses (unimodal)	17	
3	a - brown frog-like model + call 18 pulses (multimodal)	17	0.222
	b - call 18 pulses (unimodal)	26	
4	a - live male + call 18 pulses (multimodal)	15	0.761
	b - call 18 pulses (unimodal)	20	
5	a - call 18 pulses (unimodal)	43	<0.001
	b - brown frog-like model + call 6 pulses (multimodal)	1	
6	a - call 21 pulses (unimodal)	28	0.066
	b - brown frog-like model + call 15 pulses (multimodal)	15	
7	a - green frog-like model + call 18 pulses (multimodal)	23	1
	b - gray frog-like model + call 18 pulses (multimodal)	22	
8	a - gray frog-like model + call 18 pulses (multimodal)	24	0.766
	b - brown frog-like model + call 18 pulses (multimodal)	21	
9	a - green frog-like model + call 18 pulses (multimodal)	20	0.761
	b - brown frog-like model + call 18 pulses (multimodal)	23	
10	a - brown frog-like model with yellow thigh + call 18 pulses (multimodal)	16	0.073
	b - brown frog-like model with white thigh + call 18 pulses (multimodal)	29	
11	a - brown frog-like model with black throat + call 18 pulses (multimodal)	21	0.88
	b - brown frog-like model with white throat + call 18 pulses (multimodal)	23	
12	a - vocal sac movement + call 18 pulses (multimodal)	21	0.88
	b - call 18 pulses (unimodal)	23	

Set 4: Thigh color and vocal sac color as visual cue

Other body color portions might transmit information to conspecifics as secondary cues, so we tested whether the yellow thigh or black throat, respectively, increase the females' response (experiments 10 and 11; Table 1). The dorsal color of these frog models was always brown, and they were presented in multimodal condition [i.e., paired with a speaker broadcasting an 18 pulses call].

Set 5: vocal sac movement as visual cue

The dynamic aspect of an inflating vocal sac might represent a particularly salient visual cue, so we tested whether the presence of a simulated moving vocal sac would make the stimulus more attractive than a call alone (experiments 12; Table 1). Vocal sac models were presented in multimodal condition [i.e., paired with a speaker broadcasting an 18 pulses call].

Statistical analyses

We used two-tailed binomial test (computed online: <https://www.graphpad.com/quickcalcs/binomial1/>) to calculate whether the proportions of females choosing the two alternatives were significantly different. Additionally, for all experiments, as the data were nonparametric, we used the Mann-Whitney U test to calculate whether the response latency (i.e., seconds from the moment we released the females up to the time of choice) differed between the alternatives for each experiment.

Our overall analyses were based on population averages (above). Because we had tested most females in a number of experiments, we also collated their color choices and used logistic regressions to test whether a color preference in one experiment was correlated with a color choice in another experiment. This allowed us to examine whether a lack of population color preference might hide individual but diverging color preferences (i.e., if some individuals consistently prefer green, brown or gray models, the population analysis would average out to no preferences).

For the set of experiments 1, we video-recorded the trials with a camera placed directly above the females' release point, and with coverage of the entire arena. Following Bonachea & Ryan (2011), we extracted the frames from the videos, and from those image stacks, we produced a single image using the ImageJ software (Schneider et al., 2012) with the Extended Depth of Field plugin (Forster et al., 2004). From this image, we measured the length

of the female's approach path. We used Mann-Whitney U to test for differences path length. All Mann-Whitney U tests were run using R version 3.6.3 (R Core Team 2019).

From the stacked images we also measured the angle at which the female left the release area. Using the exit angle, we tested whether the visual cue could generate a more directional component in the females' response. We used the Oriana 4.02 software (Kovach Computing Services, Anglesey, Wales) to analyze females first movement angle in relation with the stimulus that it chose (multimodal stimulus or unimodal one). We provide the mean vector angle (μ) and length of the mean vector (r) for each test. The length of the mean vector ranges from 0 to 1, where $r = 0$ indicates total uniform dispersion and $r = 1$ indicates concentrated dispersion in one direction. The multimodal stimulus was established as 0° , while the unimodal one was in the 90° in the four experiments.

By employing both path length and angle analyses, we investigated whether the visual signal enhances sound source localization. This enhancement would be evident if females exhibit a more directional angle towards the multimodal option compared to the unimodal one and if they cover shorter distances in the arena when selecting the multimodal option.

Ethical note

Experiments were approved by the University of Wisconsin-Milwaukee Institutional Animal Care and Use Committee (no. 19-20 #26) and adhered to the ASAB/ABS Guidelines for the ethical treatment of animals. We obtained permission from the Wisconsin Department of Natural Resources (Scientific Research License #SRLR-22-62), and the landowners to enter their property. All frogs were released unharmed at the pond they were collected from.

Results

Set 1: Influence of the frog-like model/male presence as visual cue

Females did not prefer the multimodal stimulus. The presence of the models did not increase female response (two-tailed binomial: all $P > 0.174$; Figure 2; Table 1). Furthermore, neither response latency (U test: all $P > 0.067$; Figure 2) nor path length (U test: all $P > 0.214$; Figure 2) differed between unimodal and multimodal alternatives. Analysis of approach angles showed that females head for the chosen stimulus, independently whether it was the multimodal stimulus or the unimodal one (i.e., the exit angle was directed towards 0° when females chose the multimodal stimulus, and towards 90° when they chose the unimodal one; Figure 2; Table 2).

The trial that used a live male instead of a frog model as the visual cue showed similar results (experiment 4). The presence of the male did not increase the proportion of females responding to the multimodal alternative (two-tailed binomial: $P > 0.761$; Figure 2; Table 1) and response latency did not differ between the two alternatives (U test: $P > 0.278$; Figure 2). The path length was slightly shorter when females approached the live male alternative (U test: $P = 0.051$; Figure 2). Again, the same pattern of directional response was observed regardless of the chosen stimulus (Figure 2; Table 2) (Table S1 for U test detailed results in all experiments).

Table 2. Mean vector angle (μ) and length of the mean vector (r) for each experiment. The multimodal stimulus (call + frog-like model/live male) was established on 0° , while the unimodal one (call) was at 90° in all four experiments. The length of the mean vector ranges from 0 to 1, where $r = 0$ indicates total uniform dispersion and $r = 1$ indicates concentrated dispersion in one direction.

Experiment	1		2		3		4	
Stimulus	green model + call	call	gray model + call	call	brown model + call	call	Live male + call	call
Mean vector (μ)	17.74°	68.16°	4.84°	87.38°	13.97°	72.45°	9.91°	75.78°
Length of the mean vector (r)	0.708	0.938	0.69	0.71	0.656	0.698	0.92	0.778

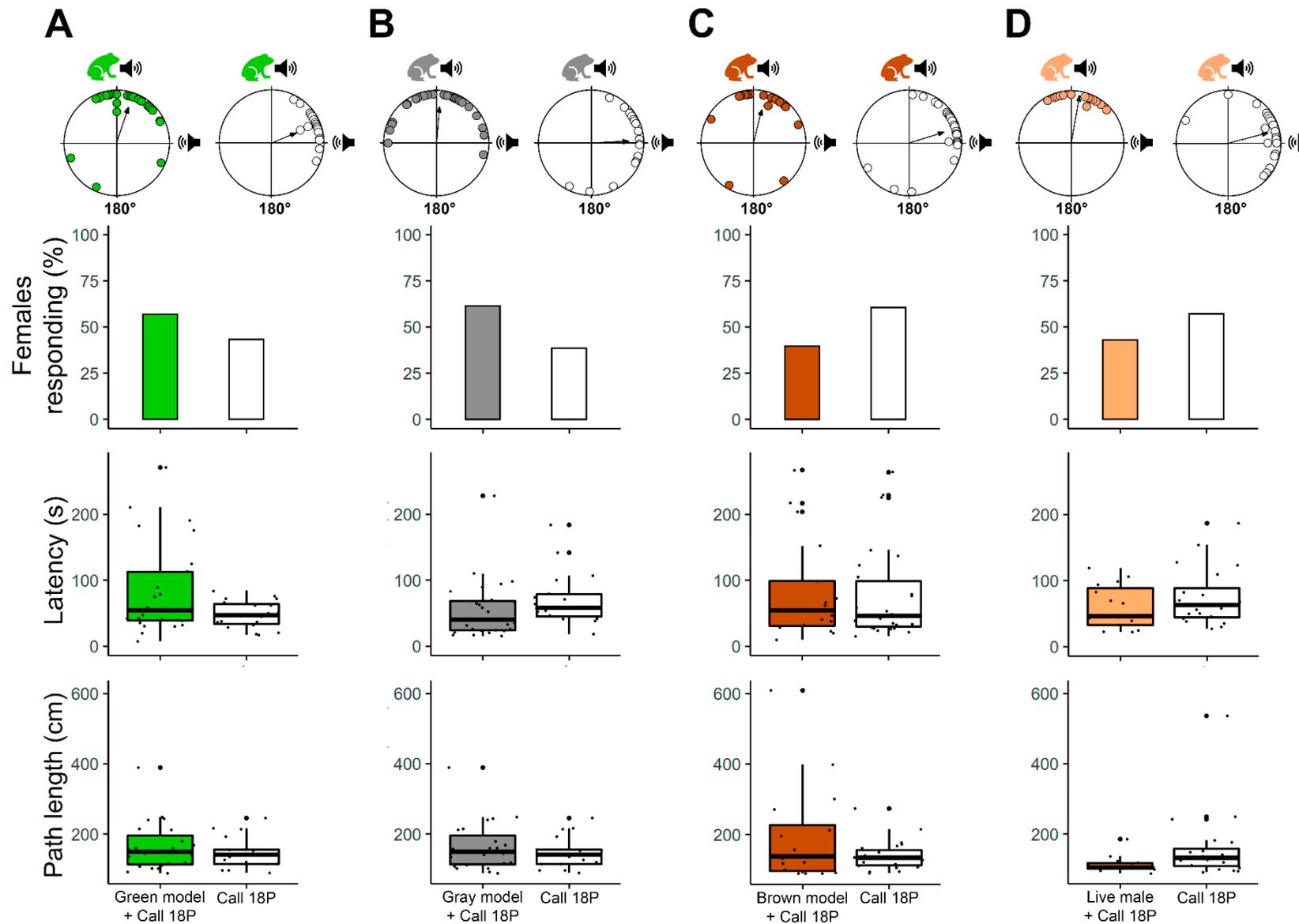


Figure 2. *Dryophytes versicolor* females' responses to two-alternative trials. The results were presented by angle direction; percentage of females responding; response latency; and path length. A) green model + average call vs. average call. B) gray model + average call vs. average call. C) brown model + average call vs. average call. D) live male + average call vs. average call. Colored (green, gray, brown, and light pink) graphics represent the results when females chose the multimodal stimulus in each experiment, while white graphics represent when females chose the unimodal stimulus. In the circular graphics, each point represents the angle of the first jump when females left the release area, and the arrows represent the mean angle direction (all females together). Boxplots indicate median, upper, and lower quartiles, and upper and lower whiskers. Table 1 for detailed results of the two-tailed binomial test and Table S1 for U test.

