Universidade Federal do Rio Grande
Instituto de Ciências Biológicas
Pós-graduação em Biologia de
Ambientes Aquáticos Continentais

Ambiente e coloração: padrões ecológicos e evolutivos da variação de coloração dorsal em anuros neotropicais

Luísa Nunes Lermen

Orientador: Daniel Loebmann

Rio Grande
2026

Universidade Federal do Rio Grande
Instituto de Ciências Biológicas
Pós-graduação em Biologia de Ambientes Aquáticos Continentais

Ambiente e coloração: padrões ecológicos e evolutivos da variação de coloração dorsal em anuros neotropicais

Aluno: Luísa Nunes Lermen
Orientador: Daniel Loebmann

Tese apresentada ao Programa de Pós-graduação em Biologia de Ambientes Aquáticos Continentais como requisito parcial para a obtenção do título de Doutora em Biologia de Ambientes Aquáticos Continentais.

Rio Grande
2026

L616a Lermen, Luísa Nunes
Ambiente e coloração: padrões ecológicos e evolutivos da variação de coloração dorsal em anuros neotropicais / Luísa Nunes Lermen - 2026.
132f.

Tese (Doutorado) – Universidade Federal do Rio Grande – Programa de Pós-Graduação em Biologia de Ambientes Aquáticos Continentais, 2026.

Orientador: Dr. Daniel Loebmann

1. Biologia de Ambientes Aquáticos Continentais. 2. Anfíbios. 3. Coloração animal. 4. Biodiversidade neotropical. 5. *Dendropsophus minutus*. 6. *Rhinella* I. Loebmann, Daniel. II. Título.

.

CDU 574.5(28)

Catalogação na fonte: Bibliotecária Brenda Sequeira CRB10/1979

UNIVERSIDADE FEDERAL DO RIO GRANDE-FURG
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA DE AMBIENTES AQUÁTICOS CONTINENTAIS - PPGBAC
Av. Itália s/n – Rio Grande/RS, Brasil. 96203-900 fone/fax: 53-3233.6848
e-mail: pgbac@furg.br
https://ppgbac.furg.br

FURG

PPGBAC

ATA DE DEFESA DE TESE DE DOUTORADO EM BIOLOGIA DE AMBIENTES AQUÁTICOS CONTINENTAIS – Nº 002/2026

Às 08h00 (oito horas) do dia 14 (quatorze) do mês de janeiro de 2026 (dois mil e vinte e seis) reuniram-se docentes, discentes e comunidade em geral, para a Defesa Pública da Tese de Doutorado da acadêmica Luísa Lermen, que ocorreu de forma híbrida. A Tese intitulada **“Ambiente e coloração: padrões ecológicos e evolutivos da variação de coloração dorsal em anuros neotropicais”** foi avaliada pela Banca Examinadora composta pelo Prof. Dr. Daniel Loebmann (Orientador), Prof. Dr. Alexandre Miranda Garcia (PPGBAC/FURG), Prof. Dr. Daniel Marques de Almeida Pessoa (UFRN) e Dr. Fernando Marques Quintela (UFFS). Após a defesa e arguição pública, a Banca Examinadora reuniu-se, para deliberação final, e considerou a acadêmica **APROVADA**. Desta forma, a acadêmica concluiu mais uma das etapas necessárias para a obtenção do grau de **DOUTOR EM BIOLOGIA DE AMBIENTES AQUÁTICOS CONTINENTAIS**. Nada mais havendo a tratar, às 12h00h (doze horas) foi lavrada a presente ata, que lida e aprovada, foi assinada pelos membros da Banca Examinadora, pela Acadêmica e pela Coordenadora do Curso.

gov.br Documento assinado digitalmente
DANIEL LOEBMANN
Data: 15/01/2026 02:27:35-0300
Verifique em https://validar.iti.gov.br

Prof. Dr. Daniel Loebmann

Dr. Fernando Marques Quintela

gov.br Documento assinado digitalmente
ALEXANDRE MIRANDA GARCIA
Data: 14/01/2026 12:29:12-0300
Verifique em https://validar.iti.gov.br

Prof. Dr. Alexandre Miranda Garcia

gov.br Documento assinado digitalmente
LUISA NUNES LERMEN
Data: 14/01/2026 16:22:12-0300
Verifique em https://validar.iti.gov.br

Luísa Lermen

gov.br Documento assinado digitalmente
DANIEL MARQUES DE ALMEIDA PESSOA
Data: 16/01/2026 15:04:27-0300
Verifique em https://validar.iti.gov.br

Prof. Dr. Daniel Marques de Almeida Pessoa

gov.br Documento assinado digitalmente
JUNIOR BORELLA
Data: 22/01/2026 13:50:25-0300
Verifique em https://validar.iti.gov.br

Profª. Drª. Camila De Martinez Gaspar Martins
Coordenadora do Curso

AGRADECIMENTOS

Agradeço o meu orientador, Prof. Dr. Daniel Loebmann, por aceitar me guiar nessa nova etapa acadêmica, pela confiança depositada em mim e por todas as oportunidades de aprendizado ao longo deste percurso.

Agradeço aos colegas, professores e funcionários do PPG Biologia de Ambientes Aquaticos Continentais, assim como aos companheiros do Laboratório de Vertebrados por todo o apoio e auxilio durante esse período.

Agradeço aos membros da banca avaliadora por terem aceitado o convite; a todos que de alguma forma me ajudaram com os trabalhos de campo; ao ICMBio pela concessão da licença necessárias para este estudo.

Agradeço aos meus queridos amigos, de perto ou de longe, que acreditaram em mim, ofereceram seu apoio e suportaram meus desabafos, mesmo sem conhecer em detalhes o trabalho aqui desenvolvido.

Agradeço à minha família pelo amor e apoio incondicionais; à minha mãe, Denise, e seu “carrinho”, pela ajuda nos deslocamentos e nas atividades de campo, infelizmente não foi desta vez que encontramos o sapo grande que você tanto queria; ao meu pai, Vitor, pelo auxílio nas coletas e nas viagens mais longas, além de enfrentar heroicamente o ataque incessante dos mosquitos.

Por fim, agradeço a Capes pelo apoio financeiro durante o período de realização deste trabalho.

A todos, meu sincero muito obrigado.

RESUMO

A coloração nos vertebrados é um traço multifuncional moldado por fatores ambientais, fisiológicos e evolutivos, desempenhando papéis essenciais na comunicação, camuflagem e termorregulação. Nos anuros, esse traço apresenta grande diversidade e plasticidade adaptativa, refletindo tanto respostas ecológicas quanto processos de convergência evolutiva em função de pressões seletivas bióticas e abióticas. Nesta tese, investigam-se como variáveis estruturais e climáticas, como tipo de habitat, temperatura e precipitação, moldam os padrões de coloração dorsal em diferentes escalas biológicas, desde populações até comunidades e linhagens evolutivas. Para isso, foram utilizados registros fotográficos *in situ* e dados ambientais obtidos em campo e em bancos de dados online, combinados a análises estatísticas e comparações filogenéticas. O primeiro capítulo avaliou a relação entre coloração e ambiente em populações de *Dendropsophus minutus* de áreas naturais de campo e floresta, observando que o brilho aumenta com a temperatura e que a saturação é modulada pela interação entre habitat e precipitação, indicando um papel termorregulatório e possivelmente reprodutivo. No segundo capítulo foi investigada a influência conjunta de variáveis ambientais sobre a coloração, mostrando que a coloração dorsal de Dendropsophus minutus é influenciada de forma distinta por temperatura, precipitação e tipo de habitat, com brilho e saturação respondendo de maneiras diferentes a esses gradientes ambientais. O terceiro capítulo analisou diferenças entre comunidades de anuros de ambientes florestais e campestres, revelando maior diversidade de cores em áreas abertas, possivelmente associada à pressão de predação e condições ambientais específicas do ambiente florestal. Por fim, o quarto capítulo explorou os padrões evolutivos da coloração em espécies do gênero *Rhinella*, demonstrando que a coloração dorsal no gênero é moldada por interações complexas entre temperatura e tipo de habitat, possivelmente relacionada à disponibilidade de carotenoides e pressões evolutivas. Em conjunto, os resultados reforçam que a coloração dos anuros é sensível às mudanças ambientais e que fatores estruturais e climáticos exercem papel central na sua modulação e evolução, reforçando a importância de integrar a variação fenotípica às estratégias de conservação de espécies frente às rápidas transformações dos ecossistemas naturais.

Palavras-chave: Anfíbios, Coloração animal, Biodiversidade neotropical, *Dendropsophus minutus*, *Rhinella*.

ABSTRACT

Coloration in vertebrates is a multifunctional trait shaped by environmental, physiological, and evolutionary factors, playing essential roles in communication, camouflage, and thermoregulation. In anurans, this trait exhibits high diversity and adaptive plasticity, reflecting both ecological responses and evolutionary convergence driven by biotic and abiotic selective pressures. In this thesis, we investigated how structural and climatic variables, such as habitat type, temperature, and precipitation, shape dorsal coloration patterns across different biological scales, from populations to communities and evolutionary lineages. To this end, in situ photographic records and environmental data obtained in the field and from online databases were combined with statistical analyses and phylogenetic comparisons. The first chapter evaluated the relationship between coloration and environment in populations of *Dendropsophus minutus* from natural grassland and forest areas, observing that brightness increases with temperature and that saturation is modulated by the interaction between habitat and precipitation, indicating a thermoregulatory and possibly reproductive role. The second chapter investigated the combined influence of environmental variables on coloration, showing that dorsal coloration in *Dendropsophus minutus* is distinctly influenced by temperature, precipitation, and habitat type, with brightness and saturation responding differently to these environmental gradients. The third chapter analyzed differences among anuran communities in forest and grassland environments, revealing greater color diversity in open areas, possibly associated with predation pressure and specific environmental conditions in forests. Finally, the fourth chapter explored the evolutionary patterns of coloration in species of the genus *Rhinella*, demonstrating that dorsal coloration is shaped by complex interactions between temperature and habitat type, possibly related to carotenoid availability and evolutionary pressures. Together, the results reinforce that anuran coloration is sensitive to environmental changes and that structural and climatic factors play a central role in its modulation and evolution, highlighting the importance of integrating phenotypic variation into species conservation strategies amid rapid transformations of natural ecosystems.

Key-words: Amphibians, Animal coloration, Neotropical biodiversity, *Dendropsophus minutus*, *Rhinella*.

APRESENTAÇÃO

Esta Tese segue o modelo de formatação sugerido pelo Programa de Pós-graduação em Biologia de Ambientes Aquáticos Continentais (PPGBAC) da Universidade Federal do Rio Grande (FURG). A Introdução Geral contém o arcabouço teórico do tema em estudo, bem como os objetivos que serão investigados nos capítulos; esta seção está formatada seguindo as normas da Associação Brasileira de Normas Técnicas (ABNT). O primeiro capítulo da tese intitulado "*Dendropsophus minutus* coloration is influenced by habitat use (Anura, Dendropsophini)" segue as normas de formatação e está sumbetido ao periódico Studies on Neotropical Fauna and Environment. Neste capítulo, investigamos a relação entre a coloração corporal e o ambiente em populações de *Dendropsophus minutus* que habitam áreas naturais de campo e de floresta. O segundo capítulo, "The influence of environmental factors on dorsal coloration patterns in a neotropical tree frog" segue as normas de formatação e está aceito para publicação no periódico Biological Journal of the Linnean Society. Neste capítulo avaliamos a influência conjunta da temperatura, da precipitação e do tipo de habitat na variação da coloração dorsal de populações de *Dendropsophus minutus*. O terceiro capítulo, intitulado "The frogs are more colorful on the other side: how habitat type affects the dorsal coloration of anuran communities" segue as normas de formatação e está submetido ao periódico Brazilian Journal of Biology. Neste capítulo avaliamos se comunidades de anuros que habitam ambientes florestais e campestres apresentam diferenças na coloração dorsal e de que maneira o ambiente influencia a diversidade de cores presentes nessas comunidades. No quarto capítulo, intitulado "Sunny grasslands and shady forests: How habitat type and temperature shape the evolution of dorsal coloration in the genus *Rhinella*" investigamos os padrões evolutivos da coloração dorsal no gênero *Rhinella*, considerando o papel do tipo de habitat e da temperatura na evolução e diversificação desse traço fenotípico. O quarto capítulo está submetido e segue as normas do periódico Canadian Journal of Zoology. Finalizamos com a seção de Considerações Finais e Perspectivas obtidas com o desenvolvimento da Tese; nesta última seção, as citações e referências bibliográficas seguem as normas da ABNT.

SUMÁRIO

LISTA DE FIGURAS

Capítulo 1

Capítulo 2

Capítulo 3

Figure 1 – Map illustrating the study locations. Top left: map of Brazil highlighting the state of Rio Grande do Sul. Bottom left: map of Rio Grande do Sul showing the study sites indicated by white dots. Right: enlarged view displaying the specific sampling locations each marked with a white dot. All sites are separated by a minimum distance of 1.000 meters.

Figure 2 – Boxplots of raw values for anuran dorsal coloration variables across forest and grassland habitats: (A) Brightness, (B) Saturation, (C) Hue, and (D) Color diversity. Asterisks denote statistically significant differences between habitats ($p < 0.05$).

Figure 3 – Visualization of anuran dorsal coloration in forest (A–C) and grassland (D–G) habitats in three-dimensional RGB space. Each point represents mean red, green, and blue (R, G, B) reflectance values obtained from standardized photographs. Lower values indicate darker colors, whereas higher values correspond to lighter colors. Panels show the same data from different perspectives, illustrating the distribution of color variation within the RGB space.

Capítulo 4

Figure 1 - Effects of habitat type and temperature on dorsal coloration in the genus *Rhinella*. Dots represent raw data, and lines indicate the fitted model predictions. (A) Hue was significantly influenced by both temperature ($p = 0.030$) and habitat type ($p = 0.008$). (B) Saturation was significantly affected only by temperature ($p = 0.035$). (C) Brightness showed no significant differences in relation to either variable. (D) Intraspecific color diversity was not directly affected by temperature or habitat type, but their interaction was significant ($p = 0.042$).

LISTA DE TABELAS

Capítulo 1

Capítulo 2

Capítulo 3

Capítulo 4

Table 1 - List of *Rhinella* species analyzed, with respective habitat type, mean annual temperature of occurrence sites (°C), and number of images used.

INTRODUÇÃO GERAL

O reconhecimento de cores ocorre a partir da interpretação dos sinais enviados ao cérebro pela retina em resposta a determinados comprimentos de onda (Crescitelli et al., 1977; Diamond; Bond, 2013). As cores podem ser divididas em três principais atributos: matiz, saturação e luminosidade (Ibraheem et al., 2012). O matiz ou "hue" estima o estímulo da cor em si (e.g.: vermelho, azul, amarelo); a saturação ou "croma" estima a pureza da cor e sua intensidade; e o brilho ou luminosidade é uma medida que estima a percepção geral da iluminação (Ibraheem et al., 2012; Stevens; Stoddard; Higham, 2009). A capacidade de percepção das cores evoluiu de forma independente diversas vezes ao longo do tempo em diferentes grupos animais (Cronin et al., 2014; Diamond; Bond, 2013). Desta forma, a cor não é percebida igualmente por todas as espécies (Cronin et al., 2014). Além disso, os órgãos dos sentidos não passam uma visão perfeita do mundo, mas sim uma representação que seleciona e ressalta aspectos importantes para os comportamentos e a sobrevivência do indivíduo (Cronin et al., 2014; Diamond; Bond, 2013; Ruxton; Sherratt; Speed, 2004).

A produção de coloração em vertebrados resulta da ação de um grupo de células especializadas chamadas cromatóforos que ficam distribuídas pela pele, escamas ou penas desses animais (Moreno-Rueda; Comas, 2023). Entre os principais tipos de cromatóforos estão os melanóforos, que produzem melanina e conferem tons escuros como preto e marrom; os xantóforos, que armazenam pigmentos carotenoides e pteridinas, resultando em colorações amareladas, alaranjadas ou avermelhadas; os eritróforos, que acumulam pigmentos semelhantes, porém mais associados a tons vermelhos e alaranjados; e os iridóforos, que contêm cristais de guanina capazes de refletir a luz, produzindo efeitos estruturais como brilho metálico e iridescência (Koneru; Caro, 2022; Moreno-Rueda; Comas, 2023). A interação entre esses diferentes tipos de cromatóforos explica parte da ampla gama de cores nos vertebrados, sendo que em aves e mamíferos a melanina desempenha papel central na variação cromática, enquanto em peixes, répteis e anfíbios a combinação de vários cromatóforos gera padrões mais complexos e dinâmicos (Moreno-Rueda; Comas, 2023; Prasetya; Peters; Delhey, 2020; Rojas; Lawrence; Márquez, 2023). Em alguns grupos, como peixes, répteis e anfíbios, mecanismos hormonais e neurais permitem ainda a redistribuição dos pigmentos dentro dos cromatóforos, possibilitando mudanças rápidas de coloração em resposta ao ambiente ou a interações sociais (Moreno-Rueda; Comas, 2023; Nilsson Sköld; Aspengren; Wallin, 2013; Rojas; Lawrence; Márquez, 2023). Além do depósito de pigmentos em cromatóforos, a coloração também pode surgir a partir de caracteristicas estruturais, resultantes da dispersão de luz por nanoestruturas que variam no índice de refração da luz, produzindo tons que vão do ultravioleta ao vermelho, especialmente azuis e iridescentes (Shawkey; D'Alba, 2017). Dessa forma, a coloração nos vertebrados é resultado de um processo complexo que une especialização celular, efeitos estruturais e mecanismos fisiológicos, sendo um traço de grande relevância adaptativa.

O padrão de cor em animais possui funções fundamentais na sobrevivência e reprodução dos indivíduos, sendo algumas delas comunicação intraespecífica, comunicação interespecífica, proteção contra radiação ultravioleta e termorregulação (Rojas; Lawrence; Márquez, 2023; Toledo; Haddad, 2009; Cuthill et al., 2017). Em um contexto de comunicação intraespecífica, a coloração auxilia no reconhecimento de outros da mesma espécie, além de atrair parceiros reprodutivos e participar na comunicação visual entre os indivíduos (Rojas; Lawrence; Márquez, 2023; Rudh; Qvarnström, 2013). Na comunicação interespecífica, a coloração pode auxiliar os organismos a passarem despercebidos por predadores e presas (Merilaita; Scott-Samuel; Cuthill, 2017; Ruxton; Sherratt; Speed, 2004) ou sinalizar a presença de estratégias de defesa secundárias, como toxinas (Barzaghi et al., 2022; Cronin et al., 2014; Rojas; Lawrence; Márquez, 2023). A coloração pode exercer função de fotoproteção ao reduzir os danos causados pela radiação ultravioleta, uma vez que pigmentos como a melanina e outros componentes da coloração absorvem ou bloqueiam a radiação UV, protegendo tecidos e estruturas biológicas sensíveis (Rudh; Qvarnström, 2013; Cuthill et al., 2017). Superfícies com diferentes colorações absorvem ou refletem distintos comprimentos de onda e, em organismos ectotérmicos como os anfíbios, a coloração corporal, em interação com as condições ambientais, modula as trocas térmicas e a retenção de água, tornando-se um componente central da termorregulação (Mack; Beaty, 2021; Laumeier et al., 2023; Rojas; Lawrence; Márquez, 2023).

As características do ambiente também podem influenciar na coloração dos organismos, uma vez que estes não são idênticos, podendo variar em sua complexidade, heterogeneidade e dispinibilidade de recursos (Cuthill; Matchette; Scott-samuel, 2019; Gomez; Théry, 2004; Hughes; Liggins; Stevens, 2019; Prasetya; Peters; Delhey, 2020). O ambiente é capaz de influenciar na detectabilidade dos indivíduos por predadores e coespecíficos, tendo um papel fundamental na transmissão de informação entre os animais (Barnett et al., 2021; Michalis et al., 2017; Roslin et al., 2017). Florestas são ambientes complexos caracterizados por elevada heterogeneidade ambiental (Carlucci; Marcilio-Silva; Torezan, 2021; Duarte et al., 2014). Essa diversidade estrutural e de micro-habitats pode sustentar um amplo espectro de estratégias defensivas, que vão desde a coloração críptica para camuflagem até exibições aposemáticas conspícuas (Dalrymple et al., 2018; Merilaita, 2003; Spaniol et al., 2020), potencialmente favorecendo uma maior diversidade geral de colorações dentro das comunidades florestais (Dalrymple et al., 2018; Delhey et al., 2023; Spaniol et al., 2020). Além disso, as condições de pouca luz em florestas de dossel fechado podem favorecer cores mais escuras ou dessaturadas que aumentam a camuflagem (Endler, 1993), já que o dossel denso atua como um filtro natural, alterando a composição espectral da luz que atinge o sub-bosque (Brelsford et al., 2022; Cronin et al., 2014). Em contraste, em habitats abertos, como campos e savanas, a luz pode permear o ambiente em maior intensidade e tende a promover cores mais claras, que auxiliam na termorregulação (Endler, 1993; Laumeier et al., 2023; Rudh; Qvarnström, 2013). Além das condições ambientais ligadas à luz e à estrutura do habitat, a coloração também está associada à disponibilidade de pigmentos no ambiente, como os carotenoides, que não podem ser produzidos pelo metabolismo dos vertebrados e, portanto, devem ser adquiridos por meio da dieta (Moreno-Rueda; Comas, 2023; Prasetya; Peters; Delhey, 2020; Umbers et al., 2016). Esses compostos têm origem primária em vegetais e podem ser adquiridos indiretamente pela predação de artrópodes (Moreno-Rueda; Comas, 2023; Rojas; Lawrence; Márquez, 2023). Como a composição e a riqueza de plantas e invertebrados diferem entre ambientes, a oferta de carotenoides também varia, o que pode impactar de forma significativa a expressão da coloração nos organismos que os habitam (Prasetya; Peters; Delhey, 2020). Dessa forma, a coloração corporal de populações e comunidades pode estar relacionada ao ambiente em que habitam apesar da influência de diversos outros fatores evolutivos.

Os anfíbios anuros exibem uma notável diversidade de padrões de coloração corporal, que influenciam a sobrevivência e o sucesso reprodutivo por meio da mediação de processos como termorregulação, sinalização visual e defesa contra predadores, constituindo um traço fenotípico fundamental moldado por processos evolutivos sob a influência combinada de pressões seletivas e restrições intrínsecas (Postema; Lippey; Armstrong-Ingram, 2023; Rojas; Lawrence; Márquez, 2023; San-Jose; Roulin, 2017). Isso é válido tanto para espécies diurnas quanto noturnas (Gomez; Théry, 2004; Stangel et al., 2015; Zamora-Camacho; Comas, 2019). Por serem organismos ectotérmicos, os anfíbios dependem de fontes externas de calor para regular a temperatura corporal, e sua coloração modula tanto as trocas térmicas quanto a retenção de água (Laumeier et al., 2023; Mack; Beaty, 2021). Em contextos reprodutivos, fêmeas de muitas espécies avaliam a coloração dos machos durante a escolha do parceiro, a qual pode atuar como um indicador do tamanho e da condição física dos indivíduos (Vásquez;

Pfennig, 2007), bem como do potencial de sobrevivência da prole (Sheldon et al., 2003). Ademais, sabe-se que os anuros apresentam uma ampla variedade de colorações crípticas e aposemáticas como estratégias de defesa contra predadores (Rojas; Lawrence; Márquez, 2023). As variedades crípticas são muito difundidas, podendo ser efetivas por si mesmas ou estar associadas a alguma estratégia comportamental (Rojas, 2017; Rojas; Lawrence; Márquez, 2023). Já as colorações aposemáticas, conspícuas, estão relacionadas a espécies com presença de toxinas na pele como forma de defesa (Rojas; Lawrence; Márquez, 2023; Rudh; Qvarnström, 2013). Além dessa diversidade funcional, os anuros ocupam uma ampla variedade de ambientes, desde florestas tropicais (Kwet; R; M, 2010) até regiões desérticas (Riddle; Bateman, 2020), o que resulta em uma distribuição que abrange habitats com diferentes propriedades espectrais e condições ecológicas. O grupo inclui ainda espécies e gêneros amplamente distribuídos (Frost, 2025; Gehara et al., 2014; Laumeier et al., 2023), dotados de grande plasticidade ecológica que lhes permite habitar e se adaptar a ambientes com características ambientais contrastantes.

Sabe-se que a coloração das populações de anuros pode variar de acordo com seu habitat (Kang; Kim; Jang, 2016; Mack; Beaty, 2021; Zamora-Camacho; Comas, 2019). Embora diversos estudos tenham investigado a influência de variáveis bióticas sobre essa variação, como as pressões seletivas exercidas por predadores (Hegna et al., 2011; Rojas; Lawrence; Márquez, 2023) ou pela seleção sexual de parceiros (Gomez et al., 2009; Richards-Zawacki; Wang; Summers, 2012), a relação entre coloração e fatores ambientais abióticos ainda é pouco explorada e, quando abordada, costuma estar associada principalmente a aspectos de termorregulação (Laumeier et al., 2023). Mack e Beaty (2021) verificaram que a coloração de indivíduos de *Anaxyrus americanus* (Holbrook, 1836) difere entre locais de acordo com a altitude, um fator abiótico com implicações termorregulatórias. Em *Rana temporaria* (Rowlands, 1952), há uma diminuição na variabilidade fenotipica da coloração em populações cujo desenvolvimento ocorreu em corpos d'água temporários, possivelmente devido a pressões ambientais de dessecação durante a fase pré-metamófica (Miramontes-Sequeiros et al., 2018). Além disso, Zamora-Camacho e Comas (2019) observaram que indivíduos de *Epidalea calamita* (Laurenti, 1768) presentes em ambientes abertos de origem antrópica apresentaram maior saturação de cor do que aqueles encontrados em áreas florestadas naturais. No entanto, a forma como variáveis ambientais abióticas influenciam a coloração dos anuros segue pouco compreendida, sobretudo ao se considerar o contraste entre diferentes tipos de ambiente.

Além disso, há ainda evidências de que algumas comunidades podem apresentar convergência fenotípica em sua coloração devido ao ambiente (Emerson; Cooper; Ehleringer, 1990; Lermen; Furtado; Hartz, 2025; Norris; Lowe, 1964). Em um estudo clássico, Norris e Lowe (1964) encontraram sobreposição na coloração de espécies e seus substratos. Enquanto que Emerson e colaboradores (1990) relataram convergência no espectro infravermelho de espécies de anuros pouco relacionadas evolutivamente. Além disso, há indícios de que o ambiente florestal pode estar afetando a coloração dos anuros residentes (Lermen; Furtado; Hartz, 2025), favorecendo colorações mais saturadas. Porém, estudos relacionando a coloração de comunidades de anuros com seu ambiente são escassos. Dessa forma, essa relação de convergência fenotípica entre a coloração de comunidades de anuros e a cobertura vegetal permanece pouco explorada.

A evolução da coloração em anfíbios anuros é influenciada por padrões ambientais e pode ser moldada ao longo do tempo por pressões seletivas associadas a mudanças nas condições do habitat (Wei et al., 2020). Além disso, Moen e colaboradores (2016) demonstraram que há convergência evolutiva em diversos atributos fenotípicos de anuros relacionados ao micro-habitat ocupado, embora a coloração corporal não tenha sido diretamente avaliada nesse contexto. Ademais, estudos indicam que a coloração corporal de anfíbios anuros não apresenta forte estruturação filogenética, sugerindo que fatores ecológicos podem exercer maior influência sobre esse traço do que as relações evolutivas históricas (Verlag et al., 2003; Wollenberg et al., 2007). Dessa forma, é plausível que o ambiente exerça um papel determinante na evolução dos padrões de coloração desses organismos. Sabe-se que alterações no habitat, decorrentes da modificação ou perda da cobertura vegetal, podem alterar a percepção visual da coloração pelos observadores naturais e, consequentemente, reduzir a eficácia de colorações defensivas originalmente adaptadas às condições ambientais prévias (Barnett et al., 2021). Assim, compreender de forma mais aprofundada a influência do ambiente sobre a evolução da coloração corporal em anuros é essencial, especialmente diante do acentuado declínio populacional que o grupo enfrenta em razão da degradação e fragmentação dos habitats naturais (Becker et al., 2007; Menéndez-Guerrero; David M. Green; Davies, 2020).

Espécies amplamente distribuídas permitem testar hipóteses em diferentes contextos ambientais sem a interferência de fortes diferenças filogenéticas. *Dendropsophus minutus* (Peters, 1872) é uma espécie abundante e generalista que ocorre em ambientes florestais e campestres, não apresenta dimorfismo sexual em coloração dorsal e mostra grande plasticidade ecológica (Gehara et al., 2014; Kwet; R; M, 2010; Señaris et al., 2014). Tais características a tornam um excelente organismo para estudos sobre como variáveis ambientais modulam a variação na coloração intraespecífica. De forma similar, gêneros taxonomicamente diversos e amplamente distribuídos, como *Rhinella*, oferecem oportunidade para avaliar se os padrões de associação entre cor, habitat e clima persistem em diferentes espécies e linhagens. O gênero reúne mais de 90 espécies reconhecidas, distribuídas por uma ampla gama de ambientes neotropicais, e apresenta grande diversidade de coloração, frequentemente associada a estratégias de defesa antipredatória como camuflagem, mascaramento e coloração disruptiva (Durán; Méndez; Correa, 2021; Frost, 2025; Pereyra et al., 2021; Sousa; Benício; Fonseca, 2022). A ampla plasticidade ecológica e o alcance ambiental do grupo, que se estende desde florestas densas até campos abertos e áreas urbanizadas (Brito et al., 2013; Durán; Méndez; Correa, 2021; Pereyra et al., 2021), reforçam sua importância como modelo para estudos sobre a evolução da coloração em resposta a condições ambientais contrastantes.