Set 2: Attractive calls vs. unattractive calls in multimodal context

Females preferred attractive calls (= calls with more pulses) over unattractive ones, even when these unattractive calls were associated with a visual stimulus. In experiment 5, with high variation between calls (18 pulses vs. 6 pulses + visual cue), only one female chose the multimodal (but shorter) stimulus (two-tailed binomial: $P < 0.001$; Figure 3; Table 1). In experiment 6, with less variation between calls (21 pulses vs. 15 pulses + visual cue), a greater number of females also showed a preference for the longer call (Figure 3), but the difference was not significant (Table 1; two-tailed binomial: $P = 0.066$). There were no differences in the response latency between stimuli (Figure 3; Table S1).

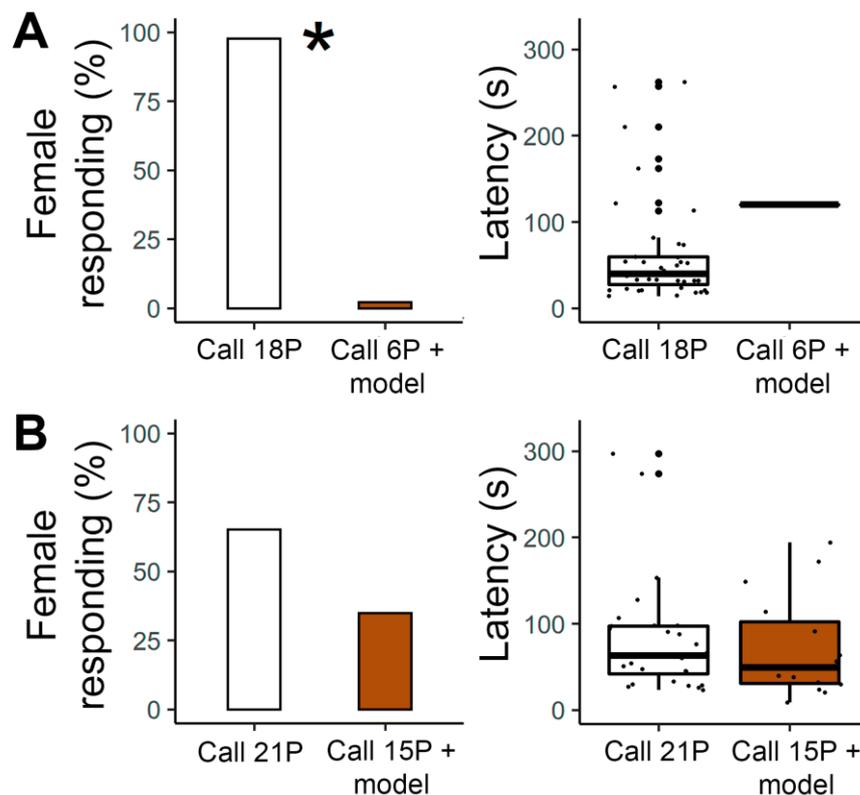


Figure 3. *Dryophytes versicolor* female responses to two-alternative choice trials. The graphs show the percentage of females that chose each stimulus and their response latency. A) Attractive calls (18 pulses; unimodal) vs. unattractive calls (6 pulses + frog model; multimodal). B) Attractive calls (21 pulses; unimodal) vs. unattractive call (15 pulses + frog model; multimodal). Boxplots indicate median, upper, and lower quartiles, and upper and lower whiskers. An asterisk indicates significant P -values. Table 1 for detailed results of the two-tailed binomial test and Table S1 for U test.

Set 3: Female preference for dorsal color pattern

Females did not show a preference for any dorsal color (two-tailed binomial: all $P > 0.761$; Figure 4; Table 1). There were no differences in the response latency between green and gray and between brown and gray models (Figure 4; Table S1), but the response latencies of females choosing the green model over the brown model were significantly shorter (U test: $P < 0.001$; Figure 4).

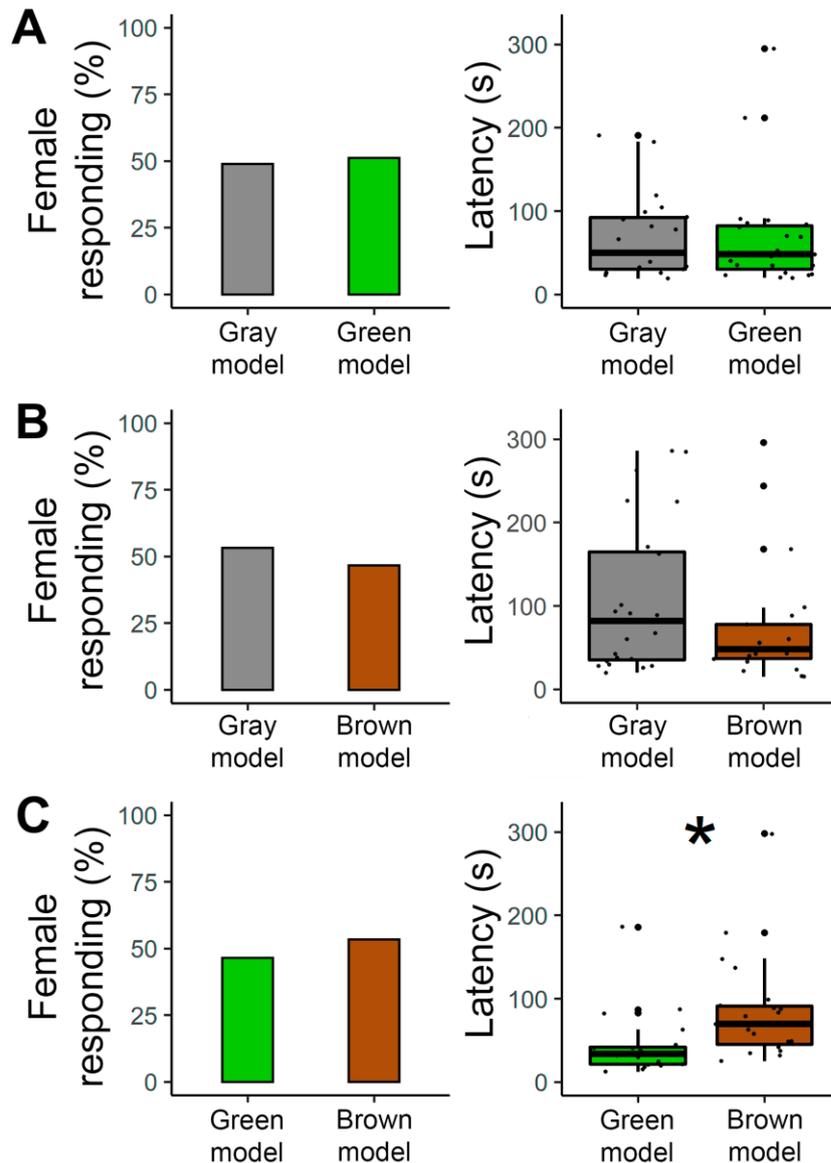


Figure 4. *Dryophytes versicolor* female responses to two-alternative choice trials. The graphs show the percentage of females that chose each stimulus and their response latency. In this set of experiments, for acoustic stimulation we broadcasted the average call with 18 pulses for all options, only changing the type of visual cues. A) gray model vs. green model. B) gray model vs. brown model. C) green model vs. brown model. Boxplots indicate median, upper, and lower quartiles, and upper and lower whiskers. An asterisk indicates significant P -values. Table 1 for detailed results of the two-tailed binomial test and Table S1 for U test.

Set 4: Thigh color and vocal sac color as visual cue

The presence of yellow thighs or black vocal sac in the models did not increase the females' preference (two-tailed binomial: $P > 0.073$; Figure 5; Table 1). Also, there were no differences in the response latency between stimuli (U test: $P > 0.213$; Figure 5; Table S1).

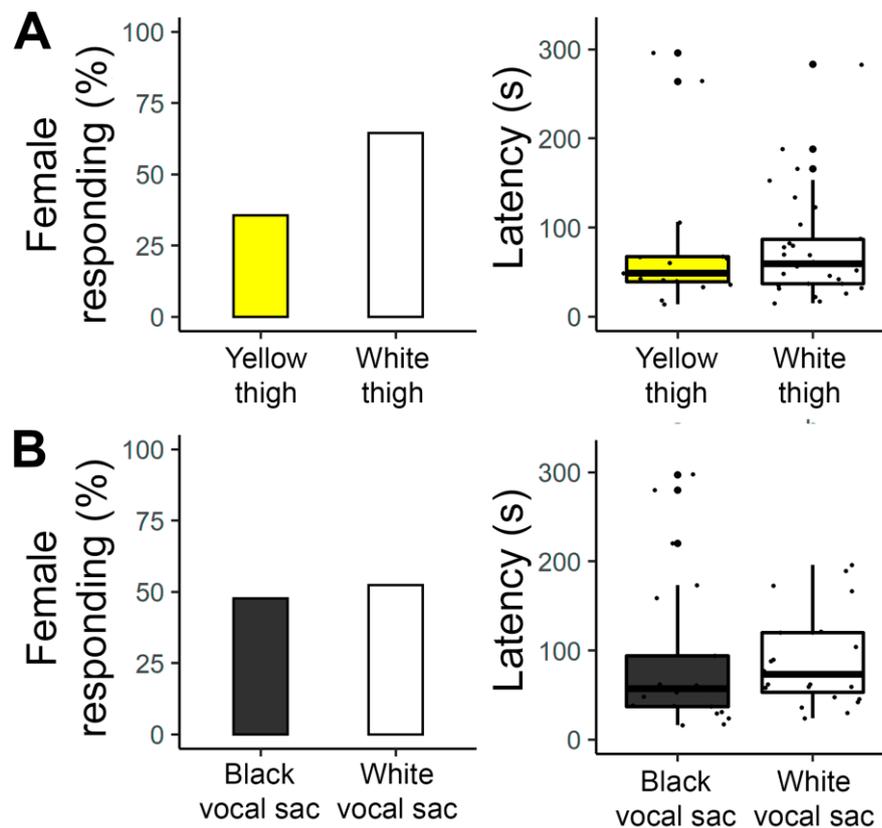


Figure 5. *Dryophytes versicolor* female responses to two-alternative choice trials. The graphs show the percentage of females that chose each stimulus and their response latency. In this set of experiments, for acoustic stimulation we broadcasted the average call with 18 pulses for all options, only changing the type of visual cues. A) frog models with yellow thighs vs. frog models with white thighs. B) frog models with black vocal sac vs. frog models with white vocal sac. Boxplots indicate median, upper, and lower quartiles, and upper and lower whiskers.

Set 5: vocal sac movement as visual cue

The vocal sac movement coupled with the call (multimodal) did not increase female response (two-tailed binomial: $P = 0.88$; Figure 6; Table 1). Also, there were no differences in the response latency between stimuli (U test: $P = 0.503$; Figure 6; Table S1).

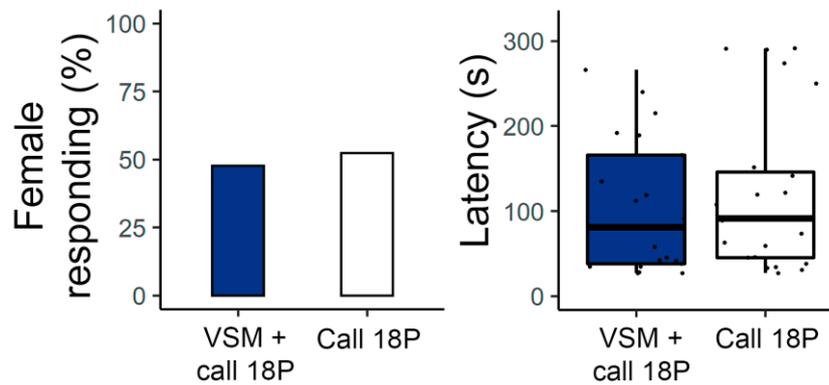


Figure 6. *Dryophytes versicolor* female responses to two-alternative choice trials of the experiment that evaluated whether the vocal sac movement (VSM) increase the females preference. The graphs display the percentage of females that chose each stimulus and their response latency. In this experiment, the average call with 18 pulses was broadcasted for both options. Boxplots indicate median, upper, and lower quartiles, and upper and lower whiskers.

Individual color preferences

Individual females did not show consistent preferences for specific colors, nor was their choice of a color in one experiment related to the choice in another experiment (Table 3). This suggests a lack of color preferences on an individual as well as population level.

Table 3. Nominal logistic regressions comparing color choices of the same females across different trials. In none of the comparisons did the color choice of a given female in one trial predict her color choice in another trial.

Response in one trial	Response in other trial	Likelihood Ratio Test
Brown model	Green model	$\chi^2_1 = 0.873$; $P = 0.350$
Brown model	Gray model	$\chi^2_1 = 0.927$, $P = 0.336$
Prefer Gray model in Gray vs. Green	Prefer Gray model in Gray vs. <i>Brown</i>	$\chi^2_1 = 0$, $P = 1$
Prefer Green model in Gray vs. Green	Prefer Green model in Green vs. <i>Brown</i>	$\chi^2_1 = 0.836$, $P = 0.360$
Prefer Brown model in Brown vs. Green	Prefer Brown model in Brown vs. <i>Gray</i>	$\chi^2_1 = 0.569$, $P = 0.451$
Brown	Brown + yellow thigh	$\chi^2_1 = 0.108$, $P = 0.742$
Green	Brown + yellow thigh	$\chi^2_1 = 0.078$, $P = 0.780$
Gray	Brown + yellow thigh	$\chi^2_1 = 1.327$, $P = 0.249$
Brown	Brown + black throat	$\chi^2_1 = 0.042$, $P = 0.837$
Green	Brown + black throat	$\chi^2_1 = 0.242$, $P = 0.623$
Gray	Brown + black throat	$\chi^2_1 = 3.288$, $P = 0.070$

Discussion

It is well established that the auditory signal component of anuran advertisement displays is necessary and sufficient to elicit mate choice behavior, and that females prefer certain call features over others (Gerhardt & Huber, 2002). The combination of bright coloration, contrasting color patches, and the ‘fixed’ multimodal cue generated by the inflating vocal sac during call emission, make it intuitively plausible that frogs should also incorporate visual cues in mediating social interactions. To date, eight species of nocturnal anurans have been tested for multimodal communication, and a detailed examination of the published literature suggests that it is less widespread than anticipated (see also discussion in Li et al., 2022). Below we summarize the results of those studies and show that our study, documenting a lack of multimodal communication in *Dryophytes versicolor*, is in fact not unusual.

Are multimodal stimuli preferred over unimodal stimuli?

A lack of female preference for the multimodal stimulus has been reported in two species (Li et al., 2022; Reichert et al., 2014, this study), while females from five species have been reported to prefer a multimodal alternative over a unimodal one (Laird et al., 2016; Taylor et al., 2007; Taylor & Ryan, 2013; Zhao et al., 2021; Zhu et al., 2021). The proportion of females that did approach the multimodal alternative in those studies ranged from 70-80%. In *Engystomops pustulosus*, the only species for which multiple replicates of the same test are available, the proportion of females approaching a dynamic multimodal alternative ranged from 60-80%, i.e., from no preference over non-significant trends favoring multimodal to statistically significant preferences for the multimodal alternative (Stange et al., 2017; Taylor et al., 2008, 2011a; Taylor & Ryan, 2013). Further, only dynamic visual cues were effective; females did not prefer a static multimodal stimulus over a unimodal stimulus (Taylor et al., 2008). Taken together, this suggests that a preference for a multimodal stimulus is not shared by all females, or easily influenced by modifying factors.

The type of visual stimulus used in the experiments did not appear to influence whether a preference was observed. Multimodal stimuli that did not elicit a positive response included static resin models (this study), dynamic models (Li et al., 2022; this study), and video playbacks (Reichert et al., 2014). Those that did elicit a positive response included dynamic models (Laird et al., 2016; Taylor et al., 2007; Taylor & Ryan, 2013) and video playbacks (Zhao et al., 2021; Zhu et al., 2021).

Are dynamic visual cues more effective than static visual cues?

The inflation and deflation of the vocal sac during call production generates a dynamic visual cue that may be particularly salient in eliciting responses. But there surprisingly is little support for this hypothesis. Three species do not show a preference for a dynamic visual component. *Dryophytes chrysoscelis* and *D. squirellus* did not prefer a dynamic model over a static one (Li et al., 2022; Taylor et al., 2007). Similarly, *D. versicolor* did not prefer a video with dynamic vocal sac over a static one (Reichert et al., 2014), and they also did not prefer a multimodal presentation of a simulated vocal sac (this study).

For two species, some data is consistent with a preference for the dynamic component. In a video playback study with *E. pustulosus*, 14 females approached the video of a male with an inflating vocal sac, while 6 approached the video of a male whose vocal sac did not inflate (Rosenthal et al., 2004). However, this difference is not statistically significant when using a two-tailed binomial test to determine whether the proportions of subjects choosing the two alternatives significantly deviate from 0.5 (two-tailed $P = 0.12$). And in a study with the same species that used three-dimensional models, female only preferred a multimodal alternative over a unimodal one if it was a dynamic model (16:4; two-tailed $P = 0.01$), but not if it was a static model (7:13, two tailed $P = 0.26$) (Taylor et al., 2008). This could be interpreted as females attending more to the dynamic visual component of a multimodal signal. In *Hyla arborea*, a dynamic model with attractive features did improve the attractiveness of an unattractive call (Richardson et al., 2010), while a similar test using static models did not (Troïanowski et al., 2014). This could also be interpreted as females attending more to the dynamic visual component of a multimodal signal.

Do nocturnal frogs have color preferences?

We conducted a comprehensive exploration of visual preferences, particularly focusing on color, in a nocturnal anuran. This was motivated by the exceptional color change ability of *D. versicolor* (Edgren, 1954), as well as the presence of their contrasting body color patches (yellow thigh, dark throat). We found that variation in male dorsal coloration did not influence female mate choice behavior, which suggests that body color change is not a component of a visual signal but may be related to thermoregulation, light intensity, or a defensive strategy involved in camouflage.

Female mate choice behavior was also not influenced by a model's yellow thigh coloration. Yellow (as well as orange and red) color in vertebrates, including frogs (Brenes-Soto et al., 2017; Suga & Munecada, 1988; Umbers et al., 2016), are usually formed by pigments belonging to the carotenoid category. These pigments are acquired from the diet (Feltl

et al., 2005; Umbers et al., 2016), and are commonly associated with an individual's foraging ability and/or food availability, which in turn may reflect their health and body condition. Therefore, they have the potential to provide honest information to conspecifics. Then again, bright coloration is also often associated with aposematism/unpalatability, and colorful patches on the thighs and flanks of otherwise cryptic frogs might represent an intermediated state between the crypsis and aposematism (Loeffler-Henry et al., 2023). Conspicuous hidden coloration is common among hylids (Loeffler-Henry et al., 2023), and Cannizzaro IV & Höbel (2023) recently documented that the black and yellow thigh color of *D. versicolor* does indeed function as an aposematic signal. While this does not preclude an additional function in conspecific communication, our data suggest that female *D. versicolor* do not attend to it during mate choice.

In *D. versicolor*, male body condition is associated with vocal sac pigmentation, and the vocal sac color of mated males is darker when compared with unmated ones (Höbel et al., 2022). Although this suggested a role of vocal sac color in mate choice, our experiments demonstrated that dark vocal sac color did not increase females' preference. This is in line with a previous study that used video playbacks to test for preferences for vocal sac color in this species (Reichert et al., 2014). Only one other species (*H. arborea*) has been tested for vocal sac color preferences, with mixed results. In a study using video playbacks, 17 of 24 females (71%) approached the alternative with a more colorful vocal sac, a difference that is not significantly different from the expected proportion of 0.5 in a two-tailed binomial test ($P = 0.06$; (Gomez et al., 2009). In a study using painted static models, only 55% approached a light red over a dark orange vocal sac (11:9; two-tailed $P = 0.82$), but 75% of females approached a dark red over a light orange vocal sac (15:5; two-tailed $P = 0.04$; Gomez et al., (2010). Gomez et al. (2010) suggested that this difference might be due to females evaluating brightness and hue cues separately. Given the pronounced phototaxis exhibited by many anurans (Hailman & Jaeger, 1974), this idea warrants further examination.

Can addition of a visual component modify preferences for the auditory component?

Assuming that visual components are attractive, adding a visual cue may improve a call with unattractive features. In two species, that was not the case. In *D. cinereus*, addition of a visual component did not change the preference for the low-frequency call (Laird et al., 2016). In our study with *D. versicolor*, females strongly discriminated against an unattractive short call that was paired with a visual cue if the difference between the two call alternatives was large (12 pulses difference; two-tailed test: $P < 0.001$), but not if the difference was smaller

(6 pulses difference; two-tailed test: $P = 0.066$). However, we interpret the latter result not as evidence for a positive effect of adding a visual cue to the unattractive call, but suggest that preferences for longer calls diminish as alternatives become more similar and/or are closer to the population average; control trials using only unimodal call presentation would be required to fully support this hypothesis.