OBJETIVO GERAL

Esta tese tem como objetivo geral investigar de que maneira variáveis ambientais estruturais e climáticas, como o tipo de vegetação (floresta ou campo), a temperatura e a umidade, influenciam os padrões de coloração dorsal em anfíbios anuros, considerando diferentes níveis biológicos, de populações a comunidades e processos evolutivos, por meio da análise das variáveis que compõem a cor, como saturação, matiz e luminosidade, bem como da diversidade de cores intra e interespecífica.

OBJETIVOS ESPECÍFICOS

- No primeiro capítulo, investigamos a relação entre a coloração corporal e o ambiente em populações de *Dendropsophus minutus* que habitam áreas naturais de campo e de floresta, com o objetivo de compreender como diferentes tipos de habitat influenciam os padrões de coloração dorsal. Hipotetizamos que anuros que ocorrem em ambientes naturais abertos apresentam coloração corporal distinta daquela observada em populações que habitam ambientes florestais, com populações de habitats fechados apresentando maior saturação.

- No segundo capítulo, avaliamos a influência conjunta de variáveis ambientais estruturais e climáticas na coloração dorsal de populações de *Dendropsophus minutus*, especificamente a temperatura, a precipitação e o tipo de habitat, com o objetivo de compreender como fatores microclimáticos e condições de luminosidade afetam a variação cromática. Hipotetizamos que a coloração dorsal em *D. minutus* varia entre os diferentes tipos de habitat, refletindo diferenças microclimáticas e de luminosidade, com indivíduos mais escuros em ambientes mais frios ou florestais, maior saturação em áreas campestres e ausência de variação no matiz da espécie.

- No terceiro capítulo, avaliamos se comunidades de anuros que habitam ambientes florestais e campestres apresentam diferenças nos parâmetros médios de coloração e de que maneira o ambiente influencia a estrutura cromática dessas comunidades. Hipotetizamos que comunidades de anuros diferem na coloração corporal entre ambientes campestres e florestais, com habitats florestais apresentando colorações mais escuras e menos saturadas, maior diversidade de cores e ausência de diferenças no matiz em nível de comunidade devido à convergência fenotípica com o fundo do habitat.

- No quarto capítulo, investigamos os padrões evolutivos da coloração dorsal em anuros neotropicais do gênero *Rhinella*, considerando o papel de variáveis ambientais estruturais e climáticas na evolução e diversificação desse traço fenotípico. Hipotetizamos que espécies do gênero que habitam ambientes mais frios exibem coloração dorsal mais escura, devido a uma vantagem termorregulatória, e que espécies de habitats florestais apresentam padrões menos saturados ou mais escuros, os quais favorecem a camuflagem em condições de baixa luminosidade.

REFERÊNCIAS

BARNETT, James B. *et al.* Habitat disturbance alters color contrast and the detectability of cryptic and aposematic frogs. **Behavioral Ecology**, v. 32, n. 5, p. 814–825, 2021.

BARZAGHI, Benedetta *et al.* Factors determining the dorsal coloration pattern of aposematic salamanders. **Scientific Reports**, v. 12, n. 1, 12 out. 2022.

BECKER, Carlos Guilherme *et al.* Habitat split and the global decline of amphibians. **Science**, v. 318, n. 5857, p. 1775–1777, 2007.

BRELSFORD, Craig C. *et al.* Understorey light quality affects leaf pigments and leaf phenology in different plant functional types. **Physiologia Plantarum**, v. 174, n. 3, p. e13723, maio 2022.

BRITO, Lucas Bezerra de Mattos *et al.* Diet, activity patterns, microhabitat use and defensive strategies of Rhinella hoogmoedi Caramaschi & Pombal, 2006 from a humid forest in northeast Brazil. v. 23, n. 1, p. 29–37, 2013.

CARLUCCI, Marcos Bergmann; MARCILIO-SILVA, Vinícius; TOREZAN, José Marcelo. The Southern Atlantic Forest: Use, Degradation, and Perspectives for Conservation. *In*: MARQUES, Marcia C. M.; GRELLE, Carlos E. V. (Orgs.). **The Atlantic Forest**. Cham: Springer International Publishing, 2021. p. 91–111.

CRESCITELLI, F. *et al.* **The Visual System in Vertebrates**. Berlin, Heidelberg: Springer Berlin Heidelberg, 1977.

CRONIN, Thomas *et al.* **Visual ecology**. Princeton (N.J.): Princeton university press, 2014.

CUTHILL, Innes C.; MATCHETTE, Samuel R.; SCOTT-SAMUEL, Nicholas E. Camouflage in a dynamic world. **COBEHA**, v. 30, p. 109–115, 2019.

CUTHILL, Innes C. *et al.* The biology of color. **SCIENCE**, v. 357, p. eaan0221, 2017.

DALRYMPLE, Rhiannon L. *et al.* Abiotic and biotic predictors of macroecological patterns in bird and butterfly coloration. **Ecological Monographs**, v. 88, n. 2, p. 204–224, 2018.

DELHEY, Kaspar *et al.* Evolutionary predictors of the specific colors of birds. **Proceedings of the National Academy of Sciences**, v. 120, n. 34, p. e2217692120, 22 ago. 2023.

DIAMOND, Judy; BOND, Alan B. **Concealing Coloration in Animals**. London: Belknap Press: An Imprint of Harvard University Press, 2013.

DUARTE, Leandro Da Silva *et al.* Phylobetadiversity among Forest Types in the Brazilian Atlantic Forest Complex. **PLoS ONE**, v. 9, n. 8, p. e105043, 14 ago. 2014.

DURÁN, Felipe; MÉNDEZ, Marco A.; CORREA, Claudio. The Atacama toad (Rhinella atacamensis) exhibits an unusual clinal pattern of decreasing body size towards more arid environments. **BMC Zoology**, v. 6, n. 1, dez. 2021.

EMERSON, S. B.; COOPER, T. A.; EHLERINGER, J. R. Convergence in Reflectance Spectra Among Treefrogs. **Functional Ecology**, v. 4, n. 1, p. 47–51, 17 jun. 1990.

ENDLER, John A. The Color of Light in Forests and It's Implications. **Ecological Monographs**, v. 63, n. 1, p. 1–27, 1993.

FROST, Darrel R. **Rhinella Fitzinger, 1826 | Amphibian Species of the World**. Disponível em: <https://amphibiansoftheworld.amnh.org/Amphibia/Anura/Bufonidae/Rhinella>. Acesso em: 24 jul. 2025.

GEHARA, Marcelo *et al.* High Levels of Diversity Uncovered in a Widespread Nominal Taxon: Continental Phylogeography of the Neotropical Tree Frog Dendropsophus minutus. **PLoS ONE**, v. 9, n. 9, p. e103958, 10 set. 2014.

GOMEZ, Doris *et al.* The role of nocturnal vision in mate choice: Females prefer conspicuous males in the European tree frog (Hyla arborea). **Proceedings of the Royal Society B: Biological Sciences**, v. 276, n. 1666, p. 2351–2358, 7 jul. 2009.

GOMEZ, Doris; THÉRY, Marc. Influence of ambient light on the evolution of colour signals: Comparative analysis of a Neotropical rainforest bird community. **Ecology Letters**, v. 7, n. 4, p. 279–284, abr. 2004.

HEGNA, Robert H. *et al.* Contrasting colors of an aposematic poison frog do not affect predation. **Annales Zoologici Fennici**, v. 48, p. 29–38, 2011.

HUGHES, Anna; LIGGINS, Eric; STEVENS, Martin. Imperfect camouflage: How to hide in a variable world? **Proceedings of the Royal Society B: Biological Sciences**, v. 286, n. 1902, 2019.

IBRAHEEM, Noor A. *et al.* Understanding Color Models: A Review. v. 2, n. 3, 2012.

KANG, Changku; KIM, Ye Eun; JANG, Yikweon. Colour and pattern change against visually heterogeneous backgrounds in the tree frog Hyla japonica. **Scientific Reports**, v. 6, 2 mar. 2016.

KONERU, Manisha; CARO, Tim. Animal Coloration in the Anthropocene. **Frontiers in Ecology and Evolution**, v. 10, p. 857317, 22 abr. 2022.

KWET, A.; R, Lingnau; M, Di-Bernardo. **Pró-Mata: Anfíbios da Serra Gaúcha, sul do Brasil/Amphibien der Serra Gaúcha, Südbrasilien/Amphibiansofthe Serra Gaúcha, South of Brazil**. Porto Alegre: EDIPUCRS, 2010.

LAUMEIER, Ricarda *et al.* The global importance and interplay of colour-based protective and thermoregulatory functions in frogs. **Nature Communications**, v. 14, n. 1, p. 8117, 19 dez. 2023.

LERMEN, Luísa; FURTADO, Raíssa; HARTZ, Sandra Maria. To be or not be seen: the influence of substrate on anuran community coloration. **Journal of Natural History**, v. 59, n. 17–20, p. 1249–1265, 4 maio 2025.

MACK, Mariah; BEATY, Lynne. The influence of environmental and physiological factors on variation in American toad (Anaxyrus americanus) dorsal coloration. **Journal of Herpetology**, v. 55, n. 2, p. 119–126, 2021.

MENÉNDEZ-GUERRERO, Pablo A.; DAVID M. GREEN; DAVIES, T. Jonathan. Climate change and the future restructuring of Neotropical anuran biodiversity. **Ecography**, v. 43, p. 222–235, 2020.

MERILAITA, Sami. Visual background complexity facilitates the evolution of camouflage. **Evolution**, v. 57, n. 6, p. 1248–1254, 1 jun. 2003.

MERILAITA, Sami; SCOTT-SAMUEL, Nicholas E.; CUTHILL, Innes C. How camouflage works. **Philosophical Transactions of the Royal Society B: Biological Sciences**, v. 372, n. 1724, 2017.

MICHALIS, Constantine *et al.* Optimal background matching camouflage. **Proceedings of the Royal Society B: Biological Sciences**, v. 284, n. 1858, p. 1–6, 2017.

MIRAMONTES-SEQUEIROS, Luz Calia *et al.* The phenotypic variability in Rana temporaria decreases in response to drying habitats. **Science of The Total Environment**, v. 612, p. 538–543, jan. 2018.

MOEN, Daniel S.; MORLON, Hélène; WIENS, John J. Testing Convergence Versus History: Convergence Dominates Phenotypic Evolution for over 150 Million Years in Frogs. **Systematic Biology**, v. 65, n. 1, p. 146–160, jan. 2016.

MORENO-RUEDA, Gregorio; COMAS, Mar. **Evolutionary Ecology of Amphibians**. 1. ed. Boca Raton: CRC Press, 2023.

NILSSON SKÖLD, Helen; ASPENGREN, Sara; WALLIN, Margareta. Rapid color change in fish and amphibians – function, regulation, and emerging applications. **Pigment Cell & Melanoma Research**, v. 26, n. 1, p. 29–38, jan. 2013.

NORRIS, Kenneth S.; LOWE, Charles H. An Analysis of Background Color-Matching in Amphibians and Reptiles. **Ecology**, v. 45, n. 3, p. 565–580, jul. 1964.

PEREYRA, Martín O. *et al.* Evolution in the Genus Rhinella: A Total Evidence Phylogenetic Analysis of Neotropical True Toads (Anura: Bufonidae). **Bulletin of the American Museum of Natural History**, v. 447, n. 1, 31 mar. 2021.

POSTEMA, Elizabeth G.; LIPPEY, Mia K.; ARMSTRONG-INGRAM, Tiernan. Color under pressure: how multiple factors shape defensive coloration. **Behavioral Ecology**, v. 34, n. 1, p. 1–13, 11 fev. 2023.

PRASETYA, Audrey Miranda; PETERS, Anne; DELHEY, Kaspar. Carotenoid-based plumage colour saturation increases with temperature in Australian passerines. **Journal of Biogeography**, v. 47, n. 12, p. 2671–2683, dez. 2020.

RICHARDS-ZAWACKI, Corinne L.; WANG, Ian J.; SUMMERS, Kyle. Mate choice and the genetic basis for colour variation in a polymorphic dart frog: Inferences from a wild pedigree. **Molecular Ecology**, v. 21, n. 15, p. 3879–3892, ago. 2012.

RIDDLE, Sidney B.; BATEMAN, Heather L. Habitat and soil associations of a fossorial toad in a Sonoran Desert riparian forest. **Journal of Arid Environments**, v. 181, n. June, p. 104239, 2020.

ROJAS, Bibiana. Behavioural, ecological, and evolutionary aspects of diversity in frog colour patterns. **Biological Reviews**, v. 92, n. 2, p. 1059–1080, 1 maio 2017.

ROJAS, Bibiana; LAWRENCE, J. P.; MÁRQUEZ, Roberto. Amphibian Coloration: Proximate Mechanisms, Function, and Evolution. *In*: MORENO-RUEDA, Gregorio; COMAS, Mar (Eds.). **Evolutionary Ecology of Amphibians**. 1. ed. Boca Raton: CRC Press, 2023. p. 219–258.

ROSLIN, Tomas *et al.* Higher predation risk for insect prey at low latitudes and elevations. **Science**, v. 356, n. 6339, p. 742–744, 1 ago. 2017.

RUDH, Andreas; QVARNSTRÖM, Anna. Adaptive colouration in amphibians. **Seminars in Cell and Developmental Biology**, v. 24, n. 6–7, p. 553–561, 2013.

RUXTON, Graeme D.; SHERRATT, Thomas N.; SPEED, Michael P. **Avoiding Attack: The Evolutionary Ecology of Crypsis, Warning Signals and Mimicry**. Oxford, NY: Oxford University Press, 2004.

SAN-JOSE, Luis M.; ROULIN, Alexandre. Genomics of coloration in natural animal populations. **Philosophical Transactions of the Royal Society B: Biological Sciences**, v. 372, n. 1724, 2017.

SEÑARIS, J. Celsa *et al.* **Guía ilustrada de los anfibios del Parque Nacional Canaima, Venezuela**. Caracas, Venezuela: Ediciones IVIC, Instituto Venezolano de Investigaciones Científicas (IVIC), 2014.

SHAWKEY, Matthew D.; D'ALBA, Liliana.. Interactions between colour-producing mechanisms and their effects on the integumentary colour palette. **Philosophical Transactions of the Royal Society B: Biological Sciences**, v. 372, n. 1724, 2017.

SHELDON, B. C. *et al.* Sire coloration influences offspring survival under predation risk in the moorfrog. **Journal of Evolutionary Biology**, v. 16, n. 6, p. 1288–1295, 2003.

SOUSA, Tiago Rafael de; BENÍCIO, Ronildo Alves; FONSECA, Mariluce Gonçalves. POLIMORFISMO EM Rhinella diptycha (ANURA: BUFONIDAE) EM UMA ÁREA DE CAATINGA, ESTADO DO PIAUÍ, NORDESTE DO BRASIL. **Biosphere Comunicações Científicas**, v. 1, n. 2, p. 16–22, 2022.

SPANIOL, Ricardo Luís *et al.* Discolouring the Amazon Rainforest: how deforestation is affecting butterfly coloration. **Biodiversity and Conservation**, p. 1–18, 3 jun. 2020.

STANGEL, Judith *et al.* Ontogenetic Change of Signal Brightness in the Foot-Flagging Frog Species *Staurois parvus* and *Staurois guttatus*. **Herpetologica**, v. 71, n. 1, p. 1–7, mar. 2015.

STEVENS, Martin; STODDARD, Mary Caswell; HIGHAM, James P. Studying primate color: Towards visual system-dependent methods. **International Journal of Primatology**, v. 30, n. 6, p. 893–917, 2009.

TOLEDO, Luís Felipe; HADDAD, Célio F. B. Colors and Some Morphological Traits as Defensive Mechanisms in Anurans. **International Journal of Zoology**, v. 2009, p. 1–12, 12 mar. 2009.

UMBERS, Kate D. L. *et al.* Dietary carotenoids change the colour of Southern corroboree frogs. **Biological Journal of the Linnean Society**, v. 119, n. 2, p. 436–444, out. 2016.

VÁSQUEZ, Tatiana; PFENNIG, Karin S. Looking on the bright side: Females prefer coloration indicative of male size and condition in the sexually dichromatic spadefoot toad, Scaphiopus couchii. **Behavioral Ecology and Sociobiology**, v. 62, n. 1, p. 127–135, nov. 2007.

VERLAG, Fischer *et al.* Convergent evolution of aposematic coloration in Neotropical poison frogs: a molecular phylogenetic perspective. **Organisms, Diversity and Evolution**, v. 3, p. 215–226, 2003.

WEI, Shichao *et al.* The roles of climate, geography and natural selection as drivers of genetic and phenotypic differentiation in a widespread amphibian *Hyla annectans* (Anura: Hylidae). **Molecular Ecology**, v. 29, n. 19, p. 3667–3683, out. 2020.

WOLLENBERG, Katharina C. *et al.* Molecular phylogeny of Malagasy reed frogs, Heterixalus, and the relative performance of bioacoustics and color-patterns for resolving their systematics. **Molecular Phylogenetics and Evolution**, v. 45, n. 1, p. 14–22, 2007.

ZAMORA-CAMACHO, Francisco Javier; COMAS, Mar. Beyond Sexual Dimorphism and Habitat Boundaries: Coloration Correlates with Morphology, Age, and Locomotor Performance in a Toad. **Evolutionary Biology**, v. 46, n. 1, p. 60–70, 2019.

CAPÍTULO 1

***Dendropsophus minutus* coloration is influenced by habitat use (Anura, Dendropsophini)**

Luísa Nunes Lermen[*1]; Daniel Loebmann[1]

[1]Programa de Pós-graduação em Ambientes Aquáticos Continentais. Laboratório de Vertebrados, Instituto de Ciências Biológicas, Universidade Federal do Rio Grande, Campus Carreiros, Av. Itália km 8, Rio Grande, RS 96203-900, Brazil

*Corresponding author. Email: lulis.lermen@hotmail.com

Este manuscrito está aceito para publicação no periódico Studies on Neotropical Fauna and Environment

DOI: https://doi.org/10.1080/01650521.2026.2620481

Abstract: Body coloration is a crucial trait in anuran biology influenced by many selective pressures, affecting their reproductive success and survival. Although studies have shown that anuran populations' coloration differs according to habitats use, the understanding of the influence of different vegetation cover is very limited. This study investigates the relationship between body coloration and environmental factors in the widely distributed species *Dendropsophus minutus*. Using *in situ* photography, we measured colorimetric variables (hue, saturation, and brightness) from individuals in forested and grassland areas and analyzed them with linear models. Our findings revealed a significant difference in color saturation between habitats, with higher saturation in grassland populations. No significant differences were found in hue or brightness. The higher saturation in open habitats may be linked to greater reproductive effort due to greater environmental stress, while lower saturation in forested areas could be a response to light conditions. These findings suggest that environmental factors such as vegetation cover exert selective pressures on anuran coloration, and have implications in the conservation of anuran populations amid environmental changes.

Keywords: Amphibians, Animal coloration, Forests, Grasslands

INTRODUCTION

Body coloration is a critical aspect of anuran biology that plays a vital role in reproduction and survival (Rojas, 2017). This is true for both diurnal and nocturnal species (Gomez et al., 2009, Stangel, et al. 2015; Zamora-Camacho & Comas, 2019). For instance, females of many species evaluate the coloration of males during mate choice, acting as an indicator of the size and condition of males (Vásquez & Pfennig, 2007), as well as potential offspring survival (Sheldon et al., 2003). This trait is also important for intraspecific visual communication (Hödl & Amezquita, 2001), e.g., adult individuals of *Staurois parvus* and *S. guttatus* presenting brighter conspicuous displays during agonistic interaction as a cue for age of the signaler (Stangel et al., 2015). Furthermore, anurans are known to exhibit a wide variety of cryptic and aposematic colorations as defense strategies against predators (Toledo & Haddad, 2009; Rojas, 2017). Consequently, body coloration is subject to many biotic and abiotic selective pressures (Gade et al., 2016; Rojas, 2017; Wei et al., 2020; Mack & Beaty, 2021).

Anuran coloration can be selected through influences resulting from environmental changes, such as shifts in climatic conditions, and is shaped by environmental patterns over time (Wei et al., 2020). The environment can influence the detectability of individuals by predators and conspecifics, thus playing a fundamental role in the transmission of information between animals (Michalis et al., 2017; Roslin et al., 2017). Besides, the canopy present in forested areas acts as a filter to the light that passes through it, changing the composition of light that reaches the understory (Cronin et al., 2014; Brelsford et al., 2022). Meanwhile, the lack of a canopy in open environments, like natural grasslands, allows light to permeate the environment in a greater intensity (Endler, 1993). Hence, it is no surprise that changes in habitat, such as deforestation, alter the perception of the color of organisms and can impair the function of defensive colors adapted to the original conditions (Barnett et al., 2021; Koneru & Caro, 2022). Moreover, different environments can provide different prey availability (Lopes-Rodrigues et al., 2010; Toldi et al., 2021), which can influence body coloration due to dietary differences (Umbers et al., 2016; Moreno-Rueda & Comas, 2023). Therefore, understanding the relationship between body coloration and the environment is important for the conservation of anurans.

Previous studies have shown that anurans in different habitats have different body coloration (Marcelino et al., 2009; Kang et al., 2017; Mack & Beaty, 2021). Nevertheless, most studies have evaluated the relationship between body coloration and the selective pressures of sexual selection (Gomez et al., 2009; Gade et al., 2016) and predation (Hegna et al., 2011; Rojas, 2017). Studies incorporating environmental variables have shown that coloration differs between populations at different altitudes (Mack & Beaty, 2021) and between temporary and permanent water bodies (Miramontes-Sequeiros et al., 2018). However, studies on the effects of vegetation cover on anuran body coloration are scarce, although they have been shown to play a role in the coloration of other animals (Rios & Álvarez-Castañeda, 2012; Dalrymple, et al. 2018; Zamora-Camacho & Comas, 2019; Spaniol et al., 2020). For instance, Zamora-Camacho and Comas (2019) found that individuals of *Epidalea calamita* (Laurenti, 1768) in manmade open environments had higher saturation than those in natural forested areas. Meanwhile, butterflies in natural forests display higher saturation and color diversity than in open areas in disturbed habitats (Spaniol et al., 2020). Likewise, in a previous study with anuran communities and substrate matching, we found evidence of higher dorsal color saturation in anurans living in natural forests, matching the forest substrate (Lermen et al., 2025). However, since differences between natural forest and natural grassland environments were not the aim of that study, this result was inconclusive.

Dendropsophus minutus (Peters, 1872) is a small treefrog widely distributed across South America (Gehara et al., 2014; Frost, 2024). It is a habitat generalist, occurring in both forested and grassland environments, and is notably abundant wherever it is found (Kwet et al., 2010; Señaris et al., 2014). Additionally, the species exhibits no sexual dimorphism in dorsal colouration (Kwet et al., 2010). These characteristics make *D. minutus* an excellent model for investigating potential differences in body coloration between natural forest and natural grassland habitats.

A deeper understanding of how the environment influences anuran body coloration could be crucial, especially given the significant decline observed in this group (Menéndez-

Guerrero et al., 2020). Therefore, the aim of this study was to investigate the relationship between body coloration and environment in anuran populations in natural grassland and forest habitats. We used *in situ* photography to obtain color measurements of widely distributed anuran *Dendropsophus minutus* in different habitats, natural grassland and natural forest habitats. We obtained colorimetric variables (hue, saturation, and brightness) for statistical analysis with linear models to compare the different habitats. We hypothesized that anurans in natural open habitats will have different body coloration than anuran populations in closed habitats. Based on previous results in studies with other organisms (Dalrymple et al., 2018; Spaniol et al., 2020) and our own observations in a previous work (Lermen et al., 2025), we expected the populations of closed habitats to have higher saturation in body coloration.

MATERIAL AND METHODS

Study species

Dendropsophus minutus (Peters, 1872) is a small, widely distributed hylid treefrog found in South America (Gehara et al. 2014; Frost, 2024). Even though *D. minutus* is probably a species complex, the study region harbors only a single lineage (Gehara et al. 2014). Species can be found living on trees and bushes in several environments, from forested to open and even anthropized habitats (Kwet et al., 2010; Señaris et al., 2014). Dorsum coloration is highly variable, ranging from orange brown, brown, yellow, or tan, usually with two broad darker parallel longitudinal bands (Kwet et al., 2010; Señaris et al., 2014). There is no sexual dimorphism or dramatic color change in dorsum coloration (Kwet et al., 2010). Males call close to the water from grasses, aquatic plants, or low shrubs during warm months (Kwet et al., 2010).

Study area and data collection

A major forest type from the Atlantic Forest in southern Brazil is the Araucaria Mixed Forest, which is dominated by the conifer *Araucaria angustifolia* emerging above a forest canopy of angiosperm trees (Carlucci et al., 2021). This forest type is characterized by a subtropical climate with well-distributed rainfall, monthly temperatures typically below 23 °C, and frosts during the coldest months (Carlucci et al., 2021; Grillo, 2011). The Araucaria Mixed Forest is not as rich in tree species as other Atlantic Forest subcategories but it harbors great phylogenetic diversity (Duarte et al., 2014; Grillo, 2011).

The Pampa region, located in southern Brazil, is characterized by its vast grasslands ecosystem with trees being scarce and mainly found along riverbanks. These grasslands also have a subtropical climate but receive lower precipitation and experience occasional hydric deficits in summer (Overbeck et al., 2024). Historically shaped by grazing, both by extinct large herbivores and livestock, the Pampa maintains its grassland physiognomy more due to land use practices than climate conditions (Overbeck et al., 2024; Verdum et al., 2019).

We collected data from forested and natural grassland areas in southern Brazil (Table 1, Fig. 1). This region consists of a transition area between the Araucaria Mixed Forest and Pampa regions. The forested areas mostly consist of evergreen subtropical moist Araucaria Mixed Forest, with a dense canopy of predominantly *Araucaria angustifolia*, an average annual rainfall of 2200 mm, and an average annual temperature of 14.5 ° C. The water bodies sampled in this area are located within two conservation units in the municipality of São Francisco de Paula, namely the Floresta Nacional de São Francisco de Paula (FLONA-SFP) and Centro de Pesquisas e Conservação da Natureza Pró-Mata (PRÓ-MATA). Grassland areas are mainly composed of species of grasses and Asteraceae with no canopy cover (Andrade et al. 2019), with an average annual rainfall of 1440 mm and average annual temperature of 17 °C. These natural low grassland areas are part of the Pampa region. The water bodies sampled in this area were located in the municipality of Eldorado do Sul inside the Estação Experimental Agronômica da Universidade Federal do Rio Grande do Sul (EEA-UFRGS) and within a private property near a low granitic ridge known as Cerro das Almas (CDA) in the municipality of Capão do Leão. The habitat type of each sampled water body was defined a priori based on the predominant vegetation within a 250-meter radius surrounding each site. Additionally, all sampled water bodies were located at least 500 meters apart from one another.

Table 1 – Number of sampled water bodies and individuals (N) of *Dendropsophus minutus* in each sample area, namely: Floresta Nacional de São Francisco de Paula (FLONA-SFP); Centro de Pesquisas e Conservação da Natureza Pró-Mata (PRÓ-MATA); Estação Experimental Agronômica da Universidade Federal do Rio Grande do Sul (EEA-UFRGS); and Cerro das Almas (CDA).

Sample area	**Geographic coordinates**	**Habitat**	**Sampled individuals (N)**	**Nº of water bodies sampled**
FLONA-SFP	S 29°25'24.4 W 50°23'12.2"	Subtropical moist forest	23	3
PRÓ-MATA	S 29°29'17,8" W 50°12'25,3"	Subtropical moist forest	7	1
EEA-UFRGS	S 30°05'31.5" W 51°40'18.6"	Grassland	22	2
CDA	S 31°47'28,3" W 52°35'35,8"	Grassland	8	1

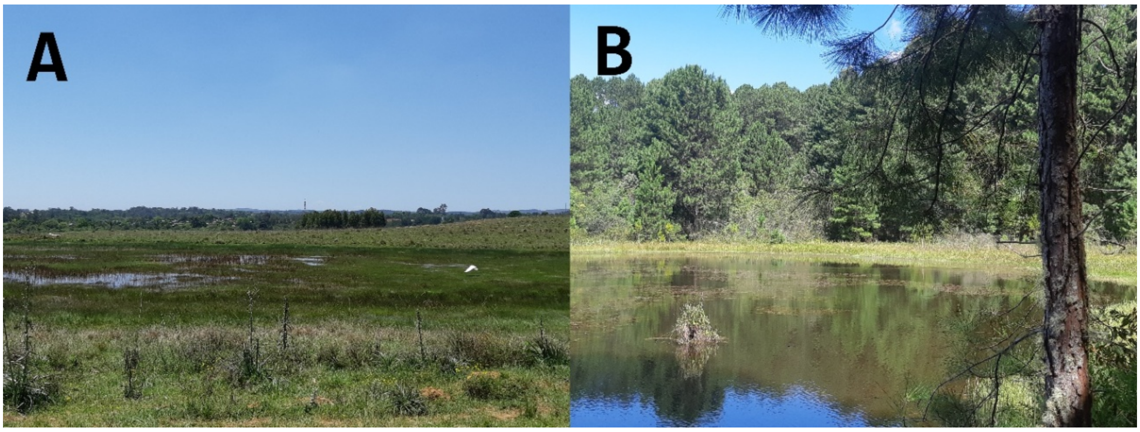

Figure 1 – Example of natural grassland (A) and forest (B) areas where the coloration of *Dendropsophus minutus* populations were sampled.