In one species (*D. squirellus*) adding a visual cue rendered an unattractive call less unattractive, but never made it more attractive than the unimodal attractive call stimulus (Taylor et al., 2011b). For two other species, different studies obtained opposing results, such that only some data is consistent with an “improving” function of adding a visual cue. In *E. pustulosus* adding a visual cue rendered an unattractive simple call less unattractive than the attractive complex call in one study (Stange et al., 2017), but in another study addition of a visual cue did not modulate female responses and the less attractive simple call remained less attractive when the alternative was a complex call (Taylor et al., 2011b). In *H. arborea*, when females had to choose between an all-attractive audiovisual stimulus and an all-unattractive audiovisual stimulus, they strongly preferred the all-attractive alternative. But when the attractive call was associated with unattractive vocal sac coloration, and opposed to an unattractive call combined with attractive coloration, half of the females now approached the alternative with the unattractive call (Richardson et al., 2010). However, in another study that also tested a combination of attractive call + unattractive vocal sac coloration against unattractive call + attractive coloration, females significantly discriminated against the alternative with the unattractive call (Troianowski et al., 2014). The latter comparison is complicated by differences in experimental design, however, as the first study used dynamic video playbacks and the latter used static resin models. Hence, addition of a visual cue does not always improve an unattractive call. And even in the cases where there is an improvement, the visual cue never renders the (bimodal) unattractive call more attractive than an (unimodal) attractive call, underscoring the dominance of the acoustic communication channel.

Conclusion

In frogs, a multimodal stimulus is sometimes more effective than a call alone, but the particular feature of the visual cue is secondary. Adding visual information to a call sometimes changes how females respond to it, and sometimes it does not. Moreover, replicate studies involving the same species frequently yield opposing results (which in part may be due to differences in the visual cue used). Hence, multimodal communication may affect mate choice decisions in some nocturnal anurans, but it is neither universally present nor are

preferences particularly strong.

In addition to mediating mate choice decisions, visual cues might facilitate communication by improving sound source localization or signal reliability. Our study was the first to test whether a visual component improved sound source localization, but failed to find support for this hypothesis. And none of the three studies that have tested whether females shift to greater reliance on the visual component if the reliability of the auditory component is compromised, for example because it is temporally degraded or masked by background noise, found evidence for it (Coss et al., 2022; Li et al., 2022; Zhu et al., 2022). Overall, visual cues may play some role in the social behavior of some species, but acoustic communication is the dominant modality mediating mate choice in nocturnal anurans (Gerhardt, 2001; Li et al., 2022).

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SUPPLEMENTARY MATERIAL

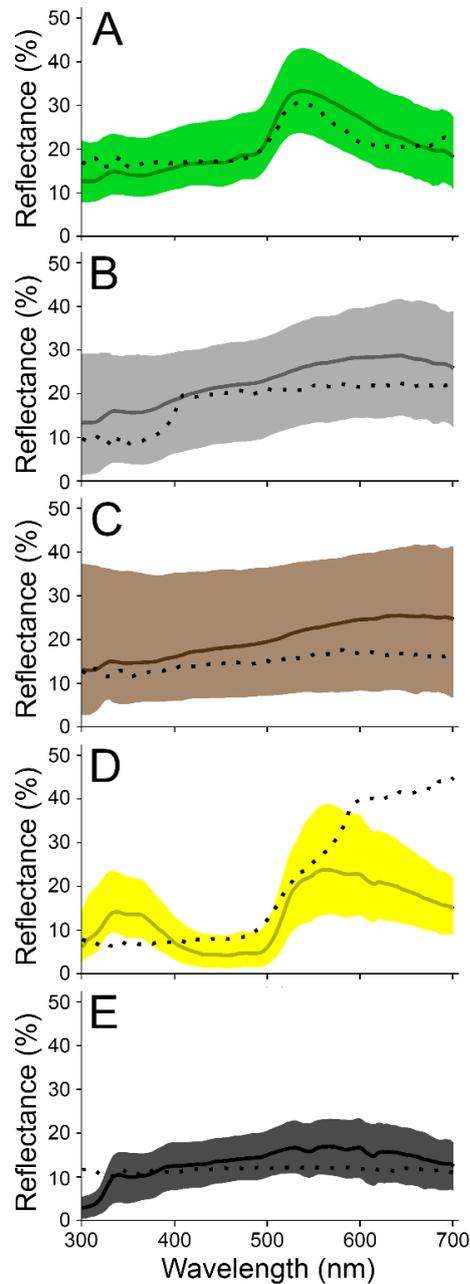


Figure S1. Reflectance spectra obtained from body color portions of *Dryophytes versicolor* and the paints used in the frog-like models. To construct the graphics, we measured 10 males in the respective body portions of interest. In all the graphics (A–E), the continuous line represents the mean reflectance, while the shaded area represents the variation among the 10 measured individuals, minimum and maximum values. The dotted line in each graphic represents the reflectance measurement of the paint used in the frog-like models. A) Reflectance of the dorsal color of males presenting the green pattern and the green paint (Sherwin Williams #146-C3); B) Reflectance of the dorsal color of males presenting the gray pattern and the gray paint (#239-C2); C) Reflectance of the dorsal color of males presenting the brown pattern and the brown paint (#209-C5); D) Reflectance of the yellow portion of the leg and the yellow paint (#131-C4); E) Reflectance of the vocal sac color and black paint (#237-C7).

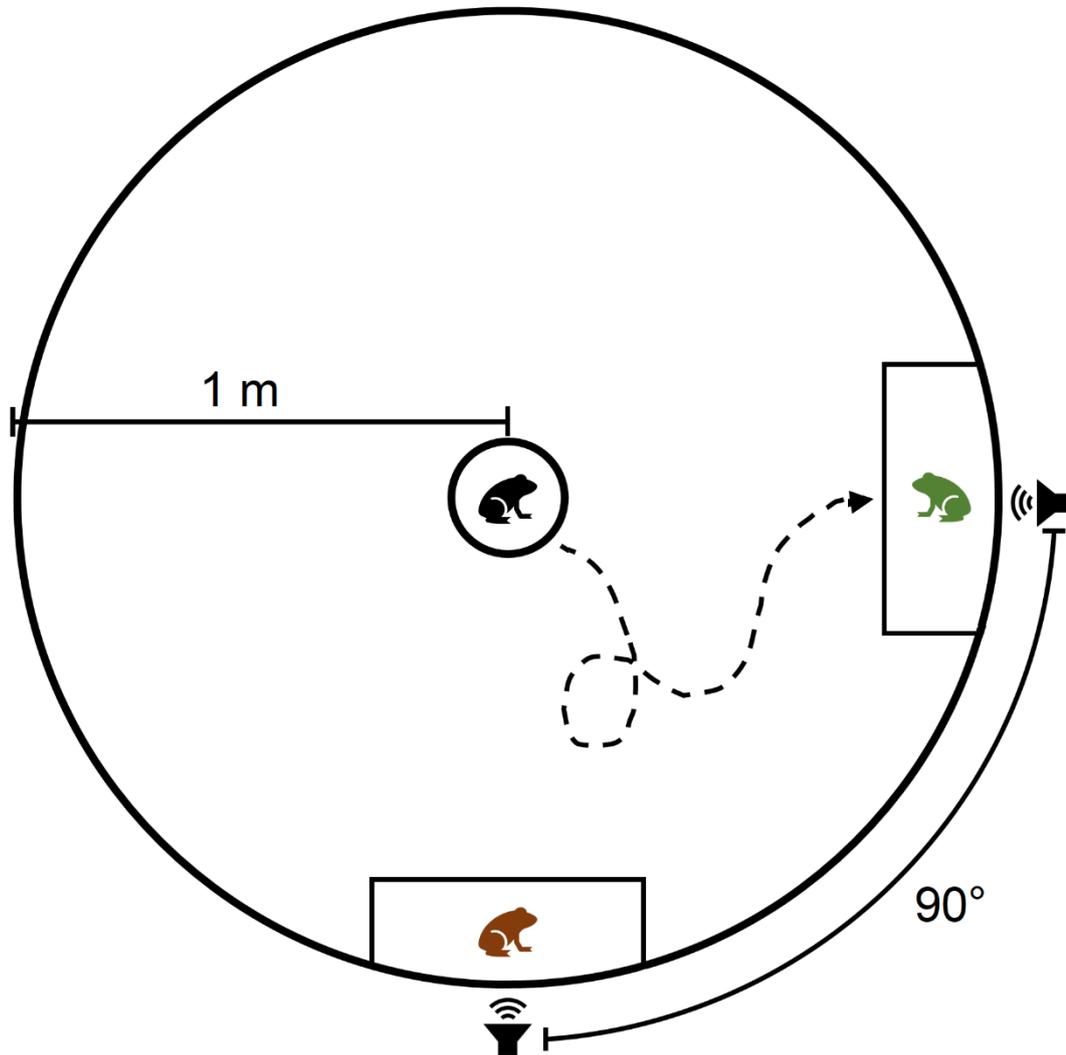


Figure S2. Representation of the arena used in the trials. The small circle in the center represents the release point where the females were placed in the beginning of each trial. The stimuli were positioned at a 90° angle from each other. The rectangles indicate where the frog-like models were placed represent the choice area. The dashed line illustrates an example of a path that the female might have taken while choosing between the two stimuli options.

Table S1. Detailed Mann-Whitney U results for all experiments. We were unable to conduct the test for latency for the experiment 5, as only one female chose the multimodal alternative, resulting in a lack of variation. Path length was not measured for experiments 5–12. Bold font indicates significant P -values.

Set of experiments	Experiment	Latency	Path length
1	1	U = 296.5, $P = 0.166$	U = 218, $P = 0.551$
	2	U = 153, $P = 0.067$	U = 163.5, $P = 0.214$
	3	U = 231.5, $P = 0.804$	U = 198, $P = 0.703$
	4	U = 117, $P = 0.278$	U = 84, $P = 0.051$
2	5	-	-
	6	U = 238, $P = 0.483$	-
	7	U = 276, $P = 0.601$	-
3	8	U = 309, $P = 0.197$	-
	9	U = 85, $P < 0.001$	-
4	10	U = 209, $P = 0.602$	-
	11	U = 188, $P = 0.213$	-
5	12	U = 212.5, $P = 0.503$	-

CONSIDERAÇÕES FINAIS

A alometria acústica, que é a relação entre o tamanho corpóreo e a frequência dos cantos, é conservada para os anuros, e a fuga dessa relação alométrica foi relatada em poucos grupos, sendo o hábito reofílico um dos motivos levantados. O Capítulo I trouxe uma revisão sobre os tamanhos corpóreos e as frequências dos cantos de todas as espécies que apresentam canto de anúncio da família Hylodidae, uma família que compreende espécies com hábitos associados a riachos. Nossos resultados confirmam a relação entre o canto e a frequência para os gêneros cantantes, *Crossodactylus* e *Hylodes*. Esses dois gêneros compreendem espécies de pequeno a médio porte que são capazes de cantar em frequências mais altas (agudas), escapando do mascaramento acústico provocado pela correnteza dos riachos. Além disso, utilizando a espécie *H. phyllodes* como modelo, demonstramos que os indivíduos aumentam o volume dos cantos quando estão em ambientes mais ruidosos, também como estratégia para fugir do mascaramento acústico. Essa família ainda conta com outros dois gêneros, *Megaelosia* e *Phantasmarana*, ambos mudos e de grande porte. Confrontando nossos dados de uma alometria acústica conservada para família, assim como, com o grande porte corpóreo das espécies desses gêneros, ainda discutimos como o ruído abiótico natural pode ter atuado como uma pressão seletiva para o surgimento de espécies mudas nesse ambiente.

Ainda nessa temática, o Capítulo II utilizou de uma abordagem observacional em campo para relatar o amplo repertório comportamental de duas populações da espécie *H. phyllodes*. Trabalhos anteriores já haviam descrito parte dos cantos e *displays* visuais utilizados por essa espécie; este capítulo mostrou que o repertório utilizado por *H. phyllodes* é ainda maior do que o conhecido previamente. O capítulo também traz a descrição detalhada de interações agonísticas entre machos, que utilizam sinais acústicos, táteis e visuais. Por fim, discutimos como a sinalização visual pode ser quantificada nessas espécies que usam amplamente essa via de sinalização; assim, esses *displays* poderiam ser utilizados em estudos comparativos a nível de indivíduos, populações e espécies, como é amplamente realizado com a comunicação acústica.

Os capítulos III e IV exploraram um outro aspecto da comunicação intraespecífica em anuros, o uso de características de coloração corpórea na transmissão de informações. O capítulo III foi construído com base em uma pesquisa multipopulacional com a espécie *Boana albomarginata*, na qual avaliamos o tamanho e condição corpórea dos machos, e relacionamos com características do canto de anúncio e de coloração corpórea. Em relação às características do canto, nossos resultados mostram que coespecíficos podem utilizar parâmetros espectrais e

temporais para prever condição corpórea dos emissores. Já os resultados em relação à coloração corpórea variaram geograficamente. Encontramos que em algumas populações as características das manchas alaranjadas são preditoras da condição corpórea dos indivíduos, enquanto essa relação não foi observada em outras populações. Estudos complementares são necessários para entender se essas características da coloração corpórea podem transmitir informações, atuando como uma pista visual em um contexto multimodal de comunicação.

Utilizando de experimentos de duas alternativas, o capítulo IV buscou entender se as pistas visuais de coloração e movimentação do saco vocal são levadas em consideração por fêmeas de *Dryophytes versicolor* durante o processo de escolha reprodutiva. Por meio de diversos testes, testamos características da coloração dorsal, das coxas, do saco vocal, além da movimentação do saco vocal durante atividade de canto. Os resultados demonstram que pistas visuais não são tomadas em consideração durante o processo de escolha de parceiros, fêmeas apenas priorizam machos com cantos mais atrativos (cantos mais longos), independente do estímulo visual oferecido. Os resultados não excluem o uso de sinais visuais em outros contextos sociais e não levaram em consideração ambientes com grandes coros (ruidosos). No entanto, demonstram que, apesar da comunicação multimodal estar presente em uma grande quantidade de espécies, esse não é um padrão geral. Algumas espécies, como é o caso de *D. versicolor*, consideram apenas as características do canto na escolha de parceiros.

A presente tese reúne os resultados que obtive durante esses anos de doutorado, nos quais dediquei minha pesquisa a temas que permeiam a ecologia comportamental em anuros. Dentre a gama de possibilidades existentes para o estudo da comunicação acústica e visual, busquei abordar diferentes complexidades em uma intersecção multimodal, que sempre me interessou. Utilizei diferentes abordagens para o levantamento de dados, incluindo revisões bibliográficas, coletas em campo e experimentação em laboratório, a fim de variar e ampliar meu aprendizado durante esses anos e me preparar para possíveis oportunidades. De uma forma geral, procurei apresentar os resultados obtidos com clareza e transparência, sempre disponibilizando os dados brutos, propiciando a replicabilidade. Espero que os dados aqui expostos possam ajudar a pautar discussões acerca das temáticas abordadas, contribuir com o avanço científico, servir de base para novos estudos e despertar o interesse acerca da comunicação deste grupo.

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ANEXOS

Anexo I – Licença de coleta: Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio #63697-2)



Ministério do Meio Ambiente - MMA
 Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio
 Sistema de Autorização e Informação em Biodiversidade - SISBIO

Autorização para atividades com finalidade científica

Número: 63697-2	Data da Emissão: 23/09/2019 15:43:53	Data da Revalidação*: 01/07/2020
De acordo com o art. 28 da IN 03/2014, esta autorização tem prazo de validade equivalente ao previsto no cronograma de atividades do projeto, mas deverá ser revalidada anualmente mediante a apresentação do relatório de atividades a ser enviado por meio do Sisbio no prazo de até 30 dias a contar da data do aniversário de sua emissão.		

Dados do titular

Nome: GUILHERME AUGUSTO ALVES	CPF: 401.739.638-03
Título do Projeto: As diferentes complexidades das vias de comunicação e influência do fungo quitrídio na sinalização acústica e visual em anuros da Mata Atlântica	
Nome da Instituição: UNIVERSIDADE ESTADUAL DE CAMPINAS	CNPJ: 46.068.425/0001-33

Cronograma de atividades

#	Descrição da atividade	Início (mês/ano)	Fim (mês/ano)
1	Trabalho em campo - levantamento dados abióticos (temperatura e umidade)	07/2018	03/2020
2	Trabalho em campo - observação comportamental dos indivíduos	07/2018	03/2020
3	Data aproximada para defesa da tese	02/2022	02/2022
4	Trabalho em campo - obtenção das gravações acústicas	07/2018	03/2020
5	Análise dos resultados e escrita da tese	04/2020	01/2022

Equipe

#	Nome	Função	CPF	Nacionalidade
1	Camila Inês Zomosa Torres	Colaboração em campo	236.898.378-39	Estrangeira
2	Carolina Lambertini	Colaboração em campo	360.051.528-40	Brasileira
3	LUÍS FELIPE DE TOLEDO RAMOS PEREIRA	Orientador do projeto de Doutorado	289.618.908-40	Brasileira
4	VICTOR FAVARO AUGUSTO	Colaboração em campo	407.555.738-38	Brasileira

Observações e ressalvas

1	Esta autorização NÃO exige o pesquisador titular e os membros de sua equipe da necessidade de obter as anuências previstas em outros instrumentos legais, bem como do consentimento do responsável pela área, pública ou privada, onde será realizada a atividade, inclusive do órgão gestor de terra indígena (FUNAI), da unidade de conservação estadual, distrital ou municipal, ou do proprietário, arrendatário, posseiro ou morador de área dentro dos limites de unidade de conservação federal cujo processo de regularização fundiária encontra-se em curso.
2	Em caso de pesquisa em UNIDADE DE CONSERVAÇÃO, o pesquisador titular desta autorização deverá contactar a administração da unidade a fim de CONFIRMAR AS DATAS das expedições, as condições para realização das coletas e de uso da infraestrutura da unidade.
3	O titular de autorização ou de licença permanente, assim como os membros de sua equipe, quando da violação da legislação vigente, ou quando da inadequação, omissão ou falsa descrição de informações relevantes que subsidiaram a expedição do ato, poderá, mediante decisão motivada, ter a autorização ou licença suspensa ou revogada pelo ICMBio, nos termos da legislação brasileira em vigor.
4	Este documento somente poderá ser utilizado para os fins previstos na Instrução Normativa ICMBio nº 03/2014 ou na Instrução Normativa ICMBio nº 10/2010, no que especifica esta Autorização, não podendo ser utilizado para fins comerciais, industriais ou esportivos. O material biológico coletado deverá ser utilizado para atividades científicas ou didáticas no âmbito do ensino superior.
5	As atividades de campo exercidas por pessoa natural ou jurídica estrangeira, em todo o território nacional, que impliquem o deslocamento de recursos humanos e materiais, tendo por objeto coletar dados, materiais, espécimes biológicos e minerais, peças integrantes da cultura nativa e cultura popular, presente e passada, obtidos por meio de recursos e técnicas que se destinem ao estudo, à difusão ou à pesquisa, estão sujeitas a autorização do Ministério de Ciência e Tecnologia.
6	O titular de licença ou autorização e os membros da sua equipe deverão optar por métodos de coleta e instrumentos de captura direcionados, sempre que possível, ao grupo taxonômico de interesse, evitando a morte ou dano significativo a outros grupos; e empregar esforço de coleta ou captura que não comprometa a viabilidade de populações do grupo taxonômico de interesse em condição in situ.

Este documento foi expedido com base na Instrução Normativa nº 03/2014. Através do código de autenticação abaixo, qualquer cidadão poderá verificar a autenticidade ou regularidade deste documento, por meio da página do Sisbio/ICMBio na Internet (www.icmbio.gov.br/sisbio).

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Página 1/3



Ministério do Meio Ambiente - MMA
 Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio
 Sistema de Autorização e Informação em Biodiversidade - SISBIO

Autorização para atividades com finalidade científica

Número: 63697-2	Data da Emissão: 23/09/2019 15:43:53	Data da Revalidação*: 01/07/2020
De acordo com o art. 28 da IN 03/2014, esta autorização tem prazo de validade equivalente ao previsto no cronograma de atividades do projeto, mas deverá ser revalidada anualmente mediante a apresentação do relatório de atividades a ser enviado por meio do Sisbio no prazo de até 30 dias a contar da data do aniversário de sua emissão.		