We sampled 60 adult male *D. minutus* individuals between October 2023 and February 2024, of which 30 were sampled in forested areas and 30 in natural grassland areas. To standardize sampling conditions, water bodies were visited at night during the species' reproductive period, minimizing color variations caused by natural changes in environmental conditions throughout the day. Sampling was carried out until there were no more males calling. Individuals were located by active searching, following male calls. Individuals were captured by hand, photographed, and kept in a container with some water and vegetation from the environment. To avoid recaptures of the same individual, frogs were released at the end of sampling at the water body. Additionally, each individual water body was sampled only once. This methodology was approved by the Ethics Committee of the Universidade Federal do Rio Grande (N° P034/2022).

Color measurement

We took pictures in the field using a Canon EOS 1200D camera with an 18-55 mm lens. Because the sampling occurred at night, the standard flash of the device was turned on and served as the standard light source. For calibration purposes, each frog was photographed alongside a scale bar and a gray card 18% to correct any variation in light conditions and the use of the built-in flash (Stevens, 2007; Spaniol et al., 2020). When using a flash, placing a grey standard with known reflectance in every image can help accurately recover reflectance values, even under variable lighting (Stevens, 2007). We took 10 pictures of the dorsum of each individual for further selection to assess the overall body coloration. Images were taken in RAW format with a fixed aperture setting and flash-specific white balance. The photographs were imported to ImageJ software with the micaToolbox version 2.2.0 add-on. We used micaToolbox photo screening to select three pictures of each individual with appropriate exposure and generate multispectral images calibrated from the gray card (Troscianko & Stevens, 2015).

For every image we drew a region of interest encompassing the dorsum of the animal and carefully removing any specular reflection on the skin. From the standardized images, we obtained color data for the dorsum corresponding to the reflectance in the three camera color channels: red, green, and blue (RGB). These RGB values were used to calculate three color variables for each individual: hue (color type, e.g., yellow or green), saturation (color intensity compared to white light), and brightness (the sum of the reflectance values along the spectral range) (Stevens et al., 2007).

Data analysis

To obtain the colorimetric variables, the standardized RGB values were tabled so that they could be recognized as an appropriate input to the colspace function from the PAVO package (v.2.9.0) (Maia et al., 2019) in R v.4.3.3 (R Core Team, 2024). Therefore, the RGB values were reorganized in a table with values of short (B), medium (G), and large (R) wavelengths and plotted into XY coordinates in a triangular color space from which we obtained the colorimetric variables hue and saturation (Figure 2). The center of the space represents the achromatic point, and the location around this point corresponds to a given color type and saturation. We estimated saturation as the magnitude of r, which is the distance from the achromatic origin in the center of the color space (Figure 2A). Hue was given by the direction of the color vector via θ, which is the angular displacement of the color vector from the positive x-axis in the color space (Figure 2B). Brightness was calculated as the average reflectance across RGB channels.

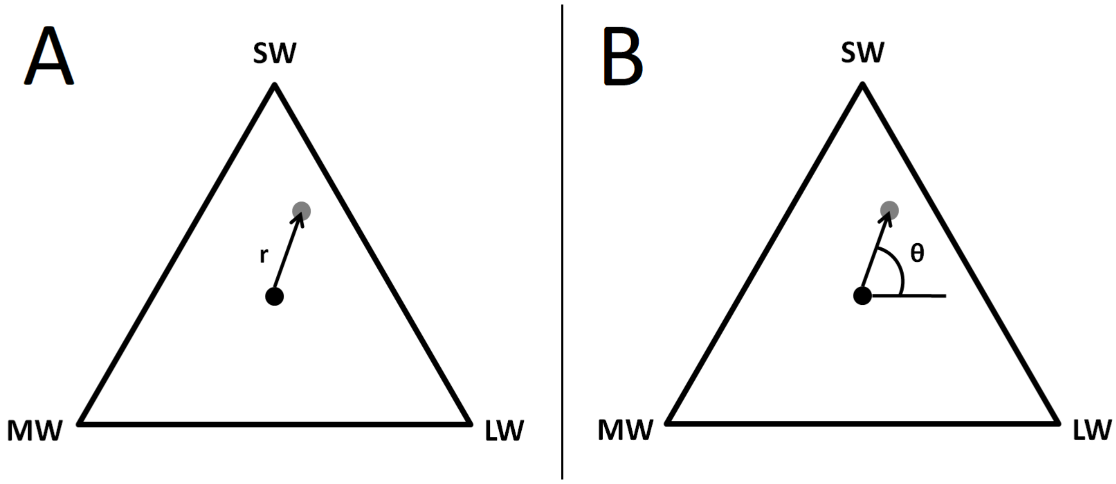

Figure 2 –A triangular color space with the indication of short (SW), medium (MW), and large (LW) wavelengths extremities. The black dot at the center represents the achromatic point and the gray dot represents a hypothetical RGB value plotted into XY coordinates in the color space. Vector r is the distance from the achromatic point to the plotted coordinate and its magnitude is the saturation of the plotted point **(A).** Hue is given by the direction of vector r via the angular rotation, represented by θ, of the vector from the positive x-axis in the color space (B).

We analyzed each colorimetric variable (hue, saturation, and brightness) individually in relation to the habitat type (open or closed) using linear models. Thus, we employed three similar models with a colorimetric variable as the response and habitat type as a categorical explanatory variable. The data were assessed for normality using Q-Q plots and the Akaike information criterion (AIC). Brightness values required logarithmic transformation to correct for skewness in the data. All statistical analyses were performed using the lmer4 package in R software (Bates et al. 2015).

RESULTS

We found significant differences in saturation of *D. minutus* between open and forested habitats (p= 0.0005; R^2= 0.172; Figure 3A). Individuals living in grassland areas displayed higher saturation than those in the forest. We found no statistical difference in the brightness (p = 0.184, Figure 3B) or hue (p = 0.905, Figure 3C) between the two habitats types.

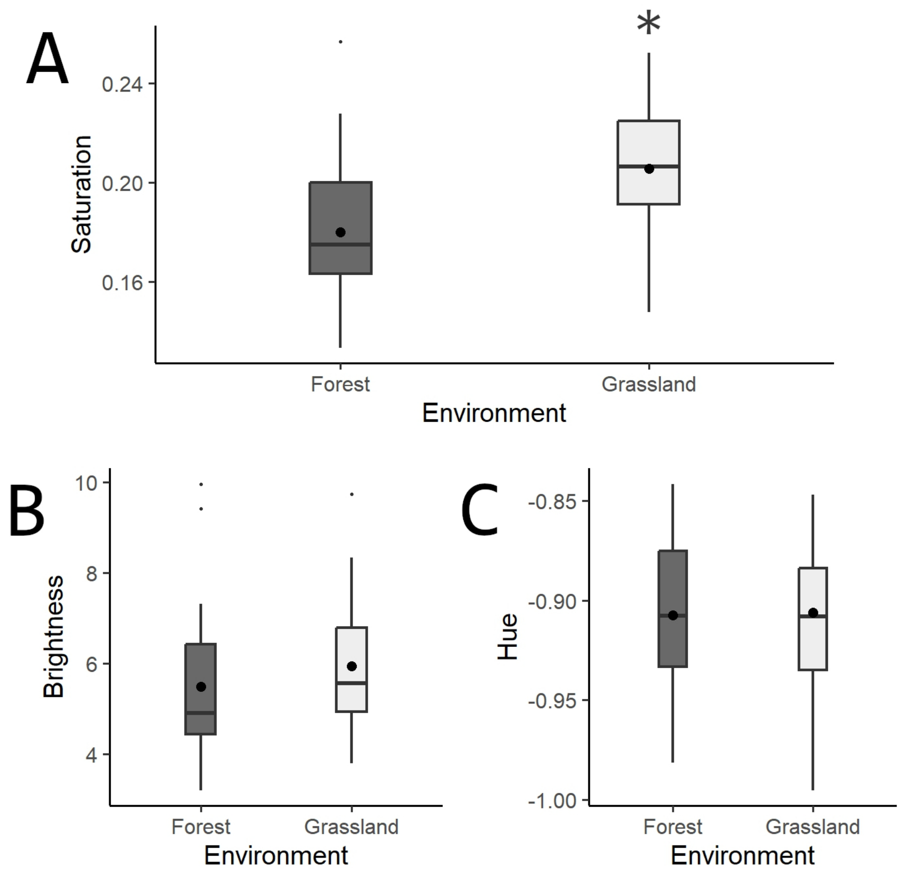

Figure 3 – Values of saturation (A), brightness (B), and hue (C) for *Dendropsophus minutus* of the different habitats, natural grassland and forest. Black dots indicate the mean of each group. The asterisk symbol indicates statistical difference between the habitat types.

DISCUSSION

This study demonstrates that dorsal coloration of *D. minutus* populations differs among natural grasslands and forests, although results contrast with our prediction. Our findings are similar to what was found in the natterjack toad *Epidalea calamita* (Laurenti, 1768), where individuals in manmade open environments had higher saturation (Zamora-Camacho & Comas, 2019), suggesting a standard response of anuran coloration both in natural and manmade open habitats. Even though agrosystem environments are widely used by amphibians (Valdez et al., 2021), the agrochemicals used in these environments bioacumulate and have negative effects leading to DNA damage (Ascoli-Morrete et al., 2022) and mortality (Wrubleswski et al., 2018). Higher color saturation in agrosystem environments may be attributed to populations in these habitats undergoing greater environmental stress, which lead to a shorter lifespan, and consequently to a greater investment in reproductive effort (Zamora-Camacho & Comas, 2017). It is possible that the natural grasslands of the Pampa region sampled in this study are contaminated with pesticides from surrounding crop plantations that have intense pesticide use (Ziliotto et al., 2023). Therefore, the same environmental stressors could be at play in both manmade and natural habitats, leading to greater reproductive investment and consequently to a higher saturation in body coloration in anurans living in these environments. However, further studies assessing the environmental pesticide contamination and impact on *D. minutus* in different habitats are required to confirm this hypothesis.

Another similarity between natural and manmade open environment is the lack of a canopy, which allows light to permeate the environment in a greater intensity (Endler, 1993). Light intensity is known to be an important factor shaping the occurrence of *Hylodes fredi*

(Canedo & Pombal, 2007), with the species showing a preference for better lit habitats (Motta-Tavares et al., 2024). The canopy present in forested areas acts as a filter to the light that passes through it, changing the composition of light that reaches the understory (Cronin et al., 2014; Brelsford et al., 2022). Light that reaches the understory is less saturated and highly colored (Endler, 1993; Théry, 2001). Recently, Sever (2024) proposed that body coloration of animals is determined mainly by abiotic factors, like the light waves radiating from the environment, instead of biotic ones. Therefore, population of *D. minutus* living in forest habitats would exhibit body coloration that is less saturated due to the aforementioned characteristics of light that reaches the understory. Moreover, cryptic coloration is widespread among anuran amphibians (Rojas, 2017) due to their susceptibility to a large number of vertebrate and invertebrate predators (Toledo et al., 2007). Hence, even if biotic factors are at play, to be more cryptic in forested environments individuals should have a less saturated coloration (Théry, 2001). Therefore, characteristics of the light in open and forested environments could yield results that would align with our findings.

Another explanation may be a dietary difference between the populations in the two environments. For instance, carotenoids are yellow-red skin pigments that cannot be synthesized by anurans and are instead acquired through dietary sources (Koneru & Caro, 2022; Moreno-Rueda & Comas, 2023). If the diet of the populations of *D. minutus* in open grassland habitats is richer in carotenoids a higher saturation in skin coloration is to be expected (Umbers et al., 2016). *Dendropsophus minutus* is a dietary generalist with a diet composed mainly of small arthropods (Leivas et al., 2018). Arthropod composition can differ between forested and open areas (Lopes-Rodrigues et al., 2010; Toldi et al., 2021) and even within grassland patches (Vilardo et al., 2018). Therefore, the idea that this difference in coloration may simply be a result of individuals consuming what is available for them cannot be completely discarded. Future research combining body coloration with an evaluation of dietary differences among these different environments would be valuable for assessing their contribution to the observed patterns.

The simple fact that body coloration correlates with habitat type can have implications for survival in light of habitat modification. Considering that forest light can be determined by the thickness of the leaves (Théry, 2001), changes in species composition could affect the functionality of anuran coloration. Indeed, Barnett et al. (2021) have already shown that changes in the habitat can impair the function of both cryptic and conspicuous defensive colors in anurans. However, the understanding of the impact of anthropocentric changes to visual environments is very limited (Koneru & Caro, 2022). Given the rapid decline in amphibians species (Alroy, 2015), this only adds one more cause for concern to the current conservation efforts.

CONCLUSION

In sum, the coloration of populations of *D. minutus* was affected by the environment, with populations of forested areas exhibiting less saturated colors than those in open areas. This seems to be related to the specific properties of each habitat, like the presence of a canopy or environmental stressors, which affect the body coloration of these organisms. In the light of the quick decline in amphibian species, our results show that alterations to habitat structure may hinder the functionality of anuran coloration, adding one more concern for their conservation.

Acknowledgement

We thank Juliana Zina, Raul Maneyro and Sonia Huckembeck for their input in an earlier version of the manuscript. We thank the Programa de Pós-Gradação em Ambientes

Aquáticos Continentais of the Universidade Federal do Rio Grande for the support during this study. We thank the Instituto Chico Mendes de Biodiversidade (ICMBio) for permitting the fieldwork in the Floresta Nacional de São Francisco de Paula. DL recieved a research fellowship from Conselho Nacional de Desenvolvimento Científico e Tecnológico (307232/2023-9). LL recieved a scholarship from Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.

Conflicts of interest: The authors declare that they have no conflict of interest.

Funding: D. Loebmann recieved a research fellowship from Conselho Nacional de Desenvolvimento Científico e Tecnológico (307232/2023-9). L. Lermen recieved a scholarship from Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.

Data Availability: The datasets used and analysed during the current study are available from the corresponding author on reasonable request. The R script used during the current study is available from the corresponding author on reasonable request.

References

Alroy, J. (2015). Current extinction rates of reptiles and amphibians. Proceedings of the National Academy of Sciences, 112(42), 13003–13008. https://doi.org/10.1073/pnas.1508681112

AmphibiaWeb (2024). https://amphibiaweb.org. University of California, Berkeley, CA, USA.

Andrade, B. O., Bonilha, C. L., Overbeck, G. E., Vélez-Martin, E., Rolim, R. G., Bordignon, S. A. L., Schneider, A. A., Vogel Ely, C., Lucas, D. B., Garcia, É. N., Dos Santos, E. D., Torchelsen, F. P., Vieira, M. S., Silva Filho, P. J. S., Ferreira, P. M. A., Trevisan, R., Hollas, R., Campestrini, S., Pillar, V. D., & Boldrini, I. I. (2019). Classification of South Brazilian grasslands: Implications for conservation. Applied Vegetation Science, 22(1), 168–184. https://doi.org/10.1111/avsc.12413

Ascoli-Morrete, T., Bandeira, N. M. G., Signor, E., Gazola, H. A., Homrich, I. S., Biondo, R., Rossato-Grando, L. G., & Zanella, N. (2022). Bioaccumulation of pesticides and genotoxicity in anurans from southern Brazil. Environmental Science and Pollution Research, 29(30), 45549–45559. https://doi.org/10.1007/s11356-022-19042-z

Backes, A., Prates, F. L., & Viola, M. G. (2006). Produção de serapilheira em Floresta Ombrófila Mista, em São Francisco de Paula, Rio Grande do Sul, Brasil. Acta Botanica Brasilica, 19(1), 155–160. https://doi.org/10.1590/s0102-33062005000100015

Barnett, J. B., Varela, B. J., Jennings, B. J., Lesbarrères, D., Pruitt, J. N., & Green, D. M. (2021). Habitat disturbance alters color contrast and the detectability of cryptic and aposematic frogs. Behavioral Ecology, 32(5), 814–825. https://doi.org/10.1093/beheco/arab032

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software, 67(1), 1–48. https://doi.org/10.18637/jss.v067.i01

Brelsford, C. C., Trasser, M., Paris, T., Hartikainen, S. M., & Robson, T. M. (2022). Understorey light quality affects leaf pigments and leaf phenology in different plant functional types. Physiologia Plantarum, 174(3), e13723. https://doi.org/10.1111/ppl.13723

Carlucci, M. B., Marcilio-Silva, V., & Torezan, J. M. (2021). The Southern Atlantic Forest: Use, Degradation, and Perspectives for Conservation. In M. C. M. Marques & C. E. V.

Grelle (Eds.), The Atlantic Forest (pp. 91–111). Springer International Publishing. https://doi.org/10.1007/978-3-030-55322-7_5

Cronin, T., Johnsen, S., Marshall, J., & Warrant, E. J. (2014). Visual ecology. Princeton university press.

Dalrymple, R. L., Flores-Moreno, H., Kemp, D. J., White, T. E., Laffan, S. W., Hemmings, F. A., Hitchcock, T. D., & Moles, A. T. (2018). Abiotic and biotic predictors of macroecological patterns in bird and butterfly coloration. Ecological Monographs, 88(2), 204–224. https://doi.org/10.1002/ecm.1287

Duarte, L. D. S., Bergamin, R. S., Marcilio-Silva, V., Seger, G. D. D. S., & Marques, M. C. M. (2014). Phylobetadiversity among Forest Types in the Brazilian Atlantic Forest Complex. PLoS ONE, 9(8), e105043. https://doi.org/10.1371/journal.pone.0105043

Endler, J. A. (1993). The Color of Light in Forests and It's Implications. Ecological Monographs, 63(1), 1–27.

Frost, D.R. (2024). Amphibian Species of the World: an Online Reference. Version 6.2. Electronic Database accessible at https://amphibiansoftheworld.amnh.org/index.php. American Museum of Natural History, New York, USA. https://doi.org/10.5531/db.vz.0001 Acessed in April 17,2024

Gade, M. R., Hill, M., & Saporito, R. A. (2016). Color Assortative Mating in a Mainland Population of the Poison Frog Oophaga pumilio. Ethology, 122(11), 851–858. https://doi.org/10.1111/eth.12533

Gehara, M., Crawford, A. J., Orrico, V. G. D., Rodríguez, A., Lötters, S., Fouquet, A., Barrientos, L. S., Brusquetti, F., De La Riva, I., Ernst, R., Urrutia, G. G., Glaw, F., Guayasamin, J. M., Hölting, M., Jansen, M., Kok, P. J. R., Kwet, A., Lingnau, R., Lyra, M., … Köhler, J. (2014). High Levels of Diversity Uncovered in a Widespread Nominal Taxon: Continental Phylogeography of the Neotropical Tree Frog

Dendropsophus minutus. PLoS ONE, 9(9), e103958. https://doi.org/10.1371/journal.pone.0103958

Gomez, D., Richardson, C., Lengagne, T., Plenet, S., Joly, P., Léna, J. P., & Théry, M. (2009). The role of nocturnal vision in mate choice: Females prefer conspicuous males in the European tree frog (Hyla arborea). Proceedings of the Royal Society B: Biological Sciences, 276(1666), 2351–2358. https://doi.org/10.1098/rspb.2009.0168

Grillo, O. (Ed.). (2011). The Dynamical Processes of Biodiversity—Case Studies of Evolution and Spatial Distribution. InTech. https://doi.org/10.5772/1830

Hegna, R. H., Saporito, R. A., Gerow, K. G., & Donnelly, M. A. (2011). Contrasting colors of an aposematic poison frog do not affect predation. Annales Zoologici Fennici, 48, 29–38.

Hödl, W., & Amezquita, A. (2001). Visual signaling in anuran amphibians. In M. J. Ryan (Ed.), Anuran communication (pp. 121–141). Smithsonian lnst. Press.

Kang, C., Sherratt, T. N., Kim, Y. E., Shin, Y., Moon, J., Song, U., Kang, J. Y., Kim, K., & Jang, Y. (2017). Differential predation drives the geographical divergence in multiple traits in aposematic frogs. Behavioral Ecology, 28(4), 1122–1130. https://doi.org/10.1093/beheco/arx076

Koneru, M., & Caro, T. (2022). Animal Coloration in the Anthropocene. Frontiers in Ecology and Evolution, 10, 857317. https://doi.org/10.3389/fevo.2022.857317

Kwet, A., R, L., & M, D.-B. (2010). Pró-Mata: Anfíbios da Serra Gaúcha, sul do Brasil/Amphibien der Serra Gaúcha, Südbrasilien/Amphibiansofthe Serra Gaúcha, South of Brazil. EDIPUCRS.

Leivas, P. T., Mayer, T. B., Leivas, F. W. T., & Fávaro, L. F. (2018). Trophic niche of Dendropsophus minutus (Anura: Hylidae) in southern Brazil. Phyllomedusa: Journal of Herpetology, 17(2), 267–272. https://doi.org/10.11606/issn.2316-9079.v17i2p267-272

Lermen, L., Furtado, R., Hartz, S.M. (2025). To be or not be seen: the influence of substrate on anuran community coloration. Journal of Natural History, 59, 1249-1265. https://doi.org/10.1080/00222933.2025.2480733

Lopes-Rodrigues, E. N., De S. Mendonça, Jr, M., L. O. Rosado, J., & Loeck, A. E. (2010). Soil spiders in differing environments: Eucalyptus plantations and grasslands in the Pampa biome, southern Brazil. Revista Colombiana de Entomología, 36(2), 277–284. https://doi.org/10.25100/socolen.v36i2.9159

Mack, M., & Beaty, L. (2021). The influence of environmental and physiological factors on variation in American toad (Anaxyrus americanus) dorsal coloration. Journal of Herpetology, 55(2), 119–126. https://doi.org/10.1670/20-093

Maia, R., Gruson, H., Endler, J. A., & White, T. E. (2019). pavo 2: New tools for the spectral and spatial analysis of colour in r. Methods in Ecology and Evolution, 10(7), 1–11. https://doi.org/10.1111/2041-210X.13174

Marcelino, V. R., Haddad, C. F. B., & Alexandrino, J. (2009). Geographic Distribution and Morphological Variation of Striped and Nonstriped Populations of the Brazilian Atlantic Forest Treefrog Hypsiboas bischoffi (Anura: Hylidae). Journal of Herpetology, 43(2), 351–361. https://doi.org/10.1670/08-050R2.1

Menéndez-Guerrero, P. A., David M. Green, & Davies, T. J. (2020). Climate change and the future restructuring of Neotropical anuran biodiversity. Ecography, 43, 222–235. https://doi.org/10.1111/ecog.04510

Michalis, C., Scott-Samuel, N. E., Gibson, D. P., & Cuthill, I. C. (2017). Optimal background matching camouflage. Proceedings of the Royal Society B: Biological Sciences, 284(1858), 1–6. https://doi.org/10.1098/rspb.2017.0709

Miramontes-Sequeiros, L. C., Palanca-Castán, N., Caamaño-Chinchilla, L., & Palanca-Soler, A. (2018). The phenotypic variability in Rana temporaria decreases in response to drying habitats. Science of The Total Environment, 612, 538–543. https://doi.org/10.1016/j.scitotenv.2017.08.261

Moreno-Rueda, G., & Comas, M. (2023). Evolutionary Ecology of Amphibians (1st ed.). CRC Press. https://doi.org/10.1201/9781003093312

Motta-Tavares, T., Ferreguetti, Á. C., Bergallo, H. D. G., & Rocha, C. F. D. (2024). Light Intensity as a Habitat Requirement for the Insular Endemic Frog Hylodes Fredi (Hylodidae) in Streams of Ilha Grande, Southeastern Brazil. South American Journal of Herpetology, 30(1). https://doi.org/10.2994/SAJH-D-22-00008.1

Overbeck, G. E., Pillar, V. D. P., Müller, S. C., & Bencke, G. A. (Eds.). (2024). South Brazilian Grasslands: Ecology and Conservation of the Campos Sulinos. Springer International Publishing. https://doi.org/10.1007/978-3-031-42580-6

R Core Team. (2024). R: A language and environment for statistical computing. R Foundation for Statistical Computing. https://www.r-project.org/

Rios, E., & Álvarez-Castañeda, S. T. (2012). Pelage color variation in pocket gophers (Rodentia: Geomyidae) in relation to sex, age and differences in habitat. Mammalian Biology, 77(3), 160–165. https://doi.org/10.1016/j.mambio.2011.12.003

Rojas, B. (2017). Behavioural, ecological, and evolutionary aspects of diversity in frog colour patterns. Biological Reviews, 92(2), 1059–1080. https://doi.org/10.1111/brv.12269

Roslin, T., Hardwick, B., Novotny, V., Petry, W. K., Andrew, N. R., Asmus, A., Barrio, I. C., Basset, Y., Boesing, A. L., Bonebrake, T. C., Cameron, E. K., Dáttilo, W., Donoso, D. A., Drozd, P., Gray, C. L., Hik, D. S., Hill, S. J., Hopkins, T., Huang, S., … Slade, E. M. (2017). Higher predation risk for insect prey at low latitudes and elevations. Science, 356(6339), 742–744. https://doi.org/10.1126/science.aaj1631

Señaris, J. C., Lampo, M., Rojas-Runjaic, F. J. M., & Barrio-Amorós, C. L. (2014). Guía ilustrada de los anfibios del Parque Nacional Canaima, Venezuela. Ediciones IVIC, Instituto Venezolano de Investigaciones Científicas (IVIC).

Sever, Z. (2024). What If I Told You Camouflage is a Myth? Animal Coloration is Mainly A-biotic and not Biotic (Camouflage): Animal coloration is mainly a-biotic and not biotic (camouflage). Research in Ecology, 6(1), 14–27. https://doi.org/10.30564/re.v6i1.6100

Sheldon, B. C., Arponen, H., Laurila, A., Crochet, P. A., & Merilä, J. (2003). Sire coloration influences offspring survival under predation risk in the moorfrog. Journal of Evolutionary Biology, 16(6), 1288–1295. https://doi.org/10.1046/j.1420-9101.2003.00606.x

Spaniol, R. L., Mendonça, M. de S., Hartz, S. M., Iserhard, C. A., & Stevens, M. (2020). Discolouring the Amazon Rainforest: How deforestation is affecting butterfly coloration. Biodiversity and Conservation, 1–18. https://doi.org/10.1007/s10531-020-01999-3

Stangel, J., Preininger, D., Sztatecsny, M., & Hödl, W. (2015). Ontogenetic Change of Signal Brightness in the Foot-Flagging Frog Species Staurois parvus and Staurois guttatus. Herpetologica, 71(1), 1–7. https://doi.org/10.1655/HERPETOLOGICA-D-14-00014

Stevens, M., Párraga, C. A., Cuthill, I. C., Partridge, J. C., & Troscianko, T. S. (2007). Using digital photography to study animal coloration. Biological Journal of the Linnean Society, 90, 211–237.

Théry, M. (2001). Forest light and its influence on habitat selection. Plant Ecology, 153, 251–261.

Toldi, M., Bizarro, G. L., Da-Costa, T., Da Silva, V. L., Jantsch Ferla, J., Johann, L., De Freitas, E. M., Da Silva, G. L., & Ferla, N. J. (2021). Mite fauna associated with different environments in the Southern Pampa, Brazil. International Journal of Acarology, 47(5), 387–395. https://doi.org/10.1080/01647954.2021.1915378

Toledo, L. F., & Haddad, C. F. B. (2009). Colors and Some Morphological Traits as Defensive Mechanisms in Anurans. International Journal of Zoology, 2009, 1–12. https://doi.org/10.1155/2009/910892

Toledo, L. F., Ribeiro, R. S., & Haddad, C. F. B. (2007). Anurans as prey: An exploratory analysis and size relationships between predators and their prey. Journal of Zoology, 271(2), 170–177. https://doi.org/10.1111/j.1469-7998.2006.00195.x

Troscianko, J., & Stevens, M. (2015). Image calibration and analysis toolbox—A free software suite for objectively measuring reflectance, colour and pattern. Methods in Ecology and Evolution, 6(11), 1320–1331. https://doi.org/10.1111/2041-210X.12439

Umbers, K. D. L., Silla, A. J., Bailey, J. A., Shaw, A. K., & Byrne, P. G. (2016). Dietary carotenoids change the colour of Southern corroboree frogs. Biological Journal of the Linnean Society, 119(2), 436–444. https://doi.org/10.1111/bij.12818

Valdez, J.W., Gould, J., Garnham, J.I. (2021). Global assessment of artificial habitat use by amphibian species. Biological Conservation , 257, 109129. https://doi.org/10.1016/j.biocon.2021.109129

Vásquez, T., & Pfennig, K. S. (2007). Looking on the bright side: Females prefer coloration indicative of male size and condition in the sexually dichromatic spadefoot toad, Scaphiopus couchii. Behavioral Ecology and Sociobiology, 62(1), 127–135. https://doi.org/10.1007/s00265-007-0446-7

Verdum, R., Vieira, L. D. F. D. S., Caneppele, J. C. G., & Gass, S. L. B. (2019). Pampa: The South Brazil. In A. A. R. Salgado, L. J. C. Santos, & J. C. Paisani (Eds.), The Physical Geography of Brazil (pp. 7–20). Springer International Publishing. https://doi.org/10.1007/978-3-030-04333-9_2

Vilardo, G., Tognetti, P. M., González-Arzac, A., & Yahdjian, L. (2018). Soil arthropod composition differs between old-fields dominated by exotic plant species and remnant native grasslands. Acta Oecologica, 91, 57–64. https://doi.org/10.1016/j.actao.2018.06.003

Wei, S., Li, Z., Momigliano, P., Fu, C., Wu, H., & Merilä, J. (2020). The roles of climate, geography and natural selection as drivers of genetic and phenotypic differentiation in a widespread amphibian Hyla annectans (Anura: Hylidae). Molecular Ecology, 29(19), 3667–3683. https://doi.org/10.1111/mec.15584

Wrubleswski, J., Reichert, F. W., Galon, L., Hartmann, P. A., & Hartmann, M. T. (2018). Acute and chronic toxicity of pesticides on tadpoles of Physalaemus cuvieri (Anura, Leptodactylidae). Ecotoxicology, 27(3), 360–368. https://doi.org/10.1007/s10646-018-1900-1

Zamora-Camacho, F. J., & Comas, M. (2017). Greater reproductive investment, but shorter lifespan, in agrosystem than in natural-habitat toads. PeerJ, 5, e3791. https://doi.org/10.7717/peerj.3791

Zamora-Camacho, F. J., & Comas, M. (2019). Beyond Sexual Dimorphism and Habitat Boundaries: Coloration Correlates with Morphology, Age, and Locomotor Performance in a Toad. Evolutionary Biology, 46(1), 60–70. https://doi.org/10.1007/s11692-018-9466-7

Ziliotto, M., Kulmann-Leal, B., Roitman, A., Bogo Chies, J. A., & Ellwanger, J. H. (2023). Pesticide Pollution in the Brazilian Pampa: Detrimental Impacts on Ecosystems and Human Health in a Neglected Biome. Pollutants, 3(2), 280–292. https://doi.org/10.3390/pollutants3020020

CAPÍTULO 2

Temperature, precipitation, and habitat influence dorsal coloration patterns in a neotropical tree frog

Luísa Nunes Lermen*; Daniel Loebmann

Programa de Pós-graduação em Ambientes Aquáticos Continentais. Laboratório de Vertebrados, Instituto de Ciências Biológicas, Universidade Federal do Rio Grande, Campus Carreiros, Av. Itália km 8, Rio Grande, RS 96203-900, Brazil

*Corresponding author. Email: lulis.lermen@hotmail.com. Telephone: +55 51 998731349

Keywords: Amphibians, Animal coloration, Southern grasslands, Atlantic forest, *Dendropsophus minutus*

Este manuscrito está publicado no periódico Biological Journal of the Linnean Society

DOI: https://doi.org/10.1093/biolinnean/blaf138

Abstract: Animal coloration is a multifunctional trait shaped by the interplay of environmental pressures and ecological functions such as thermoregulation, predator avoidance, and social signaling. In ectotherms like amphibians, dorsal coloration may be especially sensitive to local microclimatic conditions and habitat structure. This study investigated how the environmental variables of temperature, precipitation, and habitat type influence dorsal color traits in the Neotropical treefrog *Dendropsophus minutus*, a widely distributed generalist species. Coloration was quantified in situ through standardized photography and posterior image analysis, measuring hue, saturation, and brightness. Our results revealed that brightness increased significantly with ambient temperature, particularly in open habitats, suggesting a thermoregulatory role for this trait. Saturation exhibited a complex pattern, modulated by an interaction between habitat and precipitation: under wet conditions, grassland individuals showed higher saturation than those in forests, and saturation generally decreased with increasing temperature. Hue remained unaffected by any of the evaluated variables. These findings emphasize the ecological relevance of distinct color components, showing that brightness and saturation respond differently to environmental gradients. Moreover, the study highlights the potential vulnerability of amphibian color phenotypes to habitat alteration and climate change, as changes in canopy cover, rainfall regimes, and thermal environments may disrupt the selective pressures maintaining functional coloration.

Keywords: Amphibians, Animal coloration, Southern grasslands, Pampa, Atlantic forest, *Dendropsophus minutus*

INTRODUCTION

The ecological importance of coloration in amphibians stems is resulted from its role in balancing multiple selective pressures, where visual conspicuousness and crypsis operate in dynamic tension, influenced by habitat structure, microclimatic conditions, and predation pressure (Rudh & Qvarnström, 2013; Rojas, Lawrence, & Márquez, 2023). Coloration phenotypes in these animals serve as multifunctional traits connected to their ecological performance and survival, mediating interactions related to predation, thermoregulation and social signaling (Rudh & Qvarnström, 2013; Rojas *et al.*, 2023; Laumeier *et al.*, 2023). Color polymorphism and phenotypic plasticity in coloration are widely documented phenomena in amphibians, reflecting their sensitivity to environmental conditions and the complex selective pressures that influence visual traits in this group (Toledo & Haddad, 2009; Rojas, 2017). Variations in dorsal coloration among individuals and populations have been associated with differences in habitat structure, microclimatic regimes, predation risk, and reproductive behavior (Zamora-Camacho & Comas, 2019; Rojas *et al.*, 2023; Laumeier *et al.*, 2023). Moreover, as ectothermic organisms, amphibians rely on environmental heat sources to regulate body temperature, and their coloration modulates both thermal exchange and water retention (Mack & Beaty, 2021; Laumeier *et al.*, 2023). These selective pressures acting on coloration differ across environments, as variations in temperature, moisture, and habitat structure directly affect both the visibility and thermoregulatory function of color traits, leading to complex evolutionary outcomes (Barnett *et al.*, 2021; Mack & Beaty, 2021; Rojas *et al.*, 2023; Laumeier *et al.*, 2023).

Light environments, particularly in forest ecosystems, play a decisive role in modulating the perception and ecological function of coloration (Endler, 1993; Hödl & Amezquita, 2001; Cronin *et al.*, 2014). In forested environments with closed canopies, reduced light availability typically favors the evolution of darker or less saturated coloration, which improves concealment by blending with dim, heterogeneous backgrounds (Endler, 1993). The dense canopy vegetation modifies the light environment by filtering and altering the spectral quality of light that reaches the forest floor, affecting how colors are perceived and how visual signals function in these shaded habitats (Cronin *et al.*, 2014; Brelsford *et al.*, 2022). Conversely, open environments like grasslands allow for higher light intensity and broader spectral exposure, conditions under which lighter or brighter coloration may be advantageous for enhancing thermoregulatory efficiency and reducing overheating (Rudh & Qvarnström, 2013; Laumeier *et al.*, 2023). The visibility and ecological function of color patterns for both predators and conspecifics are strongly influenced by local light environments, with the same color phenotype potentially varying greatly in conspicuousness or camouflage effectiveness depending on the prevailing light conditions (Amézquita & Hödl, 2006; Barnett *et al.*, 2021). As a result, habitat disturbances such as deforestation and climate change can disrupt these environmental parameters, potentially altering the adaptive value of coloration by affecting the efficiency of camouflage, warning signals, thermoregulation and other visual traits critical for survival (Barnett *et al.*, 2021; Laumeier *et al.*, 2023). This highlights the importance of considering habitat-specific light conditions when evaluating the adaptive value of coloration in amphibians.

Among the abiotic factors influencing amphibian coloration, microclimatic factors such as temperature and humidity stand out as key selective forces, given their direct effects on physiological performance (Navas, Gomes, & Carvalho, 2008; Laumeier *et al.*, 2023). As ectothermic vertebrates, anurans are highly dependent on external thermal and hydric environments to regulate physiological functions, with color traits playing a pivotal role in both heat absorption and evaporative water loss (Navas *et al.*, 2008; Alho *et al.*, 2010; Laumeier *et al.*, 2023). In this context, dorsal coloration plays a functional role not only in visual signaling and crypsis but also in thermoregulation and desiccation resistance (Alho *et al.*, 2010; Tracy, Christian, & Tracy, 2010; Laumeier *et al.*, 2023). Darker individuals typically absorb more radiant heat, providing an advantage in cooler habitats or during periods of low environmental temperatures, whereas lighter coloration can help minimize heat gain and reduce evaporative water loss under conditions of high thermal or desiccation stress (Navas *et al.*, 2008; Tracy *et al.*, 2010; Laumeier *et al.*, 2023). Indeed, studies have shown that darker dorsal patterns can enhance heating rates in cool, high-altitude environments (Vences *et al.*, 2002; Alho *et al.*, 2010; Mack & Beaty, 2021). This pattern has been documented in species such as *Anaxyrus americanus* (Holbrook, 1836) and *Rana temporaria* (Linnaeus, 1758), where individuals from colder habitats frequently display darker dorsal pigmentation, likely as an adaptation to improve heat absorption under low-temperature conditions (Vences *et al.*, 2002; Alho *et al.*, 2010; Mack & Beaty, 2021). Consequently, amphibians living in different habitats may exhibit color traits finely tuned to local environmental conditions, balancing trade-offs between thermoregulation, predation risk, and reproductive signaling.

Despite growing recognition of the ecological and functional significance of coloration in amphibians, much of the existing research has focused on conspicuous, aposematic, or diurnally active species, while cryptic and nocturnal taxa remain comparatively underrepresented in empirical studies (Rojas *et al.*, 2023). Moreover, although ecological patterns linking melanism to climatic factors are well established, and the occurrence of color polymorphism and phenotypic plasticity in amphibians is well documented (Alho *et al.*, 2010; Rojas *et al.*, 2023; Laumeier *et al.*, 2023), quantitative research assessing the influence of environmental variables on color parameters (hue, saturation, and brightness) is still limited. This represents a critical gap, as habitat disturbances such as deforestation and climate change may modify environmental conditions in ways that disrupt the adaptive value of visual traits crucial for thermoregulation, predator avoidance, and reproductive success (Barnett *et al.*, 2021; Rojas *et al.*, 2023; Laumeier *et al.*, 2023).

In this context, *Dendropsophus minutus* (Peters, 1872) is an excellent model for testing ecological and ecophysiological hypotheses related to environmentally driven color variation. Widely distributed across South America (Gehara *et al.*, 2014), *D. minutus* is an abundant and habitat generalist species, occurring in both forested and grassland environments (Kwet, R, & M, 2010; Señaris *et al.*, 2014; Gehara *et al.*, 2014). In addition, the species has no sexual dimorphism or extreme color change in dorsal coloration (Kwet *et al.*, 2010; Señaris *et al.*, 2014).

Given this context, the present study aims to evaluate how environmental variables, specifically temperature, precipitation, and habitat type, influence dorsal coloration in anurans. To achieve this, we conducted in situ photographic sampling to measure the coloration of *Dendropsophus minutus* across distinct environmental conditions, with color quantified in terms of hue, saturation, and brightness. We hypothesized that dorsal coloration in *D. minutus* would exhibit significant variation between habitat types, reflecting differences in microclimatic and photic environments. Based on previous evidence in amphibians (Zamora-Camacho & Comas, 2019; Zhelev, Mollov, & Tsonev, 2020; Mack & Beaty, 2021; Laumeier *et al.*, 2023), we expected individuals inhabiting cooler environments or more humid forests to display darker dorsal coloration, while lighter individuals would predominate in hotter habitats or open grasslands. Additionally, we anticipated that saturation values would correlate with local microclimatic conditions, being influenced by habitat-specific light environments, with individuals from grassland habitats exhibiting higher saturation. Meanwhile, considering the particular traits of *D. minutus*, we expected hue to remain unchanged.

MATERIAL AND METHODS

Study Species

Dendropsophus minutus (Peters, 1872) is a small, widely distributed hylid treefrog native to South America (Gehara *et al.*, 2014). Although this taxon likely represents a complex of cryptic species, previous research indicates that only a single genetic lineage occurs in the region where this study was conducted (Gehara *et al.*, 2014). The species is typically found inhabiting vegetation such as trees and shrubs across a broad range of environments, including both forested and open landscapes, as well as human-altered areas (Kwet *et al.*, 2010; Señaris *et al.*, 2014). Its dorsal coloration exhibits notable intraspecific phenotypic plasticity, with individuals displaying shades of orange-brown, brown, yellow, or tan, usually marked by two prominent darker longitudinal stripes (Kwet *et al.*, 2010; Señaris *et al.*, 2014). Importantly, no sexual dimorphism in dorsal coloration or dramatic color change has been documented (Kwet *et al.*, 2010). Males vocalize from grasses, aquatic vegetation, and low shrubs near water bodies during the warmer months (Kwet *et al.*, 2010).

Study area and data collection

This study was carried out in two ecologically distinct natural landscapes within the state of Rio Grande do Sul, southern Brazil: the southern Atlantic Forest and the Southern Grasslands (Campos Sulinos). A total of 14 water bodies were sampled, comprising 8 sites situated within forested areas and 6 within open grassland habitats (Table 1). To maintain spatial independence among sampling locations, all sites were positioned at least 500 meters apart. Prior to fieldwork, habitat classification for each water body was determined according to the dominant vegetation type within a 250-meter radius surrounding each site.

The Araucaria Mixed Forest represents a distinctive forest type within the Atlantic Forest biome, characterized by the dominance of *Araucaria angustifolia* (Bertol.) O. Kuntze, an emergent conifer that rises above a canopy of broadleaf angiosperms (Carlucci, Marcilio-Silva, & Torezan, 2021). This formation occurs under a subtropical climate with well-distributed annual precipitation, average monthly temperatures typically remaining below 23 °C, and frequent frost events during the colder months (Grillo, 2011; Carlucci *et al.*, 2021). Data collection in forested areas occurred at four federally protected areas: the Floresta Nacional de São Francisco de Paula (FLONA-SFP) and the Centro de Pesquisas e Conservação da Natureza Pró-Mata (CPCN Pró-Mata) in São Francisco de Paula, the Floresta Nacional de Canela (FLONA-Canela) in Canela, and the Floresta Nacional de Passo Fundo (FLONA-PF) in Mato Castelhano.

In contrast, the Southern grasslands is predominantly composed of open grassland ecosystems, where arboreal vegetation is sparse and generally restricted to riparian corridors (Andrade *et al.*, 2019; Overbeck *et al.*, 2024). The region shares a subtropical climate with the forested areas but experiences lower annual precipitation and periodic hydric deficits during the summer (Overbeck *et al.*, 2024). Contemporary land-use practices remain a key factor in maintaining these open landscapes, often surpassing climatic conditions in shaping their present-day distribution (Verdum *et al.*, 2019; Overbeck *et al.*, 2024). Natural water bodies in these grasslands were sampled at two locations: within the Estação Experimental Agronômica da Universidade Federal do Rio Grande do Sul (EEA-UFRGS) in Eldorado do Sul and on private property near the granitic outcrop known as Cerro das Almas (CDA) in Capão do Leão.

Field data were collected between October 2022 and January 2025, corresponding to the region's warm season. To maintain consistency in environmental conditions during sampling, all surveys were carried out at night, coinciding with the reproductive period of the target species. This approach minimized potential variations in coloration influenced by fluctuating environmental factors throughout the day. However, it is important to note that these nocturnal data cannot be directly compared with diurnal coloration. Since coloration is ecologically important in both diurnal and nocturnal anurans (Gomez *et al.*, 2009; Stangel *et al.*, 2015; Zamora-Camacho & Comas, 2019), nighttime sampling is not expected to compromise the ecological relevance or interpretability of the data.

Table 1 – Geographic coordinates, habitat type, and number of sampled water bodies in each study area: Floresta Nacional de São Francisco de Paula (FLONA-SFP); Centro de Pesquisas e Conservação da Natureza Pró-Mata (PRÓ-MATA);Floresta Nacional de Canela (FLONA-Canela); Floresta Nacional de Passo Fundo (FLONA-PF); Estação Experimental Agronômica da Universidade Federal do Rio Grande do Sul (EEA-UFRGS); and Cerro das Almas (CDA).

Sample area	Geographic coordinates	Habitat	Nº of water bodies sampled
FLONA-SFP	S 29°25'24.4 W 50°23'12.2"	Araucaria Mixed Forest	3
PRÓ-MATA	S 29°29'17.8" W 50°12'25.3"	Araucaria Mixed Forest	2
FLONA-Canela	S 29°19'19.9" W 50°49'05.3"	Araucaria Mixed Forest	2
FLONA-PF	S 28°17'44.7" W 52°11'44.5"	Araucaria Mixed Forest	1
EEA-UFRGS	S 30°05'31.5" W 51°40'18.6"	Southern grasslands	5
CDA	S 31°47'28.3" W 52°35'35.8"	Southern grasslands	1

We sampled 89 adult *Dendropsophus minutus s* individuals of which 29 were sampled in wet forested areas, 21 in dry forested areas, 16 in wet grassland areas, and 23 in dry grassland areas. Individuals were located through active search techniques. Once detected, frogs were manually captured, measured, and photographed under controlled conditions. Ambient temperature was recorded at the time of capture using a digital thermohygrometer. Each specimen was temporarily housed in a container partially filled with water and natural vegetation collected from the immediate area to reduce stress. To avoid resampling, all individuals were released into their original water bodies only after the completion of each nightly session. Additionally, each individual water body was sampled only once. Precipitation data were obtained as the cumulative rainfall over the 30 days preceding each sampling event, with values extracted from the INMET (Instituto Nacional de Meteorologia) database. To account for repeated precipitation values associated with multiple individuals, specimens were classified into two categories: individuals exposed to less than 150 mm were assigned to the 'Dry' group, while those experiencing cumulative rainfall greater than 150 mm were assigned to the 'Wet' group. All procedures were conducted in accordance with approved ethical protocols and authorized by the Ethics Committee of the Universidade Federal do Rio Grande (Process No. P034/2022).

Color measurement

Photographs of each individual were taken in the field using a Canon EOS 1200D digital camera equipped with an 18–55 mm lens. As data collection took place during nighttime surveys, the camera’s built-in flash was employed to provide a consistent and standardized light source for all images. Therefore, the white balance was set to the automatic standard for flash use. Additionally, the exposure time was fixed at 1/80 s, with an f-stop of f/8 and an ISO of 400. To calibrate color data and account for lighting variations, each frog was photographed alongside a scale bar and an 18% gray card, which facilitated corrections for illumination inconsistencies and the effects of flash lighting during the image processing analysis (Stevens *et al.*, 2007). For each specimen, ten dorsal images were captured to allow for later selection for subsequent analysis. All images were recorded in RAW format with fixed aperture settings and white balance adjusted for flash exposure.

Following fieldwork, the images were processed using ImageJ software in combination with the micaToolbox 2.2.0 plugin (Troscianko & Stevens, 2015). The toolbox's screening tool was applied to select the three best-exposed images per individual, which were then calibrated based on the gray card reference to produce multispectral images for analysis. To extract color information, a region of interest (ROI) was manually delineated along the dorsum of each frog, excluding areas where specular reflection or glare was present. From these standardized, calibrated images, reflectance values for the red, green, and blue (RGB) channels were measured. These RGB data were then converted into three principal colorimetric variables: hue, representing the perceived color (e.g., yellow or green), saturation, indicating color intensity relative to white light, and brightness, corresponding to overall reflectance across the visible spectrum (Stevens *et al.*, 2007).

Data analysis

To calculate the colorimetric variables, the standardized RGB values were organized into a table formatted for compatibility with the *colspace* function in the PAVO package (v. 2.9.0) (Maia *et al.*, 2019) in R software (v. 4.3.3) (R Core Team, 2024). The RGB values were arranged according to their corresponding short (B), medium (G), and long (R) wavelength channels and plotted as XY coordinates within a triangular color space, from which hue and saturation values were derived. In this color space, the central point represents the achromatic reference, while positions surrounding this point indicate both color type and its saturation. Saturation was quantified as the magnitude of r, the distance from the achromatic origin to the color point, whereas hue was determined by the angle θ, representing the angular displacement of the color point relative to the positive x-axis. Brightness was estimated as the mean reflectance value across all three RGB channels.

Differences in body size between habitats were tested using ANOVA. The effects of habitat type (classified as open or closed environments) and cumulative precipitation (categorized as dry or wet) on ambient temperature were also assessed through type III ANOVA to account for the unequal numbers of individuals in each group. Each of the colorimetric parameters (hue, saturation, and brightness) was analyzed independently in relation to habitat type and cumulative precipitation using type III ANOVA, while their associations with ambient temperature were evaluated through linear regression models incorporating separate intercepts for each habitat type. Prior to analysis, data distributions were assessed for normality using Q–Q plots and by the Akaike Information Criterion (AIC) as an additional diagnostic tool. To address data skewness, brightness values were log-transformed before analysis. In cases where significant interactions were detected in the ANOVA, post hoc pairwise comparisons were conducted using t-tests with Bonferroni-adjusted p-values. All statistical procedures were performedusing the lmer4 package in R (Bates *et al.*, 2015).

RESULTS

Individuals sampled in forested habitats were significantly larger than those from grassland environments ($p > 0.001$; $R^2 = 0.568$; Figure 1A), with mean body sizes of 24.5 ± 1.47 mm in forested areas and 20.9 ± 1.68 mm in grassland habitats. Regarding temperature, the highest and lowest recorded values were observed in dry grassland habitats, with 24.7 °C and 10 °C, respectively. Unsurprisingly, dry grasslands exhibited the greatest thermal range among all habitat types (Table 2). Habitat type had a significant effect ($p = 0.001$; $R^2 = 0.143$) on temperature, cumulative precipitation alone was not significant, but a significant interaction was detected between habitat type and cumulative precipitation ($p = 0.001$). Post hoc analysis revealed that dry grasslands exhibited significantly higher temperatures compared to all other conditions (Figure 1B).

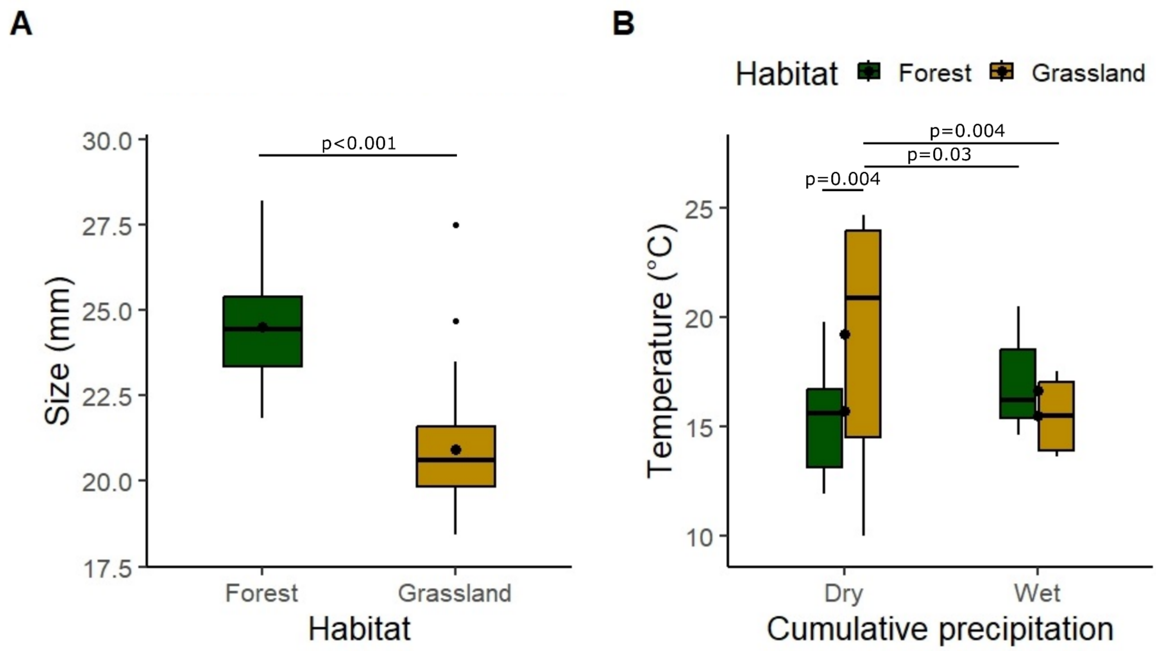

Figure 1 – Boxplots of (A) body size and (B) ambient temperature of *Dendropsophus minutus* by habitat and precipitation category. Forest individuals were larger, and post hoc analysis indicated that dry grasslands exhibited the highest temperatures. Black dots represent mean values; thick black lines indicate medians; small dots denote outliers; whiskers show the data range; boxes represent the interquartile range; and lines with p-values indicate significant differences between groups.

Table 2 – Minimum, maximum, and thermal range (°C) recorded for each habitat and precipitation category.

	Temperature (°C)		
Habitat	**Minimum**	**Maximum**	**Thermal range**
Dry grassland	10	24.7	14.7
Wet grassland	13.6	17.5	3.9
Dry forest	11.9	19.8	7.9
Wet forest	14.6	20.5	5.9

Figure 2 – Effects of habitat, precipitation, and temperature on dorsal coloration in *Dendropsophus minutus*. (A) Brightness showed no differences between categories. (B) Brightness increased with temperature. (C) Saturation varied with a significant habitat–

precipitation interaction. (D) Saturation declined with temperature, steeper in forests. (E, F) Hue was unaffected. In the boxplots, thick black lines indicate medians, the gray shading represents data density along the distribution, and p-values denote post hoc differences. In the regression plots, a single asterisk (*) indicates a significant temperature effect ($p < 0.003$) without habitat interaction, while a double asterisk (**) marks significant effects of both temperature and habitat interaction ($p < 0.001$). Colored dots represent the raw data distribution.

Neither habitat type nor cumulative precipitation had a significant effect on the brightness of *Dendropsophus minutus* dorsal coloration (Figure 2A). However, temperature showed a significant influence, with higher temperatures associated with increased brightness values in both habitat types, although the model explained a relatively small proportion of the variance ($p = 0.003$; $R^2 = 0.081$; Figure 2B). This indicates that individuals tend to exhibit lighter coloration as ambient temperature rises. Similarly, neither habitat type nor cumulative precipitation had a significant main effect on dorsal color saturation; however, a significant interaction between these two variables was detected ($p = 0.003$; $R^2 = 0.160$). Post hoc analysis revealed that individuals from wet grasslands exhibited significantly higher color saturation compared to those from wet forests (Figure 2C). Temperature had an overall negative effect on saturation ($p = 0.005$; $R^2 = 0.159$), and a significant interaction with habitat type was observed, with forest environments showing a steeper decline in saturation as temperature increased (Figure 2D). The hue of *Dendropsophus minutus* dorsal coloration was not significantly affected by any of the evaluated variables, including habitat type and cumulative precipitation (Figure 2E), or temperature (Figure 2F).

DISCUSSION

Our findings showed that *Dendropsophus minutus* exhibits notable variation in dorsal coloration traits in response to environmental factors. Brightness increased significantly with temperature, consistent with global patterns reported for anurans (Laumeier *et al.*, 2023), and partially supporting the study's initial hypotheses, as habitat effects on brightness were not significant. Meanwhile, saturation displayed a complex pattern, influenced by an interaction between habitat and precipitation, as well as temperature. Specifically, frogs in wet grasslands exhibited higher saturation than those in wet forests, partially aligning with our prediction, although only under conditions of high precipitation. Unexpectedly, temperature showed a negative correlation with saturation. As anticipated by the specific characteristics of *Dendropsophus minutus* (Kwet *et al.*, 2010; Gehara *et al.*, 2014), hue remained unaffected by any of the variables evaluated in this study. Together, these findings highlight the role of fine-scale microclimatic and habitat characteristics in shaping phenotypic traits while showing that not all color components respond equally to environmental variation.

The relationship between dorsal brightness and temperature in *Dendropsophus minutus* reinforces the functional importance of brightness as a thermoregulatory trait. This finding supports the well known Bogert's effect, which predicts lighter coloration in warmer environments as an adaptation to reduce heat absorption (Bogert, 1949; Muñoz, 2021). It is widely know that darker objects heat up faster (Pinkert & Zeuss, 2018), therefore brightness increasing with temperature is a well documented pattern in anurans and other ectotherms as a mechanism to modulate heat absorption (De Souza, Mayorquin, & Sarmiento, 2020; Laumeier *et al.*, 2023). Darker coloration can be advantageous in cooler environments or during periods of lower temperatures, while lighter pigmentation reduce heat absorption, aiding in thermal regulation and minimizing water loss under high temperatures (Alho *et al.*, 2010; Mack & Beaty, 2021; Laumeier *et al.*, 2023).