Dados do titular

Nome: GUILHERME AUGUSTO ALVES	CPF: 401.739.638-03
Título do Projeto: As diferentes complexidades das vias de comunicação e influência do fungo quitrídio na sinalização acústica e visual em anuros da Mata Atlântica	
Nome da Instituição: UNIVERSIDADE ESTADUAL DE CAMPINAS	CNPJ: 46.068.425/0001-33

Observações e ressalvas

7	Este documento não dispensa o cumprimento da legislação que dispõe sobre acesso a componente do patrimônio genético existente no território nacional, na plataforma continental e na zona econômica exclusiva, ou ao conhecimento tradicional associado ao patrimônio genético, para fins de pesquisa científica, bioprospecção e desenvolvimento tecnológico. Veja maiores informações em www.mma.gov.br/gen .
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Outras ressalvas

1	1) Recomenda-se utilização de método de marcação que seja alternativo à ablação de arelhos, desde que o mesmo ocasione injúrias menores ao animal, em comparação à ablação; 2) Caso a ablação seja o único método efetivo dentro do escopo do projeto e tendo em vista as características da espécie, deve ser observado o que segue: a) não ablação de arelhos consecutivos. b) não ablação de mais de um arelho/membro. c) não ablação de mais de três arelhos/indivíduo. d) não ablação de arelhos de importância para o comportamento do indivíduo (ex.: polegares opostos de espécies arborícolas). e) observar procedimentos de biossegurança e assepsia adequados; 3) É estimulado o desenvolvimento de estudos de métodos de marcação alternativos à ablação de arelhos.	RAN Goiânia-GO
2	A pesquisadora estrangeira Camilla Ines Zomosa Torres possui vínculo de Programa de bolsas ou auxílio à pesquisa patrocinado pela CAPES. Portanto, está dispensada de autorização do Ministério da Ciência, Tecnologia e Inovação.	COINF

Destino do material biológico coletado

#	Nome local destino	Tipo destino
1	UNIVERSIDADE ESTADUAL DE CAMPINAS	Coleção

Este documento foi expedido com base na Instrução Normativa nº 03/2014. Através do código de autenticação abaixo, qualquer cidadão poderá verificar a autenticidade ou regularidade deste documento, por meio da página do Sisbio/ICMBio na Internet (www.icmbio.gov.br/sisbio).

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Página 2/3

Anexo II – Licença de coleta: Instituto Florestal (Carta COTEC #483/2018 D98/2018 PH)



SECRETARIA DO MEIO AMBIENTE

INSTITUTO FLORESTAL

Rua do Horto, 931 - CEP 02377-000 - S. Paulo, SP - Brasil - Fone: (0xx11) 2231-8555

www.iflorestal.sp.gov.br

PROCESSO SMA N.º : 260108 - 007.257/2018
 INTERESSADO : Guilherme Augusto Alves
 ASSUNTO : Encaminha o projeto de pesquisa: "As diferentes complexidades das vias de comunicação e influência do fungo quitrídio na sinalização acústica e visual em anuros da Mata Atlântica"
 EQUIPE : Guilherme Augusto Alves, Luís Felipe de Toledo Ramos Pereira, Raoni Rebouças, Camila Inês Zornosa Torres, Mariana Retuci Pontes, Victor Augusto e Simone Aparecida Dena
 VIGÊNCIA : Março de 2018 a Março de 2022

Carta COTEC nº 483/2018 D98/2018 PH

São Paulo, 05 de Novembro de 2018.

Senhor
 Guilherme Augusto Alves
 Campinas-SP
 CEP: 13.046-545
 Tel.: (19) 3276-6313 / (19) 9-8213-4693
 E-mail: Alves.guilherme.augusto@gmail.com

Apraz-nos informar que o projeto "As diferentes complexidades das vias de comunicação e influência do fungo quitrídio na sinalização acústica e visual em anuros da Mata Atlântica", constante do processo em referência, de autoria de Guilherme Augusto Alves, Luís Felipe de Toledo Ramos Pereira, Raoni Rebouças, Camila Inês Zornosa Torres, Mariana Retuci Pontes, Victor Augusto e Simone Aparecida Dena, foi aprovado para ser executado, no período de Março de 2018 a Março de 2022, nas seguintes Unidades:

UNIDADE e RESPONSÁVEL	ENDEREÇO DA UNIDADE DE CONSERVAÇÃO	OBSERVAÇÕES
Parque Estadual da Serra do Mar - Núcleo Bertioga Ao responsável pela Unidade Chefe de Unidade: Eduardo Ferreira dos Santos Souza	a) Sede administrativa: Av. Henrique Constabile, 114 Centro Bertioga-SP CEP: 11250.000 Telefone para informações: (13) 3317-2094 (11) 9-5652-1559 E-mail: pesm.bertioga@iflorestal.sp.gov.br	<ul style="list-style-type: none"> • Com relação à realização do projeto no Parque Estadual da Serra do Mar - Núcleo Bertioga, manifestamo-nos: <u>De acordo com a execução do projeto;</u> • Com relação aos resultados do projeto, as informações geradas serão de: Alta prioridade; • Com relação ao planejamento da Unidade, o Parque Estadual da Serra do Mar - Núcleo Bertioga possui: Plano de Manejo; • Com relação às atividades previstas pelo projeto, existem restrições quanto: ao Plano de Manejo; • <u>As seguintes colocações devem ser observadas pelos autores, por ocasião da visita a esta Unidade:</u> • A administração do Parque Estadual da Serra do Mar - Núcleo Bertioga não se responsabiliza pelo transporte das equipes durante o desenvolvimento do projeto, devendo ser previstos recursos para as atividades; • O pesquisador deverá concordar e responsabilizar-se em repassar para os demais envolvidos no projeto, as normas da Unidade de Conservação; • Visitas de pesquisadores, representantes de outras instituições, convidados, amigos, fotógrafos, imprensa, etc., não relacionados no projeto original como



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PROCESSO SMA N.º : 260108 - 007.257/2018
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ASSUNTO : Encaminha o projeto de pesquisa: "As diferentes complexidades das vias de comunicação e influência do fungo quitrídio na sinalização acústica e visual em anuros da Mata Atlântica"
EQUIPE : Guilherme Augusto Alves, Luís Felipe de Toledo Ramos Pereira, Raoni Rebouças, Camila Inês Zornosa Torres, Mariana Retuci Pontes, Victor Augusto e Simone Aparecida Dena
VIGÊNCIA : Março de 2018 a Março de 2022

UNIDADE e RESPONSÁVEL	ENDEREÇO DA UNIDADE DE CONSERVAÇÃO	OBSERVAÇÕES
		<p>membro da equipe executora, devem ser previamente notificadas e autorizadas pela administração da Unidade;</p> <ul style="list-style-type: none"> • As atividades não previstas no projeto original estão vetadas, devendo ser previamente notificadas e submetidas à análise e aprovação do Instituto Florestal; • Sempre nas visitas a campo o pesquisador deverá obrigatoriamente ir acompanhado de um monitor ambiental autorizado Parque Estadual da Serra do Mar - Núcleo Bertioga; • Mandar por e-mail a lista dos nomes da equipe fixa dos colaboradores de campo, pesquisadores e estagiários; • Enviar à coordenadoria do Parque Estadual da Serra do Mar - Núcleo Bertioga, relatórios periódicos impressos e digital com fotos; • É desejável que se realize breve apresentação sobre o projeto de pesquisa para os funcionários e/ou Conselho da Unidade, a combinar com a administração; • Os pesquisadores deverão disponibilizar as publicações (dissertações, teses e outros documentos) ao Núcleo, com o intuito de valorizar a pesquisa científica e promover a integração da academia com a Gestão da Unidade de Conservação; • Os autores do projeto, ao final da realização do trabalho, deverão utilizar os dados da pesquisa para elaborar atividades de ensino, sejam na forma de mini-cursos, palestras, apostilas, folhetos, painéis explicativos para os diferentes grupos que atuam no Parque e a sociedade civil; • Relatórios parciais e final encaminhados à COTEC devem também ser remetidos à administração do Parque Estadual da Serra do Mar - Núcleo Bertioga, para serem juntados ao acervo da Unidade.
Parque Estadual da Serra do Mar - Núcleo Picinguaba Ao responsável pela Unidade	a) <u>Escritório Regional:</u> Endereço: Rua Dr. Esteves da Silva, nº. 510, Centro - Ubatuba-SP, CEP: 11.680-000 Telefones para informação: (12) 3833-6552 E-mail: pesm.picinguaba@iflorestal.sp.gov.br	<ul style="list-style-type: none"> • Com relação à realização do projeto no Parque Estadual da Serra do Mar - Núcleo Picinguaba, manifestamo-nos: <u>De acordo com a execução do projeto;</u> • Com relação aos resultados do projeto, as informações geradas serão de: Alta prioridade; • Com relação ao planejamento da Unidade, o Parque Estadual da Serra do Mar - Núcleo Picinguaba



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PROCESSO SMA N.º : 260108 - 007.257/2018
INTERESSADO : Guilherme Augusto Alves
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VIGÊNCIA : Março de 2018 a Março de 2022

UNIDADE e RESPONSÁVEL	ENDEREÇO DA UNIDADE DE CONSERVAÇÃO	OBSERVAÇÕES
Gestora: Cláudia Camila Faria de Oliveira	Dias e horário de funcionamento: de segunda-feira a sexta-feira das 8h às 17h. b) Sede Administrativa: Endereço: Rodovia BR, nº 101, km 08 Picinguaba - Ubatuba-SP Telefones para informação: (12) 3832-1397 E-mail: pesm.picinguaba@iflorestal.sp.gov.br Dias e horário de funcionamento: de segunda-feira a sexta-feira das 8h às 17h. c) Centro de Visitantes: Endereço: Rodovia BR, nº 101, km 11 Picinguaba - Ubatuba-SP E-mail: npicinguaba.agendamento@iflorestal.sp.gov.br Dias e horário de funcionamento: diariamente das 8h às 17h. Para realização de trilhas no Parque é necessário agendamento prévio.	possui: Plano de Manejo; • Com relação às atividades previstas pelo projeto: não existe restrição; • <u>As seguintes colocações devem ser observadas pelos autores, por ocasião da visita a esta Unidade:</u> • A administração da Unidade de Conservação deverá ser informada com antecedência sobre cada visita técnica e sobre o início dos trabalhos de campo; • Relatórios parciais e final encaminhados à COTEC devem também ser remetidos à administração do Parque Estadual da Serra do Mar - Núcleo Picinguaba, para serem juntados ao acervo da Unidade.

"O projeto de pesquisa se enquadra nas normas estabelecidas pela COTEC e os autores apresentaram a licença SISBIO para a captura dos espécimes e documentação referente ao cadastro SisGen.

Os autores informam que serão capturadas três espécies de anfíbios: Boana albomarginata, Hylodes phyllodes e Megaelosia goeldii. Nenhum dos espécimes capturados durante os experimentos de campo será coletado. Os autores informam que os indivíduos serão soltos no mesmo local da coleta.

Os autores deverão encaminhar à COTEC relatório parcial e final relatando as atividades desenvolvidas ao longo do projeto de pesquisa."