Interestingly, despite a significant interaction between temperature and precipitation, where the lack of rainfall resulted in higher temperatures in grasslands, this effect did not translate into significant differences in brightness between habitat categories. One might have expected that dry grasslands would select for consistently lighter individuals, resulting in significant differences in brightness between environments. However, this was not the case. The absence of a clear habitat effect suggests that factors beyond thermoregulation alone may be influencing brightness in these environments. One possible explanation is that darker dorsal coloration may provide an advantage for individuals in dry grasslands, as melanin contributes to UV protection (Rudh & Qvarnström, 2013; Burraco & Orizaola, 2022), and open habitats are subject to higher levels of solar and consequently ultraviolet exposure (Endler, 1993). Thus, although higher temperatures would favor lighter individuals, protection against intense radiation in dry grasslands could favor darker individuals, and the action of these two antagonistic forces could result in the absence of brightness differences between habitats. Interestingly, Gloger's rule, which predicts darker coloration in wetter habitats and was originally formulated for endotherms, has recently been extended to ectotherms (Kang *et al.*, 2021); however, it does not appear to apply in this case. Nevertheless, this result highlights that multiple factors may influence dorsal brightness in *Dendropsophus minutus*, and further investigation would be valuable to assess additional selective pressures contributing to the observed patterns.

Under wet conditions, individuals inhabiting grasslands displayed significantly higher dorsal color saturation than those in wet forests. This pattern parallels observations from Zamora-Camacho and Comas (2019), who reported lower saturation and smaller body sizes in forest-dwelling amphibians compared to those in open agroecosystems. It is important to note that their study was conducted during the rainy winter season (Zamora-Camacho & Comas, 2017, 2019), which could correspond to the 'wet' conditions defined in our study. In their findings, higher color saturation in open habitats was interpreted as a signal of increased reproductive effort, as individuals in those environments faced higher mortality risks and consequently shorter lifespans (Zamora-Camacho & Comas, 2017). Body size was one of several indicators of reproductive investment identified by Zamora-Camacho and Comas (2017), with larger size in open habitats attributed to faster growth rates, a costly trait associated with greater reproductive success. Interestingly, the present study diverges from those patterns: here, individuals in forest habitats were larger, while grassland frogs exhibited more saturated coloration. Despite this contrast, it remains possible that increased color saturation in grassland individuals during wet periods is related to reproductive effort. In the Southern Grasslands, individuals are susceptible to periodic hydric deficits during the summer (Overbeck *et al.*, 2024), which can restrict breeding opportunities as reproductive sites may dry out. During favorable periods of summer rainfall, individuals might increase their investment in visual signaling, as dorsal coloration is known to play a role in mate attraction for several anuran species (Sheldon *et al.*, 2003; Vásquez & Pfennig, 2007; Ries *et al.*, 2008; Rojas *et al.*, 2023).

However, smaller body size may be associated with greater vulnerability to predators, potentially placing grassland individuals at a higher risk of predation (Toledo, Ribeiro & Haddad, 2007). Moreover, open habitats such as grasslands inherently pose higher predation risks than forests (Seymoure et al., 2018; Goßmann et al., 2023). Optimal camouflage coloration results from balancing the probability of occupying a specific microhabitat with the likelihood of encountering predators within it (Merilaita & Jormalainen, 1999). Therefore, under these conditions, stronger selection for cryptic coloration would be expected. In grasslands, animals are exposed to broad-spectrum white sunlight (Endler, 1993; Amézquita & Hödl, 2006), whereas in forest habitats the light reaching the understory is less saturated and spectrally filtered by the canopy (Endler, 1993; Théry, 2001). Higher color saturation observed in grassland frogs may thus reflect enhanced background matching, favoring individuals whose coloration more closely aligns with the more saturated light environment.

Consequently, the combination of smaller body size and higher color saturation in grassland populations could represent an adaptive strategy promoting crypsis and predator avoidance through effective background matching rather than visual signaling alone. Alternatively, the pattern could reflect the effects of temperature, a factor not addressed in Zamora-Camacho's study.

In addition to the effects of habitat and precipitation, temperature had a significant influence on dorsal color saturation in *Dendropsophus minutus*, with a general tendency for saturation to decrease as temperature increased, and a notably steeper decline observed in forest habitats. The continuous canopy cover in forests contributes to maintaining lower saturation levels by acting as a natural filter, altering the spectral composition of light that reaches the understory (Brelsford et al., 2022; Cronin et al., 2014). As a result, the light in these environments tends to be less saturated (Endler, 1993; Théry, 2001), and to achieve effective crypsis under such conditions, individuals likely need to match the reduced light saturation of the understory (Théry, 2001). In contrast, in grassland environments, the negative relationship between saturation and temperature appears to be modulated by an interaction with precipitation. Rainfall events tend to lower ambient temperatures in these habitats, which in turn seem to permit an increase in dorsal color saturation. However, despite these patterns, neither canopy filtering in forests nor the interaction between temperature and rainfall in grasslands fully explain the marked effect of temperature on saturation observed in this study. In fact, while studies have assessed the influence of temperature on color brightness in anurans (Laumeier *et al.*, 2023), mainly within the context of thermoregulation by examining whether individuals lighten or darken as temperatures fluctuate (Tattersall, Eterovick, & De Andrade, 2006; Park *et al.*, 2023), and others have reported hue shifts associated with reproductive signaling in reptiles (Robinson & Gifford, 2018; Assis *et al.*, 2020), investigations specifically addressing the effects of temperature on color saturation remain scarce. This is notable given that saturation has been reported as thermally sensitive in *Pseudacris regilla* (Baird and Girard, 1852) (Stegen, Gienger, & Sun, 2004). This gap highlights the need for future studies to consider saturation as a potentially thermally sensitive color trait, especially given its ecological relevance in visual signaling and predator avoidance under variable environmental conditions.

The context-dependency of color traits observed in this study has direct implications for conservation. Habitat alterations such as deforestation, fragmentation, agricultural expansion, and climate change are rapidly transforming the environmental gradients that currently sustain these coloration patterns (Carlucci *et al.*, 2021; Koneru & Caro, 2022; Overbeck *et al.*, 2024). In fact, it has been demonstrated that habitat alterations can modify the perception of an organism's coloration, potentially impairing the effectiveness of defensive color traits that evolved under the original environmental conditions (Barnett *et al.*, 2021). Similarly, intensified temperature extremes and altered rainfall patterns resulting from climate change (Roland *et al.*, 2012; Braga & Laurini, 2024) have the potential to not only compromise defensive coloration by modifying the visual environment but also disrupt thermoregulation and affect thermally sensitive color traits. Therefore, such environmental disruptions, by reducing the effectiveness of dorsal coloration, could ultimately impact individual survival, predator avoidance, and mate attraction. Even though *Dendropsophus minutus* is a generalist species, it could still be vulnerable to these changes, potentially experiencing range contractions and local extinctions (Menéndez-Guerrero, David M. Green, & Davies, 2020).

Despite providing valuable insights into how environmental factors influence dorsal coloration in *Dendropsophus minutus*, this study has several limitations that should be acknowledged. One important consideration is that the observational nature of the study limits the ability to establish causal relationships between environmental variables and color variation. While associations between temperature, precipitation, and color traits were detected, experimental manipulations under controlled conditions would be necessary to confirm the functional roles of these variables in driving phenotypic plasticity or selection on coloration. Future research should include controlled experiments or repeated-measure studies to determine whether dorsal coloration changes within individuals in response to environmental variation. Such approaches would allow for stronger inference of causality and help disentangle the relative contributions of plasticity versus selection in shaping color traits. The relatively limited geographic scope, constrained to southern Brazil, also restricts broader generalizations about phenotypic responses in *Dendropsophus minutus* populations across its full distribution, particularly given the high levels of genetic diversity observed throughout its range (Gehara *et al.*, 2014). Incorporating broader spatial scales and additional amphibian species would help test whether the patterns observed in *Dendropsophus minutus* are widespread or taxon-specific.

CONCLUSION

In conclusion, this study demonstrates that dorsal coloration in *Dendropsophus minutus* is shaped by a complex interplay of environmental factors, with temperature, precipitation, and habitat structure influencing color brightness and saturation in ecologically meaningful ways. While brightness responded primarily to temperature fluctuations, saturation exhibited a more intricate pattern, modulated by habitat type and precipitation-driven changes in ambient temperature, particularly in open environments. The integration of multiple colorimetric parameters, along with temperature and precipitation data, provides new insights into the ecological flexibility of *Dendropsophus minutus* and offers a valuable contribution to the broader understanding of environmentally driven phenotypic variation in Neotropical amphibians. Given the increasing pace of habitat alteration and climate change, understanding how amphibians physiologically and behaviorally adjust to environmental heterogeneity is crucial for predicting species resilience. Future research integrating experimental, physiological, and visual perception approaches will be essential to clarify the functional significance of color traits.

Acknowledgement

We thank the Programa de Pós-Graduação em Ambientes Aquáticos Continentais at the Universidade Federal do Rio Grande for providing support during this study. DL received a research fellowship from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, grant number 307232/2023-9). LL was supported by a scholarship from the Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). ScholarGPT (version GPT-4-turbo) was used to assist the authors in expressing content in a non-native language, reviewing grammar, and enhancing the clarity and flow of the text.

Conflicts of interest: The authors declare that they have no conflict of interest.

Funding: D. Loebmann recieved a research fellowship from Conselho Nacional de Desenvolvimento Científico e Tecnológico (307232/2023-9). L. Lermen recieved a scholarship from Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.

Data Availability: The datasets used and analyzed during the current study are available from the corresponding author on reasonable request. The R script used during the current study is available from the corresponding author on reasonable request.

References

ALHO, Jussi S. *et al.* Increasing melanism along a latitudinal gradient in a widespread amphibian: local adaptation, ontogenic or environmental plasticity? **BMC Evolutionary Biology**, v. 10, n. 1, p. 317, 2010.

AMÉZQUITA, Adolfo; HÖDL, Walter. HOW, WHEN, AND WHERE TO PERFORM VISUAL DISPLAYS: THE CASE OF THE AMAZONIAN FROG HYLA PARVICEPS. **Herpetologica**, v. 60, n. 4, p. 420–429, 14 jul. 2006.

ANDRADE, Bianca O. *et al.* Classification of South Brazilian grasslands: Implications for conservation. **Applied Vegetation Science**, v. 22, n. 1, p. 168–184, jan. 2019.

ANDRADE, Bianca O. *et al.* 12,500+ and counting: biodiversity of the Brazilian Pampa. **Frontiers of Biogeography**, v. 15, n. 2, 6 jun. 2023.

ASSIS, Braulio A. *et al.* Plastic sexual ornaments: Assessing temperature effects on color metrics in a color-changing reptile. **PLOS ONE**, v. 15, n. 5, p. e0233221, 20 maio 2020.

BARNETT, James B. *et al.* Habitat disturbance alters color contrast and the detectability of cryptic and aposematic frogs. **Behavioral Ecology**, v. 32, n. 5, p. 814–825, 2021a.

BARNETT, James B. *et al.* Colour pattern variation forms local background matching camouflage in a leaf-mimicking toad. **Journal of Evolutionary Biology**, v. 34, n. 10, p. 1531–1540, out. 2021b.

BATES, Douglas *et al.* Fitting Linear Mixed-Effects Models Using lme4. **Journal of Statistical Software**, v. 67, n. 1, p. 1–48, 2015.

BRAGA, Adriano; LAURINI, Márcio. Spatial heterogeneity in climate change effects across Brazilian biomes. **Scientific Reports**, v. 14, n. 1, p. 16414, 16 jul. 2024a.

BRAGA, Adriano; LAURINI, Márcio. Spatial heterogeneity in climate change effects across Brazilian biomes. **Scientific Reports**, v. 14, n. 1, p. 16414, 16 jul. 2024b.

BRELSFORD, Craig C. *et al.* Understorey light quality affects leaf pigments and leaf phenology in different plant functional types. **Physiologia Plantarum**, v. 174, n. 3, p. e13723, maio 2022.

BURRACO, Pablo; ORIZAOLA, Germán. Ionizing radiation and melanism in Chornobyl tree frogs. **Evolutionary Applications**, v. 15, n. 9, p. 1469–1479, set. 2022.

CARLUCCI, Marcos Bergmann; MARCILIO-SILVA, Vinícius; TOREZAN, José Marcelo. The Southern Atlantic Forest: Use, Degradation, and Perspectives for Conservation. *In*: MARQUES, Marcia C. M.; GRELLE, Carlos E. V. (Orgs.). **The Atlantic Forest**. Cham: Springer International Publishing, 2021. p. 91–111.

COLOMBO, Patrick *et al.* Anura, Hylidae, Dendropsophus nahdereri (Lutz and Bokermann, 1963): distribution extension and new state record. **Check List**, v. 6, n. 3, p. 429, 1 ago. 2010.

CRESCITELLI, F. *et al.* **The Visual System in Vertebrates**. Berlin, Heidelberg: Springer Berlin Heidelberg, 1977.

CRONIN, Thomas *et al.* **Visual ecology**. Princeton (N.J.): Princeton university press, 2014.

DALRYMPLE, Rhiannon L. *et al.* Abiotic and biotic predictors of macroecological patterns in bird and butterfly coloration. **Ecological Monographs**, v. 88, n. 2, p. 204–224, 2018.

DE SOUZA, André R.; MAYORQUIN, Angie Z.; SARMIENTO, Carlos E. Paper wasps are darker at high elevation. **Journal of Thermal Biology**, v. 89, p. 102535, abr. 2020.

DELHEY, Kaspar *et al.* Evolutionary predictors of the specific colors of birds. **Proceedings of the National Academy of Sciences**, v. 120, n. 34, p. e2217692120, 22 ago. 2023.

DIAMOND, Judy; BOND, Alan B. **Concealing Coloration in Animals**. London: Belknap Press: An Imprint of Harvard University Press, 2013.

DUARTE, Leandro Da Silva *et al.* Phylobetadiversity among Forest Types in the Brazilian Atlantic Forest Complex. **PLoS ONE**, v. 9, n. 8, p. e105043, 14 ago. 2014.

DURÁN, Felipe; MÉNDEZ, Marco A.; CORREA, Claudio. The Atacama toad (Rhinella atacamensis) exhibits an unusual clinal pattern of decreasing body size towards more arid environments. **BMC Zoology**, v. 6, n. 1, dez. 2021.

ENDLER, John A. The Color of Light in Forests and It's Implications. **Ecological Monographs**, v. 63, n. 1, p. 1–27, 1993.

FERRÃO, Miquéias *et al.* New Species of Leaf-litter Toad of the Rhinella margaritifera Species Group (Anura: Bufonidae) from Amazonia. **Copeia**, v. 108, n. 4, 28 dez. 2020.

FROST, Darrel R. **Rhinella Fitzinger, 1826 | Amphibian Species of the World**. Disponível em: <https://amphibiansoftheworld.amnh.org/Amphibia/Anura/Bufonidae/Rhinella>. Acesso em: 24 jul. 2025.

GEHARA, Marcelo *et al.* High Levels of Diversity Uncovered in a Widespread Nominal Taxon: Continental Phylogeography of the Neotropical Tree Frog Dendropsophus minutus. **PLoS ONE**, v. 9, n. 9, p. e103958, 10 set. 2014.

GOMEZ, Doris *et al.* The role of nocturnal vision in mate choice: Females prefer conspicuous males in the European tree frog (Hyla arborea). **Proceedings of the Royal Society B: Biological Sciences**, v. 276, n. 1666, p. 2351–2358, 7 jul. 2009.

GRILLO, Oscar (ORG.). **The Dynamical Processes of Biodiversity - Case Studies of Evolution and Spatial Distribution**. *[S.l.]*: InTech, 2011.

GUMBERT, A.; KUNZE, J.; CHITTKA, L. Floral colour diversity in plant communities, bee colour space and a null model. **Proceedings of the Royal Society of London B: Biological Sciences**, v. 266, p. 1711–1716, 1999.

HADDAD, Célio F. B. (ORG.). **Guia dos anfíbios da Mata Atlântica: diversidade e biologia**. São Paulo: Anolis Books, 2013.

HÖDL, W.; AMEZQUITA, A. Visual signaling in anuran amphibians. *In*: RYAN, M. J. (Org.). **Anuran communication**. Washington: Smithsonian lnst. Press, 2001. p. 121–141.

IOP, Samanta. **Anfíbios anuros dos campos sulinos: espécies com ocorrência nas áreas campestres do Pampa e da Mata Atlântica**. *[S.l.]*: Ufrgs, 2016.

IUCN. Disponível em: <https://www.iucnredlist.org/en>. Acesso em: 24 jul. 2025.

KONERU, Manisha; CARO, Tim. Animal Coloration in the Anthropocene. **Frontiers in Ecology and Evolution**, v. 10, p. 857317, 22 abr. 2022.

KWET, A.; R, Lingnau; M, Di-Bernardo. **Pró-Mata: Anfíbios da Serra Gaúcha, sul do Brasil/Amphibien der Serra Gaúcha, Südbrasilien/Amphibiansofthe Serra Gaúcha, South of Brazil**. Porto Alegre: EDIPUCRS, 2010.

LAUMEIER, Ricarda *et al.* The global importance and interplay of colour-based protective and thermoregulatory functions in frogs. **Nature Communications**, v. 14, n. 1, p. 8117, 19 dez. 2023.

LIMA, Albertina P.; MENIN, Marcelo; DE ARAÚJO, Maria Carmozina. A new species of Rhinella (Anura: Bufonidae) from Brazilian Amazon. **Zootaxa**, v. 1663, n. 1, 17 dez. 2007.

LOPEZ, Vinicius Marques *et al.* Color lightness of velvet ants (Hymenoptera: Mutillidae) follows an environmental gradient. **Journal of Thermal Biology**, v. 100, p. 103030, ago. 2021.

MACK, Mariah; BEATY, Lynne. The influence of environmental and physiological factors on variation in American toad (Anaxyrus americanus) dorsal coloration. **Journal of Herpetology**, v. 55, n. 2, p. 119–126, 2021.

MAIA, Rafael *et al.* pavo 2: New tools for the spectral and spatial analysis of colour in r. **Methods in Ecology and Evolution**, v. 10, n. 7, p. 1–11, 2019.

MCELROY, Matthew T. Teasing apart crypsis and aposematism - evidence that disruptive coloration reduces predation on a noxious toad. **Biological Journal of the Linnean Society**, v. 117, n. 2, p. 285–294, fev. 2016.

MENÉNDEZ-GUERRERO, Pablo A.; DAVID M. GREEN; DAVIES, T. Jonathan. Climate change and the future restructuring of Neotropical anuran biodiversity. **Ecography**, v. 43, p. 222–235, 2020.

MORENO-RUEDA, Gregorio; COMAS, Mar. **Evolutionary Ecology of Amphibians**. 1. ed. Boca Raton: CRC Press, 2023.

NAVAS, Carlos A.; GOMES, Fernando R.; CARVALHO, José Eduardo. Thermal relationships and exercise physiology in anuran amphibians: Integration and evolutionary implications. **Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology**, v. 151, n. 3, p. 344–362, nov. 2008.

OVERBECK, Gerhard Ernst *et al.* (ORGS.). **South Brazilian Grasslands: Ecology and Conservation of the Campos Sulinos**. Cham: Springer International Publishing, 2024.

PARK, Chohee *et al.* Testing multiple hypotheses on the colour change of treefrogs in response to various external conditions. **Scientific Reports**, v. 13, n. 1, p. 4203, 14 mar. 2023.

PEREYRA, Martín O. *et al.* Evolution in the Genus Rhinella: A Total Evidence Phylogenetic Analysis of Neotropical True Toads (Anura: Bufonidae). **Bulletin of the American Museum of Natural History**, v. 447, n. 1, 31 mar. 2021.

PINKERT, Stefan; ZEUSS, Dirk. Thermal Biology: Melanin-Based Energy Harvesting across the Tree of Life. **Current Biology**, v. 28, n. 16, p. R887–R889, ago. 2018.

POSTEMA, Elizabeth G.; LIPPEY, Mia K.; ARMSTRONG-INGRAM, Tiernan. Color under pressure: how multiple factors shape defensive coloration. **Behavioral Ecology**, v. 34, n. 1, p. 1–13, 11 fev. 2023.

R CORE TEAM. **R: A language and environment for statistical computing**. Vienna, Austria: R Foundation for Statistical Computing, 2024.

RIES, C. *et al.* Turning blue and ultraviolet: Sex-specific colour change during the mating season in the Balkan moor frog. **Journal of Zoology**, v. 276, n. 3, p. 229–236, 2008.

ROBERTS, Sophie May; STUART-FOX, Devi; MEDINA, Iliana. The evolution of conspicuousness in frogs: When to signal toxicity? **Journal of Evolutionary Biology**, v. 35, n. 11, p. 1455–1464, nov. 2022.

ROBINSON, Christopher D.; GIFFORD, Matthew E. Covariation between Thermally Mediated Color and Performance Traits in a Lizard. **Physiological and Biochemical Zoology**, v. 91, n. 5, p. 1013–1025, set. 2018.

ROJAS, Bibiana. Behavioural, ecological, and evolutionary aspects of diversity in frog colour patterns. **Biological Reviews**, v. 92, n. 2, p. 1059–1080, 1 maio 2017.

ROJAS, Bibiana; LAWRENCE, J. P.; MÁRQUEZ, Roberto. Amphibian Coloration: Proximate Mechanisms, Function, and Evolution. *In*: MORENO-RUEDA, Gregorio; COMAS, Mar (Eds.). **Evolutionary Ecology of Amphibians**. 1. ed. Boca Raton: CRC Press, 2023. p. 219–258.

ROLAND, F. *et al.* Climate change in Brazil: perspective on the biogeochemistry of inland waters. **Brazilian Journal of Biology**, v. 72, n. 3 (suppl.), p. 709–722, 2012.

RUDH, Andreas; QVARNSTRÖM, Anna. Adaptive colouration in amphibians. **Seminars in Cell and Developmental Biology**, v. 24, n. 6–7, p. 553–561, 2013.

SAN-JOSE, Luis M.; ROULIN, Alexandre. Genomics of coloration in natural animal populations. **Philosophical Transactions of the Royal Society B: Biological Sciences**, v. 372, n. 1724, 2017.

SEÑARIS, J. Celsa *et al.* **Guía ilustrada de los anfibios del Parque Nacional Canaima, Venezuela**. Caracas, Venezuela: Ediciones IVIC, Instituto Venezolano de Investigaciones Científicas (IVIC), 2014.

SHAWKEY, Matthew D.; MOREHOUSE, Nathan I.; VUKUSIC, Peter. A protean palette: colour materials and mixing in birds and butterflies. **Journal of The Royal Society Interface**, v. 6, n. suppl_2, 6 abr. 2009.

SHELDON, B. C. *et al.* Sire coloration influences offspring survival under predation risk in the moorfrog. **Journal of Evolutionary Biology**, v. 16, n. 6, p. 1288–1295, 2003.

SOUSA, Tiago Rafael de; BENÍCIO, Ronildo Alves; FONSECA, Mariluce Gonçalves. POLIMORFISMO EM Rhinella diptycha (ANURA: BUFONIDAE) EM UMA ÁREA DE CAATINGA, ESTADO DO PIAUÍ, NORDESTE DO BRASIL. **Biosphere Comunicações Científicas**, v. 1, n. 2, p. 16–22, 2022.

SPANIOL, Ricardo Luís *et al.* Discolouring the Amazon Rainforest: how deforestation is affecting butterfly coloration. **Biodiversity and Conservation**, p. 1–18, 3 jun. 2020.

STANGEL, Judith *et al.* Ontogenetic Change of Signal Brightness in the Foot-Flagging Frog Species *Staurois parvus* and *Staurois guttatus*. **Herpetologica**, v. 71, n. 1, p. 1–7, mar. 2015.

STEGEN, James C.; GIENGER, C. M.; SUN, Lixing. The control of color change in the Pacific tree frog, *Hyla regilla*. **Canadian Journal of Zoology**, v. 82, n. 6, p. 889–896, 1 jun. 2004.

STEVENS, Martin *et al.* Using digital photography to study animal coloration. **Biological Journal of the Linnean Society**, v. 90, p. 211–237, 2007.

TATTERSALL, Glenn J.; ETEROVICK, Paula C.; DE ANDRADE, Denis V. Tribute to R. G. Boutilier: Skin colour and body temperature changes in basking *Bokermannohyla alvarengai* (Bokermann 1956). **Journal of Experimental Biology**, v. 209, n. 7, p. 1185–1196, 1 abr. 2006.

THÉRY, Marc. Forest light and its influence on habitat selection. **Plant Ecology**, v. 153, p. 251–261, 2001.

TOLEDO, L. F.; RIBEIRO, R. S.; HADDAD, C. F. B. Anurans as prey: An exploratory analysis and size relationships between predators and their prey. **Journal of Zoology**, v. 271, n. 2, p. 170–177, 2007.

TOLEDO, Luís Felipe; HADDAD, Célio F. B. Colors and Some Morphological Traits as Defensive Mechanisms in Anurans. **International Journal of Zoology**, v. 2009, p. 1–12, 12 mar. 2009.

TRACY, Christopher R.; CHRISTIAN, Keith A.; TRACY, C. Richard. Not just small, wet, and cold: effects of body size and skin resistance on thermoregulation and arboreality of frogs. **Ecology**, v. 91, n. 5, p. 1477–1484, 2010.

TROSCIANKO, Jolyon; STEVENS, Martin. Image calibration and analysis toolbox - a free software suite for objectively measuring reflectance, colour and pattern. **Methods in Ecology and Evolution**, v. 6, n. 11, p. 1320–1331, 2015.

VÁSQUEZ, Tatiana; PFENNIG, Karin S. Looking on the bright side: Females prefer coloration indicative of male size and condition in the sexually dichromatic spadefoot toad, Scaphiopus couchii. **Behavioral Ecology and Sociobiology**, v. 62, n. 1, p. 127–135, nov. 2007.

VAZ-SILVA, Wilian *et al.* Revealing Two New Species of the*Rhinella margaritifera*Species Group (Anura, Bufonidae): An Enigmatic Taxonomic Group of Neotropical Toads. **Herpetologica**, v. 71, n. 3, p. 212–222, set. 2015.

VENCES, Miguel *et al.* Field body temperatures and heating rates in a montane frog population: the importance of black dorsal pattern for thermoregulation. v. 39, p. 209–220, 2002.

VERDUM, Roberto *et al.* Pampa: The South Brazil. *In*: SALGADO, André Augusto Rodrigues; SANTOS, Leonardo José Cordeiro; PAISANI, Julio César (Orgs.). **The Physical Geography of Brazil**. Geography of the Physical Environment. Cham: Springer International Publishing, 2019. p. 7–20.

WEI, Shichao *et al.* The roles of climate, geography and natural selection as drivers of genetic and phenotypic differentiation in a widespread amphibian *Hyla annectans* (Anura: Hylidae). **Molecular Ecology**, v. 29, n. 19, p. 3667–3683, out. 2020.

ZAMORA-CAMACHO, Francisco Javier; COMAS, Mar. Greater reproductive investment, but shorter lifespan, in agrosystem than in natural-habitat toads. **PeerJ**, v. 5, p. e3791, 12 set. 2017.

ZAMORA-CAMACHO, Francisco Javier; COMAS, Mar. Beyond Sexual Dimorphism and Habitat Boundaries: Coloration Correlates with Morphology, Age, and Locomotor Performance in a Toad. **Evolutionary Biology**, v. 46, n. 1, p. 60–70, 2019.

ZHELEV, Zhivko; MOLLOV, Ivelin; TSONEV, Stefan. Body size and color polymorphism in Bufotes viridis complex (Anura: Bufonidae) inhabiting two semi-natural areas in Plovdiv City, Bulgaria. **North-Western Journal of Zoology**, v. 16, n. 2, p. 191–196, 2020.

CAPÍTULO 3

The frogs are more colorful on the other side: how habitat type affects the dorsal coloration of anuran communities

Luísa Nunes Lermen*; Daniel Loebmann

Programa de Pós-graduação em Ambientes Aquáticos Continentais. Laboratório de Vertebrados, Instituto de Ciências Biológicas, Universidade Federal do Rio Grande, Campus Carreiros, Av. Itália km 8, Rio Grande, RS, CEP 96203-900, Brazil

*Corresponding author. Email: lulis.lermen@hotmail.com. Telephone: +55 51 998731349.

Este manuscrito está submetido ao periódico Brazilian Journal of Biology

Abstract: Coloration plays essential ecological roles in anurans, contributing to camouflage, thermoregulation, and communication, and is closely influenced by environmental conditions. However, few studies have examined how coloration patterns vary at the community level across contrasting habitats. In this study, we investigated dorsal coloration in anuran communities from forest and grassland environments in southern Brazil. Using standardized in situ photography and colorimetric analysis, we measured brightness, hue, saturation, and color diversity to assess habitat-driven variation. Our results revealed that brightness, saturation, and hue did not differ significantly between habitats. However, color diversity was consistently higher in grassland communities compared to forests. Contrary to expectations of higher variation in complex vertical structured forests, open habitats showed greater color diversity, likely driven by stronger predation pressure and microhabitat heterogeneity, whereas forests may favor intermediate colorations under reduced predation and filtered light conditions. These findings demonstrate that grasslands, often undervalued in conservation planning, can sustain remarkable phenotypic variation in anuran communities.

Keywords: Amphibians, Animal coloration, Southern grasslands, Atlantic forest, Pampa

Os sapos são mais coloridos do outro lado: como o tipo de habitat afeta a coloração dorsal das comunidades de anuros

Resumo: A coloração desempenha papéis ecológicos essenciais em anuros, contribuindo para a camuflagem, a termorregulação e a comunicação, sendo fortemente influenciada pelas condições ambientais. No entanto, poucos estudos investigaram como os padrões de coloração variam em nível de comunidade entre habitats contrastantes. Neste estudo, investigamos a coloração dorsal em comunidades de anuros de florestas e campos no sul do Brasil. Utilizando fotografias padronizadas in situ e análises colorimétricas, medimos brilho, matiz, saturação e diversidade de cores para avaliar variações associadas ao habitat. Nossos resultados revelaram que brilho, saturação e matiz não diferiram significativamente entre habitats. No entanto, a diversidade de cores foi consistentemente maior em comunidades de campo em comparação com as florestas. Contrariando as expectativas de maior variação em florestas estruturalmente complexas, os ambientes abertos apresentaram maior diversidade de cores, provavelmente devido à maior pressão de predação e à heterogeneidade de micro-habitats, enquanto as florestas podem favorecer colorações intermediárias em função da menor predação e da filtragem da luz pelo dossel. Esses achados demonstram que os campos, muitas vezes subestimados no planejamento da conservação, podem sustentar uma notável variação fenotípica em comunidades de anuros.

Palavras-chave: Anfíbios, Coloração animal, Campos sulinos, Floresta Atlântica, Pampa

INTRODUCTION

Anurans display an extraordinary diversity of color, which serves critical ecological functions, including antipredator defense, thermoregulation, and communication (Rojas 2017). Since these functions are susceptible to environmental factors, it is no surprise that coloration can be influenced by climatic conditions and environmental characteristics (Zamora-Camacho and Comas 2019; Zhelev et al. 2020; Wei et al. 2020; Mack and Beaty 2021; Rojas et al. 2023). For example, *Bufotes viridis* (Laurenti, 1768) exhibit different coloration between habitats types, with darker morphotypes in shady environments (Zhelev et al. 2020). Likewise, *Anaxyrus americanus* (Holbrook, 1836) individuals in cooler environments tend to have darker dorsal coloration, enhancing heat absorption (Mack and Beaty 2021). Anurans also possess the ability to adjust their coloration to varying degrees (Rojas et al. 2023), allowing them to modify their appearance in response to both environmental conditions and behavioral contexts (Rojas 2017; Rojas et al. 2023). Such changes may improve background matching to different habitats or help regulate body temperature (Park *et al.* 2023). Hence, one can assume that selective pressures acting on coloration differ among environments.

Despite the recognized influence of habitat on anuran coloration, few studies have explored this phenomenon addressing multiple species in anuran communities, limiting our understanding of how diverse communities adapt to different habitats. In a community context, habitat characteristics can shape body coloration, as they influence how organisms perceive their surroundings (Endler 1992; Gomez and Théry 2004; Cheng et al. 2018). Research on bird and butterfly communities have shown that forests are related to darker, more saturated and more diverse colorations (Dalrymple et al. 2018; Spaniol et al. 2020). For instance, butterflies from preserved patches of the Amazon rainforest exhibit higher color diversity compared to those in more degraded habitats, likely due to the complexity of the forest environment and the need for crypsis and species-specific signaling (Spaniol et al. 2020). Similarly, birds in dense forests tend to display more saturated and darker colors, while species in open environments, such as grasslands, often evolve less saturated and brighter plumage to enhance social signaling (Dalrymple et al. 2018). In mammalian communities such as lagomorphs and rodents, coat coloration frequently matches the substrate color of their habitat, reflecting the role of camouflage as a defensive strategy (Stoner et al. 2003; Nokelainen et al. 2020). Studies of anuran communities have also shown that body coloration often converges phenotypically with habitat color (Norris and Lowe 1964; Lermen et al. 2025). Together, these patterns suggest that habitat structure may play a fundamental role in shaping coloration across taxa, including anuran communities.

Forests are complex environments characterized by high environmental heterogeneity (Duarte et al. 2014; Carlucci et al. 2021). This structural and microhabitat diversity can support a broad spectrum of defensive strategies, ranging from cryptic coloration for camouflage to conspicuous aposematic displays (Merilaita and Jormalainen 1999; Merilaita 2003; Spaniol et al. 2020), potentially fostering greater overall coloration diversity within forest-dwelling communities (Spaniol et al. 2020). In addition, the dim light conditions of closed-canopy forests may favor darker or desaturated colors that enhance crypsis (Endler 1993), while the dense canopy acts as a natural filter, altering the spectral composition of light that reaches the understory (Cronin et al. 2014; Brelsford et al. 2022). In contrast, in open habitats, such as grasslands, light can permeate the environment in a greater intensity and should promote brighter colors to aid in thermoregulation (Endler 1993; Rudh and Qvarnström 2013). These predictions align with observed patterns in *Epidalea calamita* (Laurenti, 1768), where individuals in open environments exhibit greater color saturation than their forest-dwelling counterparts (Zamora-Camacho and Comas 2019). Similarly, the fact that *Bufotes viridis* (Laurenti, 1768) individuals found in shaded environments tend to display darker coloration further support these predictions (Zhelev et al. 2020). This means that habitat disturbances, such as deforestation and climate change, can modify environmental conditions in ways that impact the adaptive value of color traits, such as by altering the effectiveness of camouflage and warning coloration in predator avoidance and survival (Barnett et al. 2021).

The southern Atlantic forest and Southern grasslands of South America provide an interesting system for investigating how environmental conditions shape anuran coloration. These two biomes present stark contrasts in vegetation structure, light availability, and thermal conditions (Carlucci et al. 2021; Overbeck et al. 2024), which may impose selective pressures on anuran communities. While the southern Atlantic Forest encompasses tropical dense, seasonal, and mixed forests that are highly biodiverse (Carlucci et al. 2021), the Southern grasslands consist of extensive open landscapes where trees are scarce and primarily restricted to riparian zones (Overbeck et al. 2024). However, both ecosystems face increasing threats due to agricultural expansion and land-use changes, which significantly alter their original environmental conditions (Carlucci et al. 2021; Overbeck et al. 2024).

Beyond ecological adaptation, identifying habitat-specific coloration patterns can aid conservation efforts, particularly for groups known to be vulnerable to habitat modification, such as many anuran species (Menéndez-Guerrero et al. 2020). Specialist species may lack the phenotypic plasticity to cope with habitat changes that alter the adaptive value of coloration traits, unlike generalist species (Spaniol et al. 2020; Barnett et al. 2021; Koneru and Caro 2022). Such changes can reduce the effectiveness of defensive coloration (Barnett et al. 2021) or impair ectotherms that rely on coloration for thermoregulation (Koneru and Caro 2022). In some cases, coloration can also be an asset for conservation, as conspicuous patterns may increase public willingness to protect a species (Prokop and Fančovičová 2013).

This study aims to investigate how anuran coloration varies between forests and grasslands by analyzing the hue, saturation, brightness, and color diversity of species inhabiting the forest and grasslands biomes of South America. We used in situ photography to obtain color measurements of multiple anuran species within the communities occupying these two contrasting environments. We hypothesized that anuran species in the grasslands would exhibit different body coloration compared to those in the forest. Based on previous studies of anuran populations (Zamora-Camacho and Comas 2019; Zhelev et al. 2020; Mack and Beaty 2021) and other organisms (Dalrymple et al. 2018; Spaniol et al. 2020), we expected anuran communities in the forest to display lower saturation, darker colors, and greater color diversity than their grassland-dwelling counterparts, while exhibiting no significant differences in overall hue at the community level due to to phenotypic convergence between body coloration and habitat background (Norris and Lowe 1964; Lermen et al. 2025) .

METHODS

Study area

This study was conducted in two distinct natural ecosystems of Rio Grande do Sul, southern Brazil: the southern Atlantic Forest and the Southern Grasslands. Fourteen water bodies were sampled, evenly distributed between forest (n = 7) and grassland (n = 7) habitats (Figure 1), with the aim of capturing the dominant coloration patterns within anuran communities in each habitat type. Sampling sites were at least 1,000 m apart to ensure spatial independence. Habitat classification followed MapBiomas land cover data, based on Landsat imagery (30 m resolution), and was verified using QGIS v.3.10.11 by generating 500 m buffers around each site to evaluate the dominant vegetation type in the surrounding area (Table 1). Sites were classified as forest or grassland when ≥75% of the buffer area consisted of the respective vegetation type. Forests included native remnants and planted stands, while grasslands encompassed native fields, temporary crops, or managed pastures. Wetlands and water bodies were excluded from cover calculations to avoid misinterpretation.

The Araucaria Forest, a major formation of the southern Atlantic Forest, is dominated by *Araucaria angustifolia* (Bertol.) O. Kuntze and occurs in subtropical highland plateaus with mild temperatures and evenly distributed rainfall (Grillo 2011; Carlucci et al. 2021). Despite lower tree species richness compared to other Atlantic Forest subtypes, it holds high phylogenetic diversity and evolutionary importance (Duarte et al. 2014). However, logging, agriculture, and exotic plantations have caused severe degradation (Carlucci et al. 2021). Sampling was conducted in four protected areas in southern Brazil: Floresta Nacional de São Francisco de Paula (F1), Centro de Pesquisas e Conservação da Natureza Pró-Mata (F3, F4), Floresta Nacional de Canela (F5, F6), and Floresta Nacional de Passo Fundo (F7).

The Atlantic Seasonal Alluvial Forest, a subtype of the Atlantic Forest, occurs along riverbanks and floodplains with fertile soils and is influenced by both tropical and subtropical climates, with low winter temperatures and reduced precipitation (Grillo 2011; Carlucci et al. 2021). Despite functioning as an ecological corridor, it is highly vulnerable to agriculture, deforestation, and hydrological alterations that threaten its biodiversity (Carlucci et al. 2021). Sampling was conducted at a single site in the Estação Ambiental Braskem (F2), a protected area within the "green belt" surrounding the Southern Petrochemical Complex of Rio Grande do Sul.

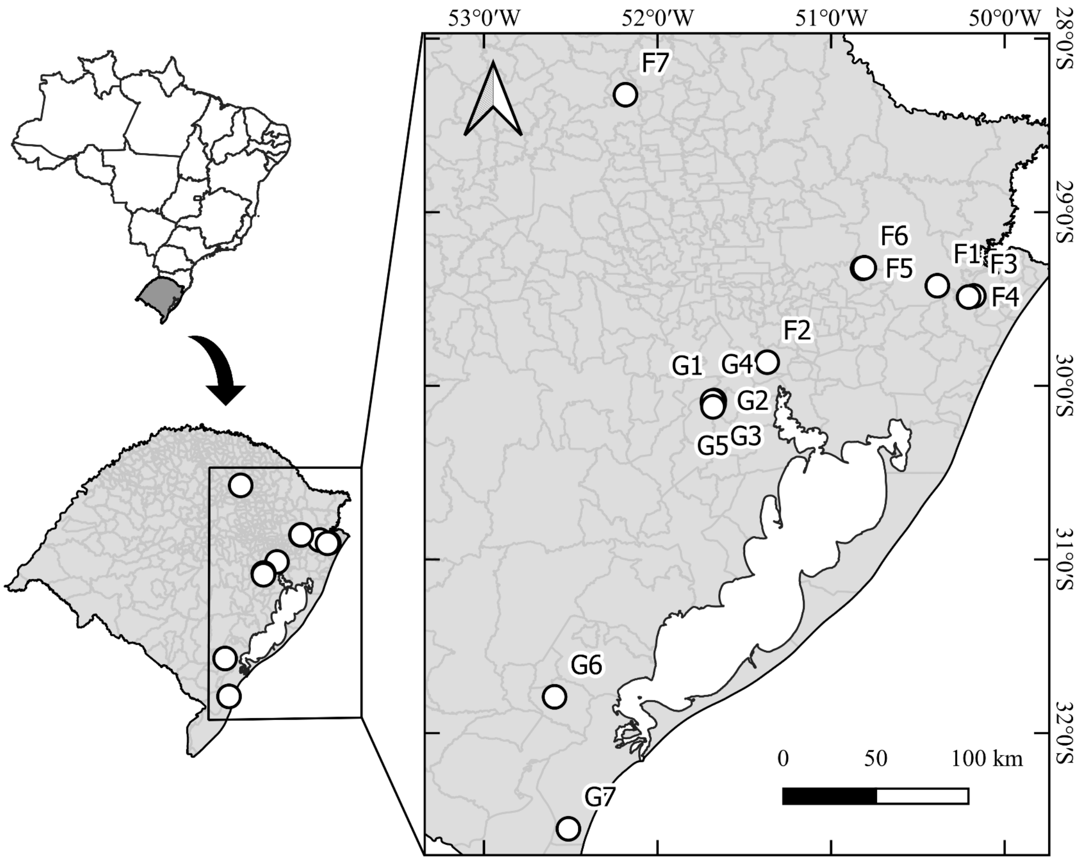

Figure 1 – Map illustrating the study locations. Top left: map of Brazil highlighting the state of Rio Grande do Sul. Bottom left: map of Rio Grande do Sul showing the study sites indicated by white dots. Right: enlarged view displaying the specific sampling locations each marked with a white dot. All sites are separated by a minimum distance of 1.000 meters.

The South Brazilian Grasslands are open ecosystems with sparse tree cover, mainly along riparian zones, and are shaped by long-term disturbance regimes such as fire and grazing (Overbeck et al. 2024). They share a subtropical climate with Araucaria forests but experience lower precipitation and summer hydric stress (Overbeck et al. 2024). Their biodiversity and productivity are sustained by the coexistence of C3 and C4 grasses adapted to seasonal variation (Verdum et al. 2019; Andrade et al. 2019; Overbeck et al. 2024). The biome faces threats from agricultural expansion, intensification, and habitat fragmentation (Verdum et al. 2019; Overbeck et al. 2024). Sampling was conducted at three locations: the Estação Experimental Agronômica of UFRGS (G1 to G5), a private property near Cerro das Almas in Capão do Leão (G6), and the protected area Estação Ecológica do Taim (G7).

Data collection

Data was collected between October 2022 and January 2025 (Table 1) during the warm months. Sampling sites were randomly selected based on the minimum distance required between the sampling points and detected vocal activity within the sampled area. Surveys were conducted at night during the reproductive period to ensure consistent environmental conditions, as coloration is ecologically relevant in both diurnal and nocturnal species (Gomez et al. 2009; Stangel et al. 2015; Zamora-Camacho and Comas 2019) we do not expect this decision to compromise the validity of our findings. Sampling effort was standardized across sites using species accumulation curves, with surveys continuing until new species were no longer detected. Distinct anuran vocalizations were also used to estimate species richness. Sessions lasted between two to five hours (Table 1). It is important to reiterate that this approach is intended to capture the dominant color patterns within the community, rather than provide a fully balanced representation of all species.

Table 1 – Study areas, corresponding percentages of vegetation cover used for habitat classification, sampling duration in each area, and the month in which sampling was conducted.

Study area	Geographic coordinates	Forest cover (%)	Grassland cover (%)	Sampling time (h:min)	Month/year of sampling
F1	S 29°25'13.28" W 50°23'13.28"	98.82	1.18	2:06	Dezember/2022
F2	S 29°51'48.4" W 51°22'03.2"	97.44	2.56	1:45	January/2023
F3	S 29°28'54.6" W 50°10'35.7"	86.19	13.81	3:15	November/2024
F4	S 29°29'17.8" W 50°12'25.3"	88.12	11.88	2:15	November/2024
F5	S 29°19'19.9" W 50°49'05.3"	94.35	5.65	2:20	December/2024
F6	S 29°19'19.9" W 50°48'30.9"	99.42	0.58	2:10	December/2024
F7	S 28°19'21.3" W 52°11'03.9"	98.29	1.71	2:55	January/2025
G1	S 30°5'43.1" W 51°40'59.5"	4.72	95.28	4:40	October/2022
G2	S 30°5'4.85" W 51°40'21.7"	18.83	81.17	3:16	October/2022
G3	S 30°06'10.9" W 51°40'25.1"	9.82	90.18	1:45	March/2023
G4	S 30°05'15.2" W 51°40'59.0"	7.49	92.51	2:49	October/2023
G5	S 30°07'14.6" W 51°40'46.6"	17.00	83.00	3:08	October/2023
G6	S 31°47'28.3" W 52°35'35.8"	20.33	79.67	2:05	December/2023
G7	S 32°33'06.1" W 52°30'48.3"	0	100	2:04	December/2023

Adult individuals were located through active search, hand-captured, measured, photographed, and kept temporarily in containers with local water and vegetation. To avoid resampling, individuals were released only after each sampling session was completed. All procedures followed ethical guidelines and were approved by the Ethics Committee of the Universidade Federal do Rio Grande (Approval No. P034/2022).

Color measurement

Photographs were taken in the field at night using a Canon EOS 1200D camera with an 18–55 mm lens and built-in flash as a standardized light source. Each individual was photographed with a scale bar and an 18% gray card, which allowed for corrections in lighting variations and the use of the built-in flash (Stevens et al. 2007; Spaniol et al. 2020). Including a grey standard with known reflectance in each photograph allows for accurate retrieval of reflectance values, even when lighting conditions are variable during image capture (Stevens et al. 2007). For each frog, ten dorsal RAW images were captured per individual under fixed aperture and flash white balance.

Images were processed in ImageJ with the micaToolbox v2.2.0 add-on for analysis. The micaToolbox screening tool was used to select three optimally exposed images per individual, ensuring they were as bright as possible while avoiding overexposure in the specific area of interest, namely the animal's dorsum. Selected images were calibrated based on the gray card to generate multispectral images (Troscianko and Stevens 2015). Color data from these images were averaged to obtain representative values for each individual.

Color data were extracted by defining a region of interest (ROI) on each frog's dorsum, carefully excluding areas with specular reflection on the skin. From the standardized images, reflectance values were obtained for the three camera color channels: red, green, and blue (RGB). These RGB values were then used to calculate three independent colorimetric variables each individual: hue (color type), saturation (color intensity), and brightness (overall reflectance) (Stevens et al. 2007; Ibraheem et al. 2012).

Data analysis

To extract colorimetric variables, we first standardized the RGB values and formatted them into a table suitable for processing with the *colspace* function from the PAVO package (v.2.9.0) (Maia et al. 2019) in R v.4.3.3 (R Core Team 2024). The RGB values were then reorganized based on short (B), medium (G), and long (R) wavelengths and plotted as XY coordinates in a triangular color space. This allowed us to derive the key colorimetric variables: hue and saturation. In this model, the achromatic point is at the center, with distance from the origin representing saturation (r) and the angle (θ) indicating hue. Brightness was computed as the mean reflectance across the RGB channels.

To calculate color diversity, we used the XY coordinates of all individuals of the same environment from the triangular color space to obtain the mean Euclidean distance of each individual to all others within the same environment, employing the *colsdist* function from the PAVO package (v.2.9.0). Consequently, each individual was assigned its own values for saturation, hue, brightness, and color diversity. The procedure was subsequently repeated for the second environment. In this framework, higher average distances indicate greater variation in coloration among individuals, reflecting higher color diversity within the community. For statistical analyses, all individuals from the seven forest sampling sites were grouped into a single category labeled as 'Forest'. The same procedure was applied to the seven grassland sampling sites, which were grouped under the 'Grassland' category. Thus, the analyses were conducted considering only two major vegetation cover types: forest and grassland.

Each variable (hue, saturation, brightness, and color diversity) was analyzed separately in relation to habitat type (forest or grassland) using Generalized Linear Mixed Models (GLMM) with a Gaussian distribution and an identity link function. We implemented four distinct models, each with a colorimetric variable as the response and the same fixed and random effects. The fixed part of each model consisted solely of a categorical predictor representing habitat type. To minimize bias, species identity was included as a random intercept to account for differences in abundance, while water body identity was included as a random intercept to control for site-specific effects on coloration. Data normality was assessed through Q-Q plots supported by the Akaike Information Criterion (AIC) as an additional diagnostic tool. Due to the skewed distribution of brightness and color diversity values, a logarithmic transformation was performed to improve data normality. We verified the assumption of homoscedasticity using residual plots. Statistical significance was assessed using the Satterthwaite method implemented in the *lmerTest* package. All statistical analyses were conducted using the lme4 package (Bates et al. 2015) in R v.4.3.3.

RESULTS

We sampled a total of 216 adult individuals representing 27 anuran species (Table 2), which corresponds to approximately 25.5% of the known species pool for the studied region (Colombo et al. 2010; Andrade et al. 2023). Of these, 113 individuals from 18 species were collected in forested areas, while 103 individuals representing 14 species were sampled in grassland habitats. Forest environments contained a greater number of unique species, with 13 recorded exclusively in these areas, while eight species were found only in grassland environments, and six species were shared between both habitat types.

Of the color variables analyzed in this study, only color diversity showed significant differences between forest and grassland environments (Table 3). Brightness (Figure 2A), saturation (Figure 2B), and hue (Figure 2C) did not differ between habitats. In contrast, color diversity was higher in grasslands (Figure 2D), reflecting a broader range of dorsal color variation among individuals in open environments (Figure 3).

Table 2 – Species identity and number of individuals photographed for dorsal coloration in each study area.

	Area													
Species	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**G1**	**G2**	**G3**	**G4**	**G5**	**G6**	**G7**
Aplastodiscus perviridis					2									
Aquarana catesbiana							*2*							
Boana bischoffi					*3*									
Boana faber	2	1	4	4	1		4							
Boana leptolineata			1	3										
Boana pulchella					2	1								
Dendropsophus microps				2										
Dendropsophus minutus	*2*		8	8	1	1	10	4	2			8	8	
Dendropsophus sanborni		7						3	1		7			3
Elachistocleis bicolor													2	
Leptodactylus gracilis					1	1								
Leptodactylus luctator	3		1				6			1		3	2	5
Leptodactylus plaumanni						*2*								
Odontophrynus maisuma														3
Ololygon aromothyella				*1*		*2*								
Physalaemus biligonigerus													2	
Physalaemus cuvieri												1		
Pseudis minuta										9		4	3	5
Pseudopaludicola falcipes													3	
Rhinella dorbignyi														1
Rhinella icterica	*3*		*4*		*3*									
Scinax fuscovarius			*3*	*1*		*3*			2		1	1		
Scinax granulatus			*2*	*3*		*1*							7	
Scinax nasicus													3	
Scinax perereca						*2*								

Scinax squalirostris		*1*	*1*				2	3		2			
Trachycephalus mesophaeus	*1*												

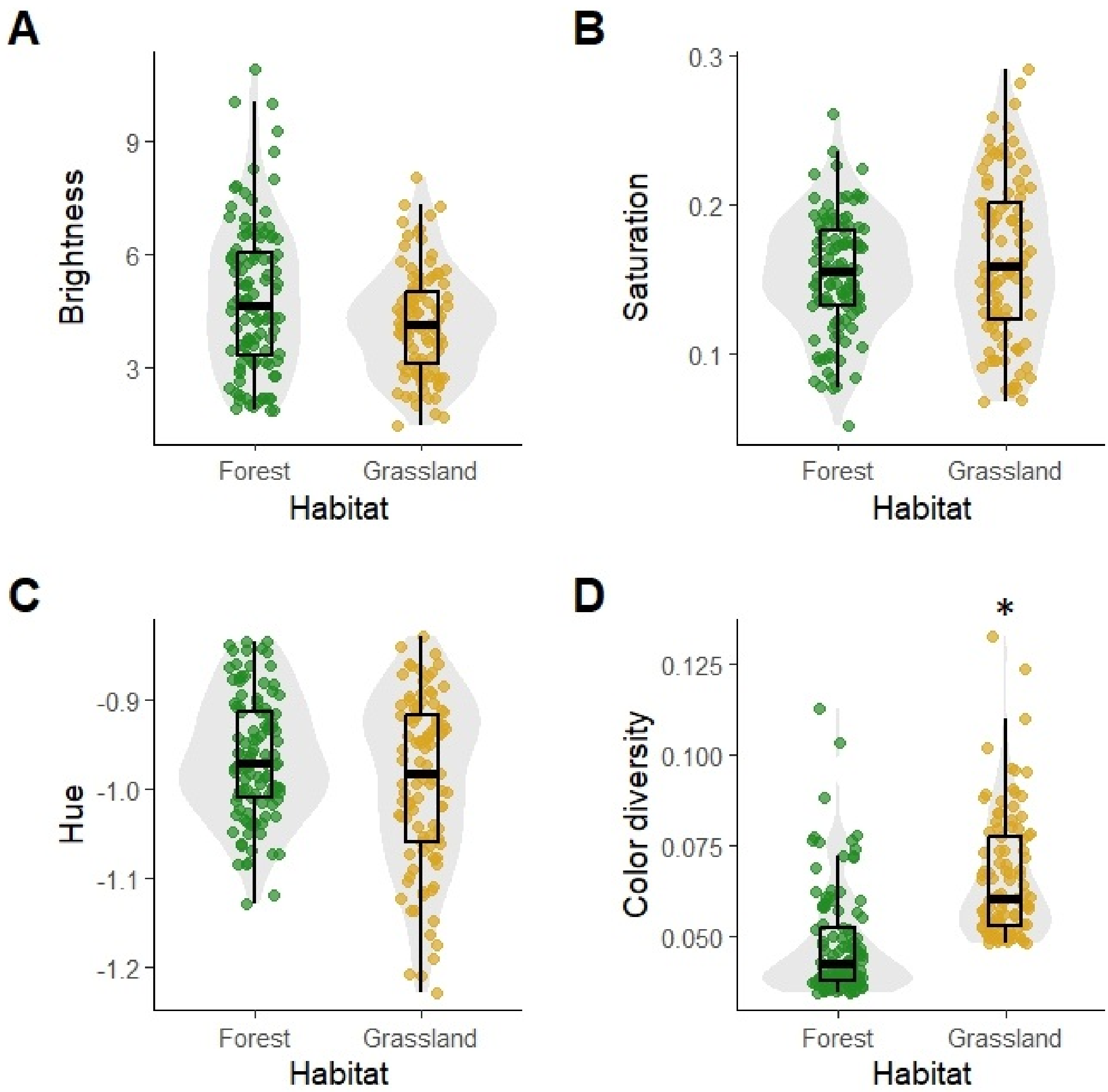

Figure 2 – Boxplots of raw values for anuran dorsal coloration variables across forest and grassland habitats: (A) Brightness, (B) Saturation, (C) Hue, and (D) Color diversity. Asterisks denote statistically significant differences between habitats ($p < 0.05$).

Table 3 – GLMM results showing difference in color variables between forest and grassland environments. Significance was obtained by the Satterthwaite method. Significant p-values for the forest environment means that the estimate value of the intercept is significantly different from zero, while significant p-values for grassland means its estimate value is significantly different than the intercept (forest).

	Factors	Estimate	Std. Error	P value	Variance Pseudo R^2
Brightness					
Fixed portion of the model	Forest (Intercept)	1.414	0.073	<0.001	0.028
	Grassland	-0.133	0.070	0.081	
Random portion of the model					0.527
Saturation					
Fixed portion of the model	Forest (Intercept)	0.132	0.011	<0.001	0.045
	Grassland	0.021	0.011	0.068	
Random portion of the model					0.691
Hue					
Fixed portion of the model	Forest (Intercept)	-0.981	0.014	<0.001	0.016
	Grassland	-0.018	0.013	0.195	
Random portion of the model					0.634
Color diversity					
Fixed portion of the model	Forest (Intercept)	-3.066	0.033	<0.001	0.337
	Grassland	0.352	0.036	<0.001	
Random portion of the model					0.119

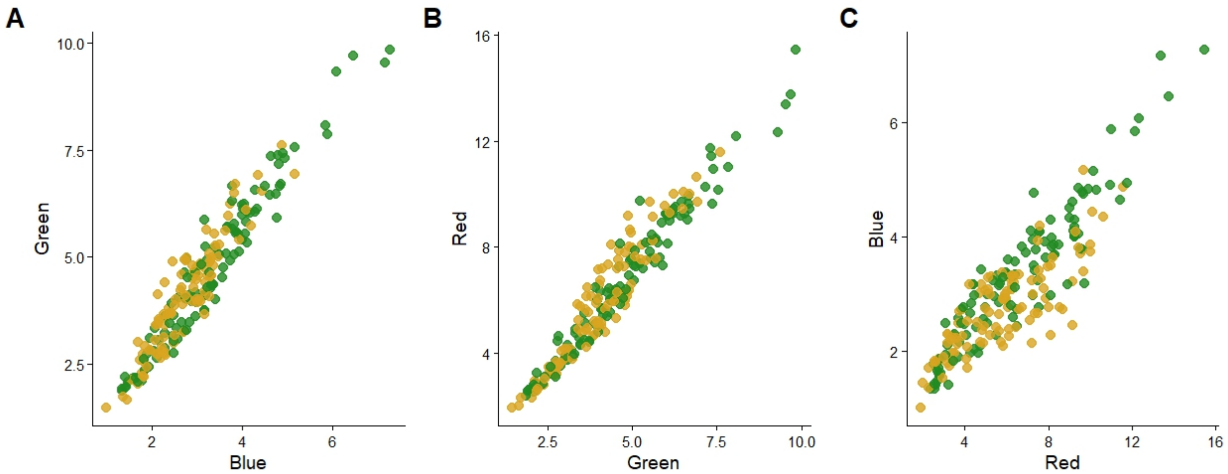

Figure 3 – Relationships among red, green, and blue (RGB) reflectance values of anuran dorsal coloration. Each point represents the mean RGB values obtained from standardized photographs, with green points representing forest habitats and yellow points representing grassland habitats. Panels show pairwise relationships between color channels: (A) Green vs. Blue, (B) Red vs. Green, and (C) Blue vs. Red. Lower values correspond to darker coloration, whereas higher values indicate lighter coloration, illustrating how dorsal color components covary across habitats.