Por ocasião das visitas nesta Unidade, solicitamos:

1. Agendar os trabalhos de campo junto à administração da Unidade, com antecedência mínima de 15 dias, fornecendo o nome de todos os membros da equipe visitante;



SECRETARIA DO MEIO AMBIENTE

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PROCESSO SMA N.º : 260108 - 007.257/2018
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 VIGÊNCIA : Março de 2018 a Março de 2022

2. Visitas de pesquisadores, representantes de outras instituições, convidados, pesquisadores estrangeiros, alunos, amigos, fotógrafos, imprensa, etc., não relacionados no projeto original como membro da equipe executora devem ser previamente notificadas e autorizadas pela administração da Unidade;
3. Permitir acompanhamento por pessoal da Unidade, quando o responsável pela Unidade assim estabelecer;
4. Atividades não previstas no projeto original estão vetadas, devendo ser previamente notificadas e submetidas à análise e aprovação do Instituto Florestal;
5. Portar a licença do SISBIO/IBAMA. Quando renovada, apresentar cópia para ser anexada ao processo;
6. Somente os autores nomeados na licença do SISBIO/IBAMA poderão efetuar coletas;
7. Questionários, formulários, entrevistas orais e outras formas de abordagem de pessoal local e do público visitante devem ter o roteiro previamente submetido à ciência do responsável pela administração da Unidade;
8. Atividades de coleta de amostras da biodiversidade estão condicionadas à apresentação de cópia da licença SISBIO/IBAMA. Quando renovada, apresentar cópia para ser anexada ao processo;
9. As intervenções a serem executadas na Unidade, como colocação de placas, pregos, faixas, distribuição de folhetos, etc. devem ser previamente e formalmente autorizadas pelo responsável pela administração da Unidade;
10. Não deixar no campo vestígios da passagem no local como resíduos, buracos, embalagens, armadilhas, tambores, etc. Trincheiras e escavações devem ser seguidas de processos de recuperação, minimizando o dano local;
11. Havendo necessidade de acompanhamento por mateiros, guarda-parques, consultar a Unidade sobre possível disponibilidade, com antecedência mínima de 15 dias e;
12. Havendo necessidade de deslocamento de equipamentos, realizar por conta própria ou consultar a Unidade sobre possível disponibilidade de auxiliares, com antecedência mínima de 15 dias.

Responsáveis por projetos com previsão de coletas devem providenciar a autorização SISBIO/IBAMA na página http://www.ibama.gov.br/sisbio/index.php?id_menu=205. Obtida a autorização, encaminhar cópia à Comissão Técnico-Científica - COTEC para ser anexado no processo respectivo. A partir de janeiro de 2008, toda e qualquer forma de coleta nas UCs deverá ser formalmente licenciada pelo SISBIO/IBAMA.

Conforme estabelece a Portaria do Diretor Geral de 23/01/90, e cientificado à V. Senhoria nos Termos de Compromisso e de Responsabilidade assinados em 22/08/2018, há necessidade de encaminhar à COTEC, um relatório anual, no mês de Dezembro de cada ano. Nos relatórios assinalar a área de estudos em GPS/coordenadas geográficas.

Relatórios parciais e final encaminhados à COTEC devem também ser remetidos à administração das Unidades de Conservação, para serem juntados ao acervo da Unidade.

Cópia da dissertação, tese, artigos, resumos em eventos científicos e outras formas de publicações podem ser apresentados como relatório parcial e final. Não havendo possibilidade de cópias, solicita-se o encaminhamento da(s) referência(s) bibliográfica(s), que possibilite(m) o acesso a todas as informações geradas no projeto.



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 VIGÊNCIA : Março de 2018 a Março de 2022

A utilização para outros fins que não seja a pesquisa científica, de fotografias, imagens, vídeos e outras mídias registradas nas Unidades a título deste projeto devem ser objetos de termo específico, conforme a Portaria CINEP, de 09/02/1999, publicada no DOE de 10/02/1999.

Esta aprovação não implica em suporte financeiro de qualquer natureza por parte do Instituto Florestal. A participação e ou auxílio financeiro por parte do Instituto Florestal, quando houver, deverá ser detalhado e formalizado através de contratos, convênios e outros instrumentos legais pertinentes, cuja cópia deve ser juntada ao presente processo.

Para qualquer informação ou eventualidade, colocamo-nos à sua inteira disposição.

Por prestigiar a nossa instituição, agradecemos.

Atenciosamente,

Israel Luiz de Lima
 COTEC - Comissão Técnico-Científica
 Instituto Florestal
 Rua do Horto, nº 931
 02377-000 - São Paulo - SP
 Fone: (011) 2231- 8555 - Ramal 2071 Fax: Ramal 2220
cotec2@gmail.com
cotec@if.sp.gov.br

Anexo III – Parecer da Comissão de Ética no Uso de Animais da Universidade Estadual de Campinas (CEUA/Unicamp #4983-1/2018)



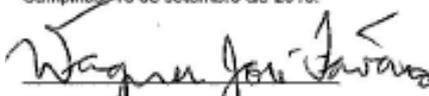
CERTIFICADO

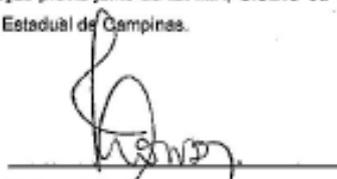
Certificamos que a proposta intitulada De uni à multimodal: as diferentes complexidades das vias de comunicação e influência do fungo quitrídio no comportamento de anuros neotropicais, registrada com o nº 4983-1/2018, sob a responsabilidade de Prof. Dr. Luís Felipe de Toledo Ramos Pereira e Guilherme Augusto Alves, que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem) para fins de pesquisa científica (ou ensino), encontra-se de acordo com os preceitos da LEI Nº 11.794, DE 8 DE OUTUBRO DE 2008, que estabelece procedimentos para o uso científico de animais, do DECRETO Nº 8.899, DE 15 DE JULHO DE 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), tendo sido aprovada pela Comissão de Ética no Uso de Animais da Universidade Estadual de Campinas - CEUA/UNICAMP, em 13 de setembro de 2018.

Finalidade:	() Ensino (X) Pesquisa Científica
Vigência do projeto:	15/08/2018-28/02/2022
Vigência da autorização para manipulação animal:	13/09/2018-28/02/2022
Espécie / linhagem/ raça:	Anfíbio / Boana albomarginata
No. de animais:	60
Idade/Peso:	01 ano / 10g
Sexo:	machos
Espécie / linhagem/ raça:	Anfíbio / Hylodes phyllodes
No. de animais:	60
Idade/Peso:	01 ano / 05g
Sexo:	machos
Espécie / linhagem/ raça:	Anfíbio / Megaelosia goeldii
No. de animais:	60
Idade/Peso:	01 ano / 30g
Sexo:	machos
Espécie / linhagem/ raça:	Anfíbio / Megaelosia apuana
No. de animais:	60
Idade/Peso:	01 ano / 30g
Sexo:	machos
Espécie / linhagem/ raça:	Anfíbio / Agalychnis callidryas
No. de animais:	60
Idade/Peso:	01 ano / 10g
Sexo:	machos
Origem:	Não se aplica (serão mantidos em seus ambientes naturais)
Biotério onde serão mantidos os animais:	Não se aplica (serão mantidos em seus ambientes naturais)

A aprovação pela CEUA/UNICAMP não dispensa autorização prévia junto ao IBAMA, SISBIO ou CIBio e é restrita a protocolos desenvolvidos em biotérios e laboratórios da Universidade Estadual de Campinas.

Campinas, 13 de setembro de 2018.


Prof. Dr. Wagner José Favaro
Coordenador


Fátima Alonso
Secretária Executiva

IMPORTANTE: Pedimos atenção ao prazo para envio do relatório final de atividades referente a este protocolo: até 30 dias após o encerramento de sua vigência. O formulário encontra-se disponível na página da CEUA/UNICAMP, área do pesquisador responsável. A não apresentação do relatório ao prazo estabelecido impedirá que novos protocolos sejam submetidos.

Anexo IV – Registro no Sistema Nacional de Gestão do Patrimônio Genético (SISGen #AE9A0E0)



Ministério do Meio Ambiente
CONSELHO DE GESTÃO DO PATRIMÔNIO GENÉTICO

SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO

Certidão

Cadastro nº AE9A0E0

Declaramos, nos termos do art. 41 do Decreto nº 8.772/2016, que o cadastro de acesso ao patrimônio genético ou conhecimento tradicional associado, abaixo identificado e resumido, no Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado foi submetido ao procedimento administrativo de verificação e não foi objeto de requerimentos admitidos de verificação de indícios de irregularidades ou, caso tenha sido, o requerimento de verificação não foi acatado pelo CGen.

Número do cadastro: AE9A0E0
 Usuário: Guilherme Augusto Alves
 CPF/CNPJ: 401.739.638-03
 Objeto do Acesso: Patrimônio Genético
 Finalidade do Acesso: Pesquisa

Espécie

Batrachochytrium dendrobatidis

Hylodes phyllodes

Megaelosia goeldii

Boana albomarginata

Título da Atividade: As diferentes complexidades das vias de comunicação e influência do fungo quitrídio na sinalização acústica e visual em anuros da Mata Atlântica

Equipe

Guilherme Augusto Alves

UNICAMP

Luís Felipe de Toledo Ramos Pereira

Universidade Estadual de Campinas

Data do Cadastro: 24/08/2018 14:49:11

Situação do Cadastro: Concluído

Conselho de Gestão do Patrimônio Genético
 Situação cadastral conforme consulta ao SisGen em 10:57 de 22/09/2023.



SISTEMA NACIONAL DE GESTÃO
 DO PATRIMÔNIO GENÉTICO
 E DO CONHECIMENTO TRADICIONAL
 ASSOCIADO - SISGEN

Anexo V – Declaração de Bioética e Biossegurança

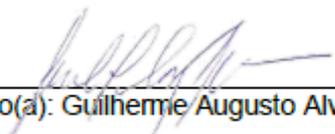


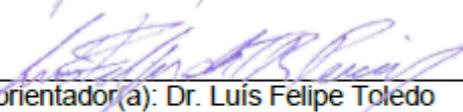
COORDENADORIA DE PÓS-GRADUAÇÃO
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DECLARAÇÃO

Em observância ao §5º do Artigo 1º da Informação CCPG-UNICAMP/001/15, referente a Bioética e Biossegurança, declaro que o conteúdo de minha Tese de Doutorado, intitulada "**Comunicação acústica e visual em anuros, seus multicomponentes e implicações no contexto multimodal**", desenvolvida no Programa de Pós-Graduação em Ecologia do Instituto de Biologia da Unicamp, não versa sobre pesquisa envolvendo seres humanos, animais ou temas afetos a Biossegurança.

Assinatura: 
Nome do(a) aluno(a): Guilherme Augusto Alves

Assinatura: 
Nome do(a) orientador(a): Dr. Luis Felipe Toledo

Data: 25 de setembro de 2023

Anexo VI – Declaração de Direitos Autorais**Declaração**

As cópias de artigos de minha autoria ou de minha co-autoria, já publicados ou submetidos para publicação em revistas científicas ou anais de congressos sujeitos a arbitragem, que constam da minha Dissertação/Tese de Mestrado/Doutorado, intitulada **Comunicação acústica e visual em anuros, seus multicomponentes e implicações no contexto multimodal**, não infringem os dispositivos da Lei n.º 9.610/98, nem o direito autoral de qualquer editora.