DISCUSSION

While we anticipated that grassland-dwelling species would display distinct dorsal coloration when compared to their forest counterparts, only color diversity differed significantly between habitats, whereas the other color variables showed no variation. Since our color diversity values are derived from a trichromatic color space, from which hue and saturation are obtained, even though individually hue and saturation did not differ significantly between habitats, when considered together the resulting variation was sufficient to influence the color diversity metric of the environments. Notably, species in forest habitats exhibited lower color diversity, which contrasts with the predicted pattern of diverse colorations in these shaded and more heterogeneous environments (Duarte et al. 2014; Carlucci et al. 2021).

Given that forest environments, with their complex, patchy lighting and heterogeneous backgrounds, are often expected to support higher color diversity at the community level, as documented in birds and butterflies (Dalrymple et al. 2018; Spaniol et al. 2020; Delhey et al. 2023), our results revealed an opposite trend. Color diversity was actually higher in grassland areas communities compared to forest habitats and, notably, habitat type accounted for a substantial proportion of the variance observed in color diversity. This pattern could, in part, be explained by the species pool available in each environment. The Araucaria forests, while part of the broader Atlantic Forest biome (Carlucci et al. 2021), host a smaller subset of its overall species richness and consequently color variation potential (Kwet et al. 2010; Haddad 2013), which might limit the dorsal color diversity expressed at the community level. However, it is interesting to note that in our study a greater total number of species, as well as a greater number of exclusive species, were recorded in forests. One might therefore expect more color variety in this environment, but this was not the case.

One possible explanation for the difference in coloration observed between forest and grassland-dwelling anurans relates to the distinctive environment beneath the forest canopy. The canopy in forested areas acts as a filter for incoming light, altering its composition before it reaches the understory, making it less saturated and shifted toward greener wavelengths (Endler 1993; Théry 2001; Cronin et al. 2014; Brelsford et al. 2022). Considering the importance of cryptic coloration as a defense strategy in anurans, given their vulnerability to a wide range of vertebrate and invertebrate predators (Toledo et al. 2007; Rojas 2017), it is plausible that the light environment of forest understories favors individuals with greener and less saturated dorsal coloration, thereby enhancing crypsis in these habitats and constraining color diversity (Endler 1993; Théry 2001).

Predation pressure may help explain why color diversity was higher in grassland communities than in forests, since open habitats such as grasslands can pose a higher predation risk than forests (Seymoure et al. 2018; Goßmann et al. 2023). Optimal camouflage coloration arises from balancing the relative probability of occupying a microhabitat with the likelihood of encountering a predator there (Merilaita and Jormalainen 1999). Additionally, color diversity in butterfly communities has been shown to increase with a higher number of bird species, potential predators of butterflies (Dalrymple et al. 2018), suggesting that greater predation pressure can drive higher variability in coloration. Thus, the higher predation risk in open habitats may favor individuals whose coloration more closely matches the different microhabitats available, thereby increasing overall color variation within the community (Merilaita and Jormalainen 1999; Merilaita 2003). In contrast, anurans inhabiting forested environments may rely on more intermediate dorsal colorations that provide sufficient camouflage across multiple microhabitats without requiring high variability (Hughes et al. 2019), especially considering that most anuran species in the region remain primarily in the understory rather than exploiting the full vertical structure of forests (Kwet et al. 2010; Iop 2016). These results highlight that, although grasslands may lack the complex vertical stratification of multi-layered environments (Overbeck et al. 2024), they are still capable, particularly when naturally occurring, of supporting a wide range of colorations.

Moreover, anurans may exhibit striking coloration on body parts that remain concealed while at rest, which can be displayed in contexts of intraspecific communication or as a defense strategy (Rojas 2017; Rojas et al. 2023). It is important to consider that while overall community color diversity was lower in forest areas, individual anurans in these habitats may exhibit greater within-individual color variability. This phenomenon, observed in other taxa such as birds and butterflies (Dalrymple et al. 2018; Spaniol et al. 2020), could similarly occur in anurans. Although this study did not assess within-individual color diversity, this possibility merits further investigation. In any case, although forests may promote greater number of colors within-individual, this does not necessarily result in a broader range of dorsal coloration at the community level.

Although the restricted sampling period at each site may have limited the detection of certain species within the local anuran communities, particularly rare or low-density species, this is unlikely to have substantially influenced the overall trends observed. It is important to highlight that the present study sampled approximately 25% of all anuran species reported for the region (Colombo et al. 2010; Andrade et al. 2023), which represents a considerable portion of the local species pool and reinforces the reliability of the general patterns detected. Furthermore, Gumbert et al. (1999), in a study on flower coloration in plant communities from the perspective of pollinators, found that although rare species occasionally exhibited distinct color traits compared to sympatric species, the overall coloration pattern of the community did not differ from what would be expected at random. Taken together, the substantial representativeness of the species sampled in our study and the pattern observed by Gumbert et al. (1999) suggest that the absence of rare species is unlikely to affect the general trends in coloration patterns within these anuran communities, although these less frequent species might occasionally deviate from the dominant patterns.

In conclusion, our study shows that while most coloration variables did not differ significantly between habitats, color diversity was consistently higher in grassland communities than in forests. This pattern likely reflects the combined influence of habitat structure, species pools, and ecological pressures such as predation risk, which together shape community-level patterns of anuran coloration. Our results emphasize that grasslands, often undervalued in conservation, can harbor remarkable variation in phenotypic traits, underscoring their ecological importance. Future research incorporating broader spatial and temporal sampling, assessments of intra-individual color variation, and experimental manipulations of light environments will be essential to clarify the ecological and evolutionary mechanisms driving color diversity in anurans and to better predict how these processes may respond to ongoing habitat change.

Acknowledgement

We thank the Programa de Pós-Graduação em Ambientes Aquáticos Continentais at the Universidade Federal do Rio Grande for providing support during this study. We are also grateful to the Braskem S.A. for granting permission to conduct fieldwork in the Estação Ambiental Braskem. DL received a research fellowship from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, grant number 307232/2023-9). LL was supported by a scholarship from the Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). ScholarGPT (version GPT-4-turbo) was used to assist the authors in expressing content in a non-native language, reviewing grammar, and enhancing the clarity and flow of the text.

Conflicts of interest: The authors declare that they have no conflict of interest.

Data Availability: The datasets used and analysed during the current study are available from the corresponding author on reasonable request. The R script used during the current study is available from the corresponding author on reasonable request.

References

ANDRADE, B.O., BONILHA, C.L., OVERBECK, G.E., VÉLEZ-MARTIN, E., ROLIM, R.G., BORDIGNON, S.A.L., SCHNEIDER, A.A., VOGEL ELY, C., LUCAS, D.B., GARCIA, E.N., DOS SANTOS, E.D., TORCHELSEN, F.P., VIEIRA, M.S., SILVA FILHO, P.J.S., FERREIRA, P.M.A., TREVISAN, R., HOLLAS, R., CAMPESTRINI, S., PILLAR, V.D. and BOLDRINI, I.I., 2019. Classification of South Brazilian grasslands: Implications for conservation. *Applied Vegetation Science*, vol. 22, no. 1, pp. 168–184. DOI 10.1111/avsc.12413

ANDRADE, B.O., DRÖSE, W., AGUIAR, C.A.D., AIRES, E.T., ALVARES, D.J., BARBIERI, R.L., CARVALHO, C.J.B.D., BARTZ, M., BECKER, F.G., BENCKE, G.A.,

BENEDUZI, A., SILVA, J.B., BLOCHTEIN, B., BOLDRINI, I.I., BOLL, P.K., BORDIN, J., SILVEIRA, R.M.B.D., MARTINS, M.B., BOSENBECKER, C., BRACCINI, J., BRAUN, B., BRITO, R., BROWN, G.G., BÜNEKER, H.M., BUZATTO, C.R., CAVALLERI, A., CECHIN, S.Z., COLOMBO, P., CONSTANTINO, R., COSTA, C.F.D., DALZOCHIO, M.S., OLIVEIRA, M.G.D., DIAS, R.A., SANTOS, L.A.D., DUARTE, A.D.F., DUARTE, J.L.P, DURIGON, J., DA SILVA, M.E., FERREIRA, P.P.A., FERREIRA, T., FERRER, J., FERRO, V.G., FONTANA, C.S., FREIRE, M.D., FREITAS, T.R.O., GALIANO, D., GARCIA, M., DOS SANTOS, T.G., GOMES, L.R. P., GONZATTI, F., GOTTSCHALK, M.S., GRACIOLLI, G., GRANADA, C.E., GRINGS, M., GUIMARÃES, P.S., HEYDRICH, I., IOP, S., JARENKOW, J.A., JUNGBLUTH, P., KÄFFER, M.I., KAMINSKI, L.A., KENNE, D.C., KIRST, F.D., KROLOW, T.K., KRÜGER, R.F., KUBIAK, B.B., LEAL-ZANCHET, A.M., LOEBMANN, D., LUCAS, D.B., LUCAS, E.M., LUZA, A.L., MACHADO, I.F., MADALOZZO, B., MAESTRI, R., MALABARBA, L.R., MANEYRO, R., MARINHO, M.A.T., MARQUES, R., MARTA, K.D.S., MARTINS, D.D.S., MARTINS, G.D.S., MARTINS, T.R., MELLO, A.S.D., MELLO, R.L., MENDONÇA JUNIOR, M.D.S., MORAIS, A.B.B.D., MOREIRA, F.F.F., MOREIRA, L.F.B., MOURA, L.D.A., NERVO, M.H., OTT, R., PALUDO, P., PASSAGLIA, L.M.P., PÉRICO, E., PETZHOLD, E.S., PIRES, M.M., POPPE, J.L., QUINTELA, F.M., RAGUSE-QUADROS, M., PEREIRA, M.J.R, RENNER, S., RIBEIRO, F.B., RIBEIRO, J.R.I., RODRIGUES, E.N.L., RODRIGUES, P.E.S., ROMANOWSKI, H.P., RUSCHEL, T.P., SACCOL, S.D.S.A., SAVARIS, M., SILVEIRA, F.S., SCHMITZ, H.J., SIEGLOCH, A.E., SIEWERT, R.R., SILVA FILHO, P.J.S.D., SOARES, A.G., SOMAVILLA, A., SPEROTTO, P., SPIES, M.R., TIRELLI, F.P., TOZETTI, A.M., VERRASTRO, L., VOGEL ELY, C., DA SILVA, A.Z., ZANK, C., ZEFA, E. and OVERBECK, G.E., 2023. 12,500+ and counting: biodiversity of the Brazilian Pampa. *Frontiers of Biogeography*, vol. 15, no. 2, pp. 1-14. DOI 10.21425/F5FBG59288

BARNETT, J.B., VARELA, B.J., JENNINGS, B.J., LESBARRÈRES, D., PRUITT, J. N. and GREEN, D. M., 2021. Habitat disturbance alters color contrast and the detectability of cryptic and aposematic frogs. *Behavioral Ecology*, vol. 32, no. 5, pp. 814–825. DOI 10.1093/beheco/arab032

BATES, D., MÄCHLER, M., BOLKER, B. and WALKER, S., 2015. Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software,* vol. 67, no. 1, pp. 1–48. DOI 10.18637/jss.v067.i01

BRELSFORD, C.C., TRASSER, M., PARIS, T., HARTIKAINEN, S.M. and ROBSON, T.M., 2022. Understorey light quality affects leaf pigments and leaf phenology in different plant functional types. *Physiologia Plantarum*, vol. 174, no. 3, pp. e13723. DOI 10.1111/ppl.13723 PMid: 35606930

CARLUCCI, M.B., MARCILIO-SILVA, V. and TOREZAN, J.M., 2021. The Southern Atlantic Forest: Use, Degradation, and Perspectives for Conservation. In: M.C.M. MARQUES and C.E.V. GRELLE eds. *The Atlantic Forest*. Cham: Springer International Publishing. pp. 91–111.

CHENG, W., XING, S., CHEN, Y., LIN, R., BONEBRAKE, T.C. and NAKAMURA, A., 2018. Dark butterflies camouflaged from predation in dark tropical forest understories. *Ecological Entomology*, vol. 43, no. 3, pp. 304–309. DOI 10.1111/een.12499

COLOMBO, P., ZANK, C., BÜHLER, D., SCHOSSLER, M., BALESTRIN, R.L., SANTOS, R.R., LEONARDI, S.B., BORGES-MARTINS, M. and VERRASTRO, L., 2010. Anura, Hylidae, Dendropsophus nahdereri (Lutz and Bokermann, 1963): distribution extension and new state record. *Check List*, vol. 6, no. 3, pp. 429-431. DOI 10.15560/6.3.429

CRONIN, T., JOHNSEN, S., MARSHALL, J. and WARRANT, E.J., 2014. *Visual ecology*. Princeton: Princeton university press. 432 p.

DALRYMPLE, R.L., FLORES-MORENO, H., KEMP, D.J., WHITE, T.E., LAFFAN, S.W., HEMMINGS, F.A., HITCHCOCK, T.D. and MOLES, A.T., 2018. Abiotic and biotic predictors of macroecological patterns in bird and butterfly coloration. *Ecological Monographs,* vol. 88, no. 2, pp. 204–224. DOI 10.1002/ecm.1287

DELHEY, K., VALCU, M., MUCK, C., DALE, J. and KEMPENAERS, B., 2023. Evolutionary predictors of the specific colors of birds. *Proceedings of the National Academy of Sciences*, vol. 120, no. 34, pp. e2217692120. DOI 10.1073/pnas.2217692120 PMid: 37579151

DUARTE, L.D.S., BERGAMIN, R.S., MARCILIO-SILVA, V., SEGER, G.D.D.S. and MARQUES, M.C.M., 2014. Phylobetadiversity among Forest Types in the Brazilian Atlantic Forest Complex. *PLoS ONE*, vol. 9, no. 8, pp. e105043. DOI 10.1371/journal.pone.0105043 PMid: 25121495

ENDLER, J.A, 1992. Signals, Signal Conditions, and the Direction of Evolution. *The American Naturalist*, vol. 139, pp. S125–S153.

ENDLER, J.A, 1993. The Color of Light in Forests and It's Implications. *Ecological Monographs*, vol. 63, no. 1, pp. 1–27.

GOMEZ, D., RICHARDSON, C., LENGAGNE, T., PLENET, S., JOLY, P., LÉNA, J.P. and THÉRY, M., 2009. The role of nocturnal vision in mate choice: Females prefer conspicuous males in the European tree frog (Hyla arborea). *Proceedings of the Royal Society B: Biological Sciences*, vol. 276, no. 1666, pp. 2351–2358. DOI 10.1098/rspb.2009.0168. PMid: 19324736

GOMEZ, D. and THÉRY, M., 2004. Influence of ambient light on the evolution of colour signals: Comparative analysis of a Neotropical rainforest bird community. *Ecology Letters*, vol. 7, no. 4, pp. 279–284. DOI 10.1111/j.1461-0248.2004.00584.x

GOSSMANN, A., AMBROŽOVÁ, L, CIZEK, L., DRAG, L., GEORGIEV, K., NEUDAM, L., PERLÍK, M., SEIDEL, D. and THORN, S., 2023. Habitat openness and predator abundance determine predation risk of warningly colored longhorn beetles (Cerambycidae) in temperate forest. *Journal of Insect Science*, vol. 23, no. 2. DOI 10.1093/jisesa/iead027

GRILLO, O. (ed), 2011. *The Dynamical Processes of Biodiversity - Case Studies of Evolution and Spatial Distribution*. Online. InTech.

GUMBERT, A, KUNZE, J and CHITTKA, L, 1999. Floral colour diversity in plant communities, bee colour space and a null model. *Proceedings of the Royal Society of London B: Biological Sciences,* vol. 266, p. 1711–1716.

HADDAD, C.F.B. (ed.), 2013. *Guia dos anfíbios da Mata Atlântica: diversidade e biologia*. São Paulo: Anolis Books.

HUGHES, A., LIGGINS, E. and STEVENS, M., 2019. Imperfect camouflage: How to hide in a variable world? *Proceedings of the Royal Society B: Biological Sciences*, vol. 286, no. 1902. DOI 10.1098/rspb.2019.0646 PMid: 31088268

IBRAHEEM, N.A, HASAN, M.M, KHAN, R.Z. and MISHRA, P.K., 2012. Understanding Color Models: A Review. *ARPN Journal of Science and Technology*, vol. 2, no. 3, pp. 265-275.

IOP, S., 2016. *Anfíbios anuros dos campos sulinos: espécies com ocorrência nas áreas campestres do Pampa e da Mata Atlântica*. Porto Alegre: Rede Campos Sulinos: Ufrgs. 22 p.

KONERU, M. and CARO, T., 2022. Animal Coloration in the Anthropocene. *Frontiers in Ecology and Evolution,* vol. 10, p. 857317. DOI 10.3389/fevo.2022.857317

KWET, A., LINGNAU, R., and DI-BERNARDO, M., 2010. *Pró-Mata: Anfíbios da Serra Gaúcha, sul do Brasil/Amphibien der Serra Gaúcha, Südbrasilien/Amphibiansofthe Serra Gaúcha, South of Brazil*. Porto Alegre: EDIPUCRS. 148 p.

LERMEN, L., FURTADO, R. and HARTZ, S.M., 2025. To be or not be seen: the influence of substrate on anuran community coloration. *Journal of Natural History*, vol. 59, no. 17–20, pp. 1249–1265. DOI 10.1080/00222933.2025.2480733

MAIA, R., GRUSON, H., ENDLER, J.A. and WHITE, T.E., 2019. pavo 2: New tools for the spectral and spatial analysis of colour in r. *Methods in Ecology and Evolution*, vol. 10, no. 7, pp. 1–11. DOI 10.1111/2041-210X.13174

MENÉNDEZ-GUERRERO, P.A., GREEN, D.M., and DAVIES, T.J, 2020. Climate change and the future restructuring of Neotropical anuran biodiversity. *Ecography*, vol. 43, pp. 222–235. DOI 10.1111/ecog.04510

MERILAITA, S., 2003. Visual background complexity facilitates the evolution of camouflage. *Evolution*, vol. 57, no. 6, pp. 1248–1254. DOI 10.1111/j.0014-3820.2003.tb00333.x PMid: 12894933

MERILAITA, S. and JORMALAINEN, V., 1999. Optimization of cryptic coloration in heterogeneous habitats. *Biological Journal of the Linnean Society*, vol. 67, pp. 151–161. DOI 10.1006/bijl.1998.0298

NOKELAINEN, O., BRITO, J.C., SCOTT-SAMUEL, N.E., VALKONEN, J.K. and BORATYŃSKI, Z., 2020. Camouflage accuracy in Sahara–Sahel desert rodents. PLAISTOW. *Journal of Animal Ecology*, vol. 89, no. 7, pp. 1658–1669. DOI 10.1111/1365-2656.13225 PMid: 32227336

NORRIS, K.S. and LOWE, C.H., 1964. An Analysis of Background Color-Matching in Amphibians and Reptiles. *Ecology*, vol. 45, no. 3, pp. 565–580. DOI 10.2307/1936109

OVERBECK, G.E., PILLAR, V.D.P., MÜLLER, S.C. and BENCKE, G.A. (eds.), 2024. *South Brazilian Grasslands: Ecology and Conservation of the Campos Sulinos*. Online. Cham: Springer International Publishing. 555 p.

PROKOP, P. and FANČOVIČOVÁ, J., 2013. Does colour matter? The influence of animal warning coloration on human emotions and willingness to protect them. *Animal Conservation*, vol. 16, no. 4, pp. 458–466. DOI 10.1111/acv.12014

R CORE TEAM, 2024. *R: A language and environment for statistical computing*. Online. Vienna, Austria: R Foundation for Statistical Computing. Available from: https://www.r-project.org/

ROJAS, B., 2017. Behavioural, ecological, and evolutionary aspects of diversity in frog colour patterns. *Biological Reviews,* vol. 92, no. 2, pp. 1059–1080. DOI 10.1111/brv.12269 PMid: 27020467

ROJAS, B., LAWRENCE, J.P. and MÁRQUEZ, R., 2023. Amphibian Coloration: Proximate Mechanisms, Function, and Evolution. In: MORENO-RUEDA, G. and COMAS, M. (eds), *Evolutionary Ecology of Amphibians*. Online. Boca Raton: CRC Press. pp. 219–258.

RUDH, A. and QVARNSTRÖM, A., 2013. Adaptive colouration in amphibians. *Seminars in Cell and Developmental Biology*, vol. 24, no. 6–7, pp. 553–561. DOI 10.1016/j.semcdb.2013.05.004 PMid: 23664831

SEYMOURE, B.M., RAYMUNDO, A., MCGRAW, K.J., MCMILLAN, W. O. and RUTOWSKI, R.L., 2018. Environment-dependent attack rates of cryptic and aposematic butterflies. *Current Zoology*, vol. 64, no. 5, pp. 663–669. DOI 10.1093/cz/zox062 PMid: 30323845

SPANIOL, R.L., MENDONÇA, M.S., HARTZ, S.M., ISERHARD, C.A. and STEVENS, M., 2020. Discolouring the Amazon Rainforest: how deforestation is affecting butterfly coloration. *Biodiversity and Conservation*, vol. 29, pp. 2821–2838. DOI 10.1007/s10531-020-01999-3

STANGEL, J., PREININGER, D., SZTATECSNY, M. and HÖDL, W., 2015. Ontogenetic Change of Signal Brightness in the Foot-Flagging Frog Species *Staurois parvus* and *Staurois guttatus*. *Herpetologica*, vol. 71, no. 1, pp. 1–7. DOI 10.1655/HERPETOLOGICA-D-14-00014 PMid: 25983337

STEVENS, M., PÁRRAGA, C.A., CUTHILL, I.C, PARTRIDGE, J.C. and TROSCIANKO, T.S., 2007. Using digital photography to study animal coloration. *Biological Journal of the Linnean Society,* vol. 90, p. 211–237. DOI 10.1111/j.1095-8312.2007.00725.x

STONER, C.J., BININDA-EMONDS, O.R.P. and CARO, T., 2003. The adaptive significance of coloration in lagomorphs. *Biological Journal of the Linnean Society*, vol. 79, pp. 309–328. DOI 10.1046/j.1095-8312.2003.00190.x

THÉRY, M., 2001. Forest light and its influence on habitat selection. *Plant Ecology,* vol. 153, pp. 251–261.

TOLEDO, L.F., RIBEIRO, R.S. and HADDAD, C.F.B., 2007. Anurans as prey: An exploratory analysis and size relationships between predators and their prey. *Journal of Zoology*, vol. 271, no. 2, p. 170–177. DOI 10.1111/j.1469-7998.2006.00195.x

TROSCIANKO, J. and STEVENS, M., 2015. Image calibration and analysis toolbox - a free software suite for objectively measuring reflectance, colour and pattern. *Methods in Ecology and Evolution,* vol. 6, no. 11, p. 1320–1331. DOI 10.1111/2041-210X.12439 PMid: 27076902

VERDUM, R., VIEIRA, L.D.F.D.S., CANEPPELE, J.C.G. and GASS, S.L.B., 2019. Pampa: The South Brazil. In: A.A.R. SALGADO, L.J.C. SANTOS, and J.C. PAISANI (eds.), *The Physical Geography of Brazil*. Online. Cham: Springer International Publishing. p. 7–20.

WEI, S., LI, Z., MOMIGLIANO, P., FU, C., WU, H. and MERILÄ, J., 2020. The roles of climate, geography and natural selection as drivers of genetic and phenotypic differentiation in a widespread amphibian *Hyla annectans* (Anura: Hylidae). *Molecular Ecology*, vol. 29, no. 19, p. 3667–3683. DOI 10.1111/mec.15584 PMid: 32762086

ZAMORA-CAMACHO, F.J. and COMAS, M., 2019. Beyond Sexual Dimorphism and Habitat Boundaries: Coloration Correlates with Morphology, Age, and Locomotor Performance in a Toad. *Evolutionary Biology*, vol. 46, no. 1, pp. 60–70. DOI 10.1007/s11692-018-9466-7

ZHELEV, Z., MOLLOV, I. and TSONEV, S., 2020. Body size and color polymorphism in Bufotes viridis complex (Anura: Bufonidae) inhabiting two semi-natural areas in Plovdiv City, Bulgaria. North-Western Journal of Zoology, vol. 16, no. 2, pp. 191–196.

CAPÍTULO 4

Sunny grasslands and shady forests: How habitat type and temperature shape the evolution of dorsal coloration in the genus *Rhinella*

Luísa Nunes Lermen*; Daniel Loebmann

Programa de Pós-graduação em Ambientes Aquáticos Continentais. Laboratório de Vertebrados, Instituto de Ciências Biológicas, Universidade Federal do Rio Grande, Campus Carreiros, Av. Itália km 8, Rio Grande, RS 96203-900, Brazil

*Corresponding author. Email: lulis.lermen@hotmail.com. Telephone: +55 51 998731349

Este manuscrito está submetido ao periódico Canadian Journal of Zoology

Abstract: Color patterns are multifunctional traits shaped by ecological functions and environmental constraints. In ectothermic organisms such as amphibians, dorsal coloration is strongly influenced by habitat structure and climatic conditions. Understanding the drivers of color variation is essential for disentangling the selective pressures underlying phenotypic diversity. In this study, we examined the effects of temperature and habitat type on dorsal coloration in species of the genus *Rhinella*. We analyzed variation in hue, saturation, brightness, and intraspecific color diversity across multiple species. Our results show that hue and saturation increased with temperature, with hue also differing consistently between habitats, being higher in forests than in open environments. Brightness showed no significant association with either variable. Intraspecific color diversity was explained by the interaction between temperature and habitat, increasing under warmer conditions in open environments but decreasing in warmer forests. The observed association between warmer climates and redder, more saturated coloration may be linked to dietary carotenoid availability, camouflage strategies, and disease resistance. Forest environments, such as the Amazon, may constrain color variability through selection for specialized background matching, whereas open habitats permit greater variability. These findings highlight the complex interplay between climate, ecology, and evolutionary history in shaping coloration in *Rhinell*a.

Keywords: Amphibians, Animal coloration, Macroecology, Tropical biodiversity

INTRODUCTION

Animal coloration exhibits striking intra- and interspecific diversity and constitutes a key phenotypic trait shaped by evolutionary processes under the combined influence of selective pressures and intrinsic constraints (San-Jose and Roulin 2017; Postema et al. 2023; Rojas et al. 2023). In anurans, dorsal coloration can influence both survival and reproductive fitness by mediating thermal regulation, background matching, and visual signaling (Rojas

2017; Laumeier et al. 2023). Among the environmental factors affecting dorsal coloration, habitat structure (e.g., forested vs. open environments) and ambient temperature are particularly relevant (Endler 1993; Rojas 2017; Rojas et al. 2023; Laumeier et al. 2023). The ecological function of color patterns is context dependent, as the same coloration may appear conspicuous or cryptic depending on the surrounding environment (Barnett et al. 2021b). In forested habitats, closed canopies reduce light availability and modify spectral conditions, promoting the evolution of darker or less saturated coloration that enhances crypsis against dim, heterogeneous backgrounds by altering color perception and signal efficacy (Endler 1993; Cronin et al. 2014; Brelsford et al. 2022). By contrast, open habitats expose individuals to greater solar radiation and higher visual detectability, potentially selecting for lighter, less saturated dorsal coloration (Endler 1993; Rudh and Qvarnström 2013; Laumeier et al. 2023). Moreover, different environments can provide different prey availability (Lopes-Rodrigues et al. 2010; Toldi et al. 2021), which can influence body coloration due to dietary differences (Umbers et al. 2016; Moreno-Rueda and Comas 2023).