Campinas, 25 de setembro de 2023

Assinatura : _____

Nome do(a) autor(a): **Guilherme Augusto Alves**
RG n.º 36.011.933-5

Assinatura : _____

Nome do(a) orientador(a): **Dr. Luis Felipe Toledo**
RG n.º 28.465.361.5

Anexo VII – comprovante submissão do capítulo III

BEAS: Submission Confirmation for BEAS-D-23-00309R1 - [EMID:c6aec08c6c6b44fa]

1 mensagem

Behavioral Ecology and Sociobiology Editorial Office <em@editorialmanager.com>

2 de janeiro de 2024 às
10:16

Responder a: Behavioral Ecology and Sociobiology Editorial Office <besedass@uni-bonn.de>

Para: Guilherme Augusto-Alves <alves.guilherme.augusto@gmail.com>

CC: "Gerlinde Höbel" hoebel@uwm.edu, "Luís Felipe Toledo" toledosapo@gmail.com

Ref.: Ms. No. BEAS-D-23-00309R1

Geographic variation in acoustic and visual cues and their potential to signal body condition in the Brazilian treefrog, *Boana albomarginata*

Dear Dr. Augusto-Alves,

Behavioral Ecology and Sociobiology has received your revised submission.

You may check the status of your manuscript by logging onto Editorial Manager at <https://www.editorialmanager.com/beas/>.

Kind regards,

Editorial Office
Behavioral Ecology and Sociobiology

Anexo VIII – Comprovante submissão do capítulo IV

Behavioral Ecology - MS BEHECO-2024-0008

1 mensagem

Behavioral Ecology <onbehalf@manuscriptcentral.com>

9 de janeiro de 2024 às 11:02

Responder a: beheco.edoffice@oup.com

Para: alves.guilherme.augusto@gmail.com

*** THIS IS AN AUTOMATICALLY GENERATED MESSAGE ***

Dear Guilherme Augusto-Alves,

Your manuscript entitled:

Visual cues do not function in a multimodal signaling context for mate attraction in Gray Treefrogs

has been received by 'Behavioral Ecology' and has been assigned manuscript number: BEHECO-2024-0008

Your manuscript is currently awaiting assignment to an editor. You will be contacted by the assigned editor once a decision has been made regarding your manuscript. Meanwhile you can track the progress of your manuscript by looking in the Submitted Manuscripts section of your Author Center in Manuscript Central (<https://mc.manuscriptcentral.com/beheco>).

OPTIONAL OPEN ACCESS – Please note that if your manuscript is accepted for publication in 'Behavioral Ecology', you will now have the option, at a charge payable by you, to make your paper freely available online immediately upon publication, under the Oxford Open initiative (see <http://www.oxfordjournals.org/oxfordopen/>), Applicable Oxford Open charges can be found in the Authors Instructions (http://www.oxfordjournals.org/our_journals/beheco/for_authors/general.html). You will be asked to decide whether you wish to opt for Open Access for your paper when and if your manuscript receives final acceptance.

Dryad Data Repository:

Archiving of data in the Dryad Data Repository (<http://datadryad.org>) is a condition of publishing in the journal. In the event that your manuscript is accepted for publication, you will be required to submit your data before it can be forwarded to production using the link below.

Advantages of depositing data in Dryad include:

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- * Workload reduction: if you receive individual requests for data, you can simply direct them to the items in Dryad.
- * Preservation: your data files will be permanently and safely archived in perpetuity.
- * Impact: you will garner citations through the reuse of your data, and you can monitor the use of your data through Dryad's usage statistics.

The link below will take you to the Dryad record for your article, so you won't have to re-enter its bibliographic information, and can upload your files directly. More information about depositing data in Dryad is available at <http://www.datadryad.org/depositing>. Please use the following link:

<http://datadryad.org/submit?journalID=BEHECO&manu=BEHECO-2024-0008>,

Once you deposit your data package, it receives a unique and stable DOI identifier, which is immediately sent back to you and to the journal for inclusion in the published article. Depositing your data in good time for this to happen, is optimal; if the Dryad DOI doesn't appear in the final published article, that of course greatly weakens its connection to the underlying data.

Thank you for submitting your paper to 'Behavioral Ecology'.

Best wishes

Jenny Jekyll

Editorial Office, Behavioral Ecology

beheco.edoffice@oup.com

Anexo IX– Produção científica durante a vigência do curso de doutorado

Artigos científicos

- Augusto-Alves, G.; das Neves-da-Silva, D.; Checchinato, J.; de Carvalho-e-Silva, A.M.P.T.; Toledo, L.F. 2023. Giant and phantom frogs in the Atlantic Forest: historical distribution and conservation implications. *Journal for Nature Conservation*, 74: 126460.
- Augusto-Alves G. & Toledo, L.F. 2022. Communication across multiple sensory modes: quantifying the rich behavioural repertoire of a Neotropical torrent frog Behaviour. *Behaviour*, 159: 351-375.
- Moroti, M. de T.; Pedrozo, M; Severgnini, M.R.; Augusto-Alves, G.; Dena, S.; Martins, I.A.; Nunes, I. & Muscat, E. 2022. A new species of *Odontophrynus* (anura, Odontophrynidae) from the southern portion of the mantiqueira mountains. *European Journal of Taxonomy*, 847:160-193.
- Forti, L.R.; Pontes, M.R.; Augusto-Alves, G.; Martins, A.; Hepp, F.; Szabo, J.K. 2022. Data collected by citizen scientists reveal the role of climate and phylogeny on the frequency of shelter types used by frogs across the americas. *Zoology*, 155: 126052.
- Vittorazzi, S.E.; Augusto-Alves, G.; Neves-da-Silva, D.; Carvalho-e-Silva, A.M.P.T.; Recco-Pimentel, S.M.; Toledo, L.F.; Lourenco, L.B. & Bruschi, D.P. 2021. Paraphyly in the giant torrent-frogs (Anura: Hylodidae: *Megaelosia*) and the description of a new genus. *Salamandra*, 57: 274-284.
- Leal, F.; Zornosa-Torres, C.; Augusto-Alves, G.; Dena, S.; Pezzuti, T.; Leite, F.; Bolsoni, L.; Garcia, P. & Toledo, L.F. 2021. Head in the clouds: a new dwarf frog species of the *Physalaemus signifer* clade (Leptodactylidae, Leiuperinae) from the top of the brazilian Atlantic Forest. *European Journal of Taxonomy*, 764: 119-151.
- Augusto-Alves G.; Dena S. & Toledo L.F. 2021. Acoustic allometry, background stream noise and its relationship with large-bodied and voiceless rheophilic frogs. *Zoologischer Anzeiger*, 295: 156-162.
- Bardier, C.; Székely, D.; Augusto-Alves, G.; Matínez-Latorraca, N.; Schmidt, B.R. & Cruickshank, S.S. 2021. Performance of visual vs. software-assisted photo-identification in mark-recapture studies: a case study examining different life stages of the pacific horned frog (*Ceratophrys stolzmanni*). *Amphibia-Reptilia*, 42: 17-28.

- Dena, S.; Rebouças, R.; Augusto-Alves, G.; Zornosa-Torres, C.; Pontes, M.R. & Toledo, L.F. 2020. How much are we losing in not depositing anuran sound recordings in scientific collections? *Bioacoustics*, 29: 590-601.
- Augusto-Alves, G.; Ruggeri-Gomes, J.; Martins, A.G.S.; Domingos, A.H.R.; Santos, I. & Toledo, L.F. 2020. *Leptodactylus flavopictus*: temporal calling activity and tadpole re-description. *Salamandra*, 56: 123-134.
- Bovolon, J.P.; Zornosa-Torres, C.; Augusto-Alves, G.; Almeida, A.P.; Gasparini, J.L. & Toledo, L.F. 2020. Advertisement calls of two species of the *Sphaenorhynchus platycephalus* group and the aggressive call of *S. bromelicola* (Anura: Hylidae: Scinaxinae). *Salamandra*, 56: 401-404.
- Muscat, E.; Stuginski, D.R.; Nunes, I.; Martins, I.; Augusto-Alves, G.; Vittorazzi, S.E.; Toledo, L.F. & Moroti, M.T. 2020. Update on the geographic distribution of three poorly known frog species in the mantiqueira mountain range. *Herpetology notes*, 13: 573-577.
- Zornosa-Torres, C.; Augusto-Alves, G.; Lyra, M.L.; Júnior, J.C. de S.; Garcia, P.C.A; Leite, F.; Verdade, V.; Rodrigues, M.T.; Gasparini, J.L; Haddad, C.F.B; Toledo, L.F. 2020. Anurans of the caparaó national park and surroundings, southeast Brazil. *Biota Neotropica*, 20; e20190882.
- Rebouças, R.; Augusto-Alves, G. & Toledo, L.F. 2020. Evolution of treefrogs' calls in tropical islands might be under directional selection. *Journal of Zoology*, 312: 43-52.
- Zornosa-torres, C.; Augusto-Alves, G.; Martins, A.G.S.; Domingos, A.H.R. & Toledo, L.F. 2019. *Scinax imbegue*. Predation. *Herpetological Review*, 50: 552-552.
- Muscat, E.; Augusto-Alves, G.; Toledo, L.F.; Tanaka, R.M. & Stuginski, D.R. 2019. Multimale amplexus, amplexant and advertisement calls, and tadpole development in *Ololygon perpusilla* (lutz and lutz, 1939). *Herpetology Notes*, 12: 1067-1072.

Apresentação de trabalhos em eventos científicos

- Augusto-Alves, G.; Feagles, O.S.; Toledo, L.F. & Höbel, G. 2023. Comunicação multimodal em anuros: explorando o caso de *Dryophytes versicolor*. Comunicação oral – X Congresso Brasileiro de Herpetologia, Porto Seguro, Bahia, Brasil.
- Augusto-Alves, G.; Höbel, G. & Toledo, L.F. 2022. Males treefrog body color traits are related with call parameters and may transmit information about individual body condition. Comunicação oral - Annual Conference of Animal Behavior Society - ABS2022, San José, Costa Rica.

Augusto-Alves, G.; Reboucas, R.; Bardier, C. & Toledo, L.F. 2019. Taxa de crescimento, tempo de desenvolvimento larval e padrão de atividade em um anuro mudo de corredeira, *Megaelosia apuana* (Anura; Hylodidae). Comunicação oral – IX Congresso Brasileiro de Herpetologia, Campinas, São Paulo, Brasil.