As ectotherms, anurans depend on dorsal pigmentation to regulate body temperature under fluctuating environmental conditions (Rojas et al. 2023; Laumeier et al. 2023). Empirical evidence shows that darker dorsal coloration provides a thermoregulatory advantage in colder habitats, while lighter hues are favoured in warmer environments to reduce the risk of overheating (Alho et al. 2010; Mack and Beaty 2021; Laumeier et al. 2023). For instance, individuals of *Anaxyrus americanus* (Holbrook, 1836) and *Rana temporaria* (Linnaeus, 1758) from colder regions often exhibit increased dorsal melanization, which likely enhances solar heat absorption in low-temperature conditions (Vences et al. 2002; Alho et al. 2010; Mack and Beaty 2021). Altogether, these patterns suggest that geographic variation in anuran dorsal coloration reflects localized adaptations to the thermal and structural characteristics of their local environments.

With 97 recognized species, the genus *Rhinella* exhibits remarkable morphological and biological diversity, including broad variation in body size, dorsal coloration, and ecological traits (Kwet et al. 2010; Pereyra et al. 2021; Durán et al. 2021; Frost 2025). Dorsal coloration in the genus appears to be associated with specific antipredator strategies, such as masquerade and disruptive coloration, which help reduce detection by visually oriented predators (McElroy 2016; Barnett et al. 2021a). In addition to interspecific differences, many *Rhinella* species also exhibit notable intraspecific variation in dorsal coloration (Lima et al. 2007; Vaz-Silva et al. 2015; Ferrão et al. 2020; Sousa et al. 2022). Species within the genus occupy a wide range of Neotropical environments, from densely forested lowlands to open montane grasslands and urban areas, encompassing substantial variation in ambient temperature, canopy cover, and substrate type (Pereyra et al. 2021; Frost 2025; IUCN 2025). This ecological breadth makes *Rhinella* a valuable model system for investigating how dorsal coloration evolves in response to habitat structure and thermal regimes, especially in species with cryptic coloration.

Given the expected changes in vegetation cover and temperatures driven by the global environmental crisis (Menéndez-Guerrero et al. 2020; Braga and Laurini 2024), understanding how these variables influence the evolution of anuran coloration, a trait with clear ecological relevance for survival, may be critical in the context of the steep population declines currently affecting the group (Menéndez-Guerrero et al. 2020; IUCN 2025). Therefore, we aim to test whether coloration patterns in anurans are associated with temperature and habitat structure, and whether these associations persist after accounting for shared evolutionary history. In this study, we examine variation in dorsal coloration across multiple species of the genus *Rhinella* using image data to extract quantitative color measurements (hue, saturation, and brightness) for statistical analysis. We hypothesize that species inhabiting colder environments will exhibit darker dorsal coloration (lower brightness), consistent with a thermoregulatory advantage, and that species from forested habitats will display less saturated or darker patterns that enhance crypsis under low-light conditions.

MATERIAL AND METHODS

Data collection

Photographic records of *Rhinella* species were compiled from multiple sources, including the online image databases iNaturalist.com and CalPhotos.com, the collection of the Museo de Zoología at the Pontificia Universidad Católica del Ecuador, peer-reviewed scientific articles, and direct contributions from researchers. For images obtained from citizen science platforms, species identifications were verified to ensure accuracy. While the photographs used in this study were not standardized, they still offer ecologically relevant and biologically meaningful colour data suitable for broad-scale comparative analyses (Roberts et al. 2022). We acknowledge that non-standardized images do not capture the ultraviolet component of visual signals and may, in some cases, exaggerate color contrast; however, this approach is particularly relevant in our context, as frog coloration is not preserved in museum specimens, and obtaining standardized images of live individuals across numerous species distributed throughout the Neotropics is logistically unfeasible.

To quantify dorsal coloration, we included only species with at least five high-quality photographs available for analysis. A maximum of 20 images per species was used to calculate mean values of the colorimetric variables: hue, saturation, and brightness. To estimate intraspecific variation in coloration, we retained only those species for which at least 10 images were available. In total, 676 images representing 51 species were evaluated, covering approximately 53% of the known diversity within the genus (Table 1). While all 51 species were included in the analysis of mean colorimetric values, only 38 met the criteria for the intraspecific variation analysis.

Table 1 - List of *Rhinella* species analyzed, with respective habitat type, mean annual temperature of occurrence sites (°C), and number of images used.

Species	Habitat	Mean Annual Temperature (°C)	N° of images
Rhinella achalensis (Cei, 1972)	open	11.06	12
Rhinella achavali (Maneyro, Arrieta, and de Sá, 2004)	cover	18.55	20
Rhinella acutirostris (Spix, 1824)	cover	26.64	20
Rhinella alata (Thominot, 1884)	cover	26.75	20
Rhinella arenarum (Hensel, 1867)	open	16.84	20
Rhinella arunco (Molina, 1782)	open	13.41	20
Rhinella atacamensis (Cei, 1962)	open	15.09	20
Rhinella beebei (Gallardo, 1965)	open	25.94	20
Rhinella bergi (Céspedez, 2000)	open	21.85	20
Rhinella castaneotica (Caldwell, 1991)	cover	25.96	20
Rhinella centralis (Narvaes and Rodrigues, 2009)	open	26.27	16
Rhinella cerradensis (Maciel, Brandão, Campos, and Sebben, 2007)	open	23.01	13
Rhinella crucifer (Wied-Neuwied, 1821)	cover	23.4	20
Rhinella dapsilis (Myers and Carvalho, 1945)	cover	26.21	20
Rhinella diptych (Cope, 1862)	open	22.33	20
Rhinella dorbignyi (Duméril and Bibron, 1841)	open	16.73	20
Rhinella festae (Peracca, 1904)	cover	22.17	20
Rhinella granulose (Spix, 1824)	open	24.5	20

Rhinella henseli (Lutz, 1934)	cover	18.2	20
Rhinella hoogmoedi (Caramaschi and Pombal, 2006)	cover	23.39	20
Rhinella icterica (Spix, 1824)	cover	20.65	20
Rhinella iserni (Jiménez de la Espada, 1875)	cover	23.03	8
Rhinella justinianoi (Harvey and Smith, 1994)	cover	18.81	7
Rhinella leptoscelis (Boulenger, 1912)	cover	20.05	7
Rhinella lescurei (Fouquet, Gaucher, Blanc, and Vélez-Rodriguez, 2007)	cover	25.88	12
Rhinella limensis (Werner, 1901)	open	17.3	12
Rhinella macrorhina (Trueb, 1971)	cover	16.92	12
Rhinella magnussoni (Lima, Menin, and Araújo, 2007)	cover	26.11	13
Rhinella manu (Chaparro, Pramuk, and Gluesenkamp, 2007)	cover	19.07	6
Rhinella margaritifera (Laurenti, 1768)	cover	25.42	20
Rhinella marina (Linnaeus, 1758)	cover	25.36	20
Rhinella ocellata (Günther, 1858)	open	24.49	11
Rhinella ornate (Spix, 1824)	cover	20.29	20
Rhinella piraguas (Grant and Bolívar-Garcías, 2014)	cover	17.19	11
Rhinella poeppigii (Tschudi, 1845)	cover	23.3	16
Rhinella proboscidea (Spix, 1824)	cover	26.3	20
Rhinella pygmaea (Myers and Carvalho, 1952)	cover	22.43	11
Rhinella quechua (Gallardo, 1961)	cover	17.6	7
Rhinella rostrata (Noble, 1920)	cover	16.15	7
Rhinella rubescens (Lutz, 1925)	open	23.23	20

Rhinella rubropunctata (Guichenot, 1848)	cover	9.3	6
Rhinella ruizi (Grant, 2000)	cover	15.71	10
Rhinella rumbolli (Carrizo, 1992)	cover	19.07	5
Rhinella spinulosa (Wiegmann, 1834)	open	7.85	20
Rhinella stanlaii (Lötters and Köhler, 2000)	cover	20.46	11
Rhinella sternosignata (Günther, 1858)	cover	22.52	18
Rhinella tacana (Padial, Reichle, McDiarmid, and De la Riva, 2006)	cover	22.72	5
Rhinella tenrec (Lynch and Renjifo, 1990)	cover	22.56	5
Rhinella veraguensis (Schmidt, 1857)	cover	19.48	8
Rhinella veredas (Brandão, Maciel, and Sebben, 2007)	open	23.45	7
Rhinella yanachaga (Lehr, Pramuk, Hedges, and Córdova, 2007)	cover	15	5

Mean environmental temperature across each species' range was estimated using bioclimatic data obtained from the WorldClim 2.1 database (Fick and Hijmans 2017). The raster layers were cropped to match the extent of species distribution shapefiles provided by the IUCN Red List spatial database (IUCN 2025), allowing calculation of the mean environmental temperature for each species. Habitat type was classified as either forested or open based on species' habitat preferences, even when a species occurred in both environments, following the ecological descriptions available in the IUCN species accounts (IUCN 2025). This binary classification was applied to reduce the number of predictor variables in the statistical models, thereby minimizing the risk of overfitting given our sample size.

Color measurement

The photographic records of *Rhinella* species were processed in ImageJ software with the micaToolbox version 2.2.0 add-on, which allows standardized color analysis of digital images. Calibration was performed using a reference photograph containing three gray standard cards with reflectance values of 2%, 18%, and 34%, captured with a Canon EOS 1200D camera equipped with an 18–55 mm lens and built-in flash. This procedure aimed to minimize variation among images during multispectral image generation. For each photograph, a region of interest (ROI) was manually outlined on the dorsal surface of the frog, avoiding areas affected by glare or shadow. From these standardized images, reflectance values were extracted for the red, green, and blue (RGB) channels. These measurements were then converted into three independent colorimetric variables: hue, representing the dominant wavelength or perceived color; saturation, indicating the intensity of the color relative to white light; and brightness, corresponding to the total reflectance across the measured spectrum (Stevens et al. 2007; Ibraheem et al. 2012).

To obtain the colorimetric variables, the RGB values extracted from the standardized images were normalized and organized into a table compatible with the *colspace* function of the PAVO package (v.2.9.0) (Maia et al. 2019) in R v.4.3.3 (R Core Team 2024). The RGB channels were reordered to correspond to short (B), medium (G), and long (R) wavelengths and subsequently projected as XY coordinates within a triangular color space. This representation enabled the calculation of two of the primary colorimetric metrics: hue and saturation. In this framework, the central point of the triangle corresponds to an achromatic condition, with increasing distances from the center representing higher saturation and distinct color types. Saturation was quantified as the distance *r* from the achromatic origin, while hue was defined by the angular displacement θ of the color vector from the positive x-axis. Brightness was estimated as the mean reflectance across the three color channels, calculated by summing the RGB values and dividing the result by three. Before proceeding, we generated graphical representations of each colorimetric variable to identify and remove outliers from the dataset.

To calculate color diversity, we used the XY coordinates from the triangular color space to estimate the mean Euclidean distance of each individual to all others within the same species, employing the *colsdist* function from the PAVO package (v.2.9.0). To standardize sample sizes across species, we randomly selected ten individuals without replacement and calculated the species mean. This procedure was repeated 999 times, and the resulting means per species were averaged once more to obtain the final color diversity value for each species. Under this approach, species with more than ten individuals had a final mean composed of 999 different resampled averages, whereas species with exactly ten individuals produced identical averages across all iterations. In this framework, larger mean distances reflect greater variation in coloration among individuals, thereby indicating higher intraspecific color diversity.

Data analysis

We evaluated hue, saturation, brightness, and color diversity independently in relation to two predictors: habitat type (forest vs. open) and mean annual temperature across each species' distribution. Analyses were conducted using Phylogenetic Generalized Least Squares (PGLS), which corrects for the lack of independence among species due to shared ancestry, applying Pagel's correlation structure. Four separate models were fitted, one for each colorimetric variable, with the same set of fixed and random effects. Habitat type was included as a categorical predictor, while mean annual temperature was treated as a continuous predictor. The potential interaction between predictors was also tested; however, it proved significant only for color diversity. For hue, saturation, and brightness, models were fitted without interaction terms. Phylogenetic relationships were incorporated through a published time-calibrated consensus tree (Portik et al. 2023) and used maximum likelihood to estimate the phylogenetic signal (lambda) of each model. Model assumptions were assessed by examining data normality with Q-Q plots and the Akaike Information Criterion (AIC) as an additional diagnostic criterion, following Lermen et al. (2025). Homoscedasticity was verified using residual plots. All analyses were performed in R v.4.3.3 (R Core Team 2024) using the nlme package (Pinheiro et al. 1999).

RESULTS

The phylogenetic signal (λ) varied among the colorimetric traits. For hue, λ was estimated at 0.503. Hue was significantly influenced by both temperature and habitat type (Figure 1A). Higher mean annual temperatures were associated with increased hue values ($\beta = 0.011$, $t = 2.231$, $p = 0.030$), indicating a shift toward redder tones, whereas species from open habitats exhibited lower hue values, corresponding to more yellowish coloration, compared to those from forests ($\beta = -0.120$, $t = -2.750$, $p = 0.008$). For saturation, λ was 0.437, and this trait was significantly affected only by temperature ($\beta = 0.007$, $t = 2.169$, $p = 0.035$), with higher temperatures leading to greater saturation values (Figure 1B). Brightness exhibited a lower phylogenetic signal ($\lambda = 0.182$) and was not significantly influenced by either temperature or habitat type (Figure 1C). Intraspecific color diversity showed the weakest phylogenetic signal ($\lambda = 0.032$). While no direct effect of temperature or habitat type was detected, there was a significant interaction between these variables ($\beta = 0.010$, $t = 2.111$, $p = 0.042$). Specifically, color diversity increased with rising temperatures in open habitats but decreased with temperature in forest habitats (Figure 1D).

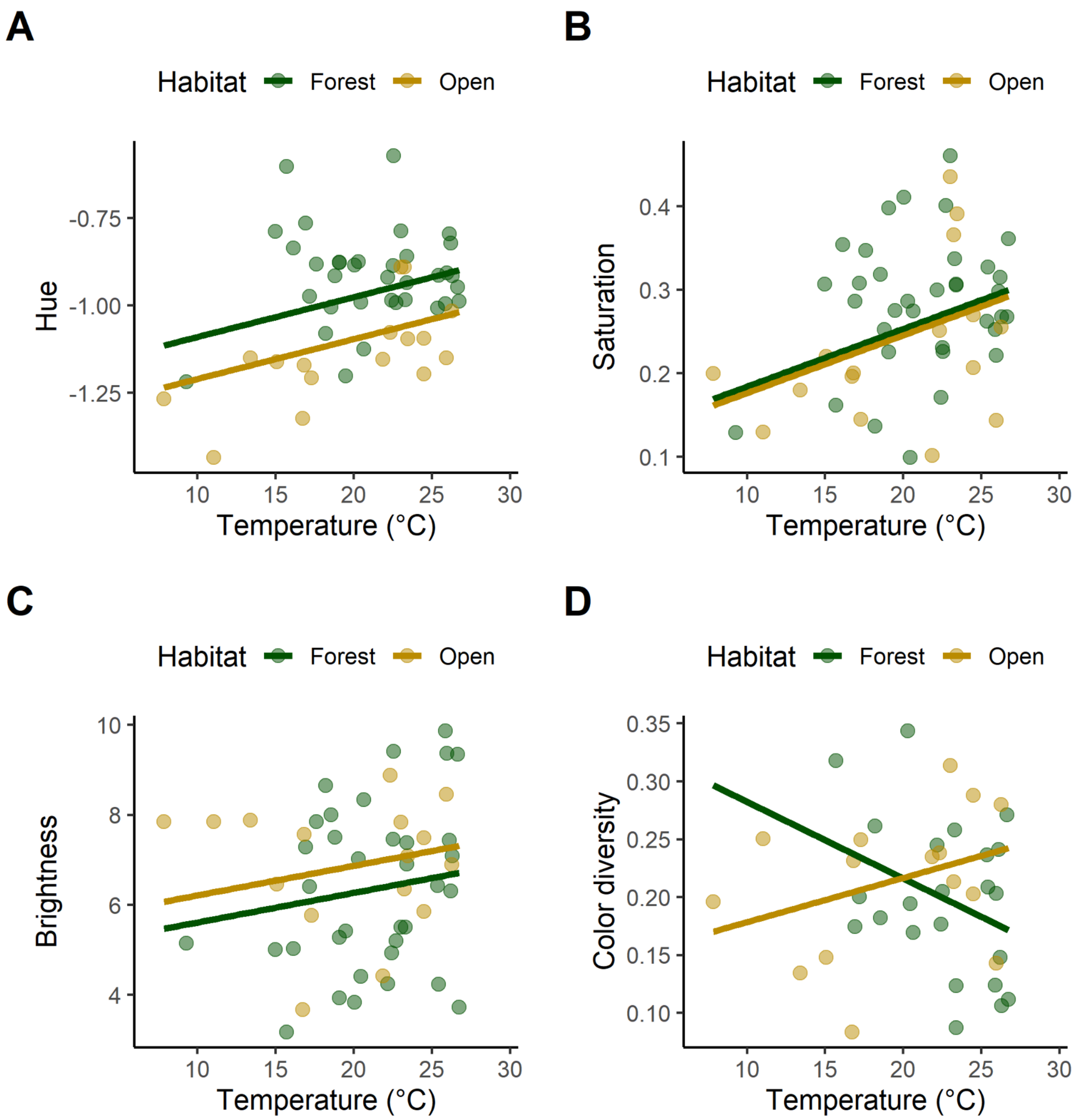

Figure 1 - Effects of habitat type and temperature on dorsal coloration in the genus *Rhinella*. Dots represent raw data, and lines indicate the fitted model predictions. (A) Hue was significantly influenced by both temperature ($p = 0.030$) and habitat type ($p = 0.008$). (B) Saturation was significantly affected only by temperature ($p = 0.035$). (C) Brightness showed no significant differences in relation to either variable. (D) Intraspecific color diversity was not directly affected by temperature or habitat type, but their interaction was significant ($p = 0.042$).

DISCUSSION

Our results show that dorsal coloration in *Rhinella* is shaped by both climatic and ecological factors. Hue and saturation increased with temperature, while hue also differed consistently between habitats, being lower in open environments compared to forests. In contrast, brightness exhibited no association with either predictor, suggesting that this trait may not be strongly influenced by macroclimatic conditions, which is a surprising outcome given evidence from previous studies (Alho et al. 2010; Mack and Beaty 2021; Laumeier et al. 2023). This unexpected result suggests that brightness may be under weaker selective pressures in *Rhinella* or shaped by microhabitat-level factors not captured in our analyses like solar radiation. Finally, intraspecific color diversity was best explained by the interaction between habitat type and temperature, with diversity increasing under warmer conditions in open habitats but decreasing in warmer forests. Overall, these findings emphasize the complex interplay between environment and evolutionary history in determining dorsal coloration.

Higher hue values observed in forest habitats suggest that *Rhinella* species inhabiting these environments tend to display more reddish dorsal coloration, whereas species from open habitats show more yellowish tones. The difference in hue between forested and open environments can be explained by camouflage strategies. Species of the genus *Rhinella* are primarily terrestrial and rely on background matching as a defense mechanism against predators (Ferreira et al. 2019). In forest habitats, individuals may resemble dead leaves on the forest floor, thereby reducing predation risk (Toledo and Haddad 2009; Brito et al. 2013). This can be seen in several species of leaf-mimicking toads, including *Rhinella hoogmoedi* (Caramaschi & Pombal, 2006), *Rhinella margaritifera* (Laurenti, 1768), and *Rhinella alata* (Thominot, 1884) (Brito et al. 2013; McElroy 2016; Barnett et al. 2021a). The accumulation of leaf litter and organic matter on the forest floor, together with soil moisture, alters the background coloration, often producing darker reddish-brown tones (Santos et al. 2016; Sirisathitkul and Sirisathitkul 2025). This environmental setting likely exerts selective pressure on forest-dwelling individuals to develop more reddish hues, enhancing their ability to blend with the substrate and improving camouflage efficiency. This interpretation is supported by evidence showing that forest species are often well camouflaged against the forest floor (Barnett et al. 2021a).

Our results show a positive effect of temperature on dorsal color saturation and hue in *Rhinella*. Previous research has primarily examined the effects of temperature on brightness in anurans, focusing on thermoregulatory processes and how individuals lighten or darken in response to thermal fluctuations (Tattersall et al. 2006; Park et al. 2023; Laumeier et al. 2023). In contrast, the influence of temperature on color saturation and hue has received far less attention, despite evidence that saturation can respond to thermal variation in *Pseudacris regilla* (Baird and Girard, 1852) (Stegen et al. 2004) and reported hue shifts associated with reproductive signaling in reptiles (Robinson and Gifford 2018; Assis et al. 2020). In our study, species inhabiting warmer environments were generally associated with regions closer to the equator and the Amazon rainforest, whereas lower temperatures were observed at higher altitudes, such as in the Andes, or in areas near the southern tropics. This pattern indicates that *Rhinella* species occurring at lower altitudes and closer to the equator tend to exhibit a redder and more saturated dorsal coloration.

This trend is consistent with patterns observed for carotenoid-based coloration in Australian birds, where higher saturation is associated with lower latitudes and warmer temperatures (Prasetya et al. 2020). Carotenoids, which are yellow-red pigments that cannot be synthesized by anurans and must be obtained through diet, have been documented in othe bufonid species such as *Melanophryniscus rubriventris* (Vellard, 1947) (Bonansea et al. 2017; Koneru and Caro 2022; Moreno-Rueda and Comas 2023). Arthropods, an important component of the diet of *Rhinella* species (Maragno and Souza 2011; Sabagh et al. 2012; Brito et al. 2013; de Oliveira et al. 2017), are a primary dietary source of carotenoids for anurans (Umbers et al. 2016; Rojas et al. 2023). It is therefore possible that greater availability of carotenoid-rich prey, or a higher quality of carotenoids in warmer regions, enhances dietary intake and consequently leads to changes in coloration in species inhabiting these environments (Umbers et al. 2016; Prasetya et al. 2020), ultimately resulting in a more saturated and redder dorsal coloration. In addition to their role in coloration, carotenoids are also important in disease and parasite resistance, as they can enhance immune responses (Szuroczki et al. 2019). Since parasite and pathogen abundance and diversity tend to increase at lower latitudes (Harvell et al. 2002), the pattern observed in our study, where coloration is more saturated and redder closer to the equator, may also be linked to protective functions against parasites and diseases. However, further studies are needed to directly test the role of dietary carotenoids in shaping dorsal coloration patterns in *Rhinella*.

An interesting pattern emerged in color diversity, with intraspecific variation increasing under warmer conditions in open habitats but decreasing in warmer forests. Warm forests, such as those in the Amazon, are characterized by a highly developed canopy that strongly influences light conditions in the understory, where *Rhinella* species typically occur (Morton et al. 2016; Barnett et al. 2021a). Camouflage in these environments can be achieved through two strategies: a generalist approach, in which an individual's coloration resembles the average background sufficiently to avoid detection, or a specialist approach, in which coloration closely matches a specific component of the substrate (Merilaita and Jormalainen 1999; Merilaita et al. 2017). There is evidence that Amazonian *Rhinella* species employ a specialist camouflage strategy, closely resembling the leaf litter of the forest floor (Barnett et al. 2021a). This suggests that environmental conditions in such forests may constrain intraspecific color diversity when compared to temperate forests. In contrast, open habitats may allow greater intraspecific variability in dorsal coloration due to higher light exposure and greater visibility under warmer conditions (Endler 1993; Amézquita and Hödl 2006). Another possibility is that open environments associated with colder regions, such as the Atacama Desert, provide more homogeneous backgrounds, which could limit dorsal color variation within species (Norris and Lowe 1964; Lermen et al. 2025).

However, it is important to acknowledge the methodological limitations of this study. Because the images used were not standardized, the results should be interpreted with caution. Many photographs may have been edited prior to publication to enhance their perceived aesthetic appeal, potentially altering color balance and RGB values and introducing bias into the dataset. In addition, images taken under different lighting conditions, such as daylight, nighttime, or artificial studio lighting, may further affect color measurements, particularly brightness. Despite these limitations, we argue that this study represents a valid and informative first step toward identifying broad-scale patterns and trends in dorsal coloration, encompassing an entire anuran genus with wide geographic distribution (Pereyra et al. 2021; Frost 2025; IUCN 2025). This approach is especially relevant given that body coloration in anuran amphibians is not preserved in museum specimens, which severely restricts large-scale comparative studies of coloration in this group. Future research using standardized photographic protocols will be essential to more rigorously test and refine the coloration patterns observed in the genus *Rhinella*.

In conclusion, our findings demonstrate that dorsal coloration in *Rhinella* is shaped by both climatic and ecological factors, with temperature and habitat type exerting distinct influences on hue, saturation, and intraspecific color diversity. While hue and saturation increased with temperature, hue also differed consistently between forested and open habitats, supporting the role of background matching and camouflage in driving coloration patterns. Intraspecific color diversity, in turn, was modulated by the interaction between habitat and temperature, highlighting the importance of considering environmental context when interpreting evolutionary responses. Taken together, these results emphasize the complex interplay between ecological pressures, climatic conditions, and evolutionary history in shaping amphibian coloration, while also underscoring the vulnerability of these traits to ongoing global environmental change.

Acknowledgement

We thank the Programa de Pós-Graduação em Ambientes Aquáticos Continentais at the Universidade Federal do Rio Grande for providing support during this study. DL received a research fellowship from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, grantnumber 307232/2023-9). LL was supported by a scholarship from the Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). ScholarGPT (version GPT-4-turbo) was used to assist the authors in expressing content in a non-native language, reviewing grammar, and enhancing the clarity and flow of the text.

Conflicts of interest: The authors declare that they have no conflict of interest.

Author Contributions: LNL conceived and designed the methodology, LNL analyzed the data, LNL wrote the manuscript, and DL provided editorial advice.

Funding: D. Loebmann recieved a researchfellowshipfrom Conselho Nacional de Desenvolvimento Científico e Tecnológico (307232/2023-9). L. Lermen recieved a scholarship from Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.

Data Availability: The datasets used and analyzed during the current study are available from the corresponding author on reasonable request. The R script used during the current study is available from the corresponding author on reasonable request.

References

Alho, J.S., Herczeg, G., Söderman, F., Laurila, A., Jönsson, K., and Merilä, J. 2010. Increasing melanism along a latitudinal gradient in a widespread amphibian: local adaptation, ontogenic or environmental plasticity? BMC Evol Biol **10**(1): 317. doi:10.1186/1471-2148-10-317.

Amézquita, A., and Hödl, W. 2006. HOW, WHEN, AND WHERE TO PERFORM VISUAL DISPLAYS: THE CASE OF THE AMAZONIAN FROG HYLA PARVICEPS. Herpetologica **60**(4): 420–429. Herpetologists League. doi:10.1655/02-51.

Assis, B.A., Jarrett, B.J.M., Koscky, G., Langkilde, T., and Avery, J.D. 2020. Plastic sexual ornaments: Assessing temperature effects on color metrics in a color-changing reptile. PLoS ONE **15**(5): e0233221. doi:10.1371/journal.pone.0233221.

Barnett, J.B., Michalis, C., Scott-Samuel, N.E., and Cuthill, I.C. 2021a. Colour pattern variation forms local background matching camouflage in a leaf-mimicking toad. J of Evolutionary Biology **34**(10): 1531–1540. Oxford University Press (OUP). doi:10.1111/jeb.13923.

Barnett, J.B., Varela, B.J., Jennings, B.J., Lesbarrères, D., Pruitt, J.N., and Green, D.M. 2021b. Habitat disturbance alters color contrast and the detectability of cryptic and aposematic frogs. Behavioral Ecology **32**(5): 814–825. doi:10.1093/beheco/arab032.

Bonansea, M.I., Heit, C., and Vaira, M. 2017. Pigment composition of the bright skin in the poison toad Melanophryniscus rubriventris (Anura: Bufonidae) from Argentina. SALAMANDRA **53**(1): 142–147.

Braga, A., and Laurini, M. 2024. Spatial heterogeneity in climate change effects across Brazilian biomes. Sci Rep **14**(1): 16414. doi:10.1038/s41598-024-67244-x.

Brelsford, C.C., Trasser, M., Paris, T., Hartikainen, S.M., and Robson, T.M. 2022. Understorey light quality affects leaf pigments and leaf phenology in different plant functional types. Physiologia Plantarum **174**(3): e13723. doi:10.1111/ppl.13723.

Brito, L.B. de M., Moura-Neto, C., Zucco, C.A., and Cascon, P. 2013. Diet, activity patterns, microhabitat use and defensive strategies of Rhinella hoogmoedi Caramaschi & Pombal, 2006 from a humid forest in northeast Brazil. **23**(1): 29–37.

Cronin, T., Johnsen, S., Marshall, J., and Warrant, E.J. 2014. Visual ecology. Princeton university press, Princeton (N.J.).

Durán, F., Méndez, M.A., and Correa, C. 2021. The Atacama toad (Rhinella atacamensis) exhibits an unusual clinal pattern of decreasing body size towards more arid environments. BMC Zool **6**(1). Springer Science and Business Media LLC. doi:10.1186/s40850-021-00090-w.

Endler, J.A. 1993. The Color of Light in Forests and It's Implications. Ecological Monographs **63**(1): 1–27.

Ferrão, M., Lima, A.P., Ron, S., Santos, S.P.D., and Hanken, J. 2020. New Species of Leaf-litter Toad of the Rhinella margaritifera Species Group (Anura: Bufonidae) from Amazonia. Copeia **108**(4). American Society of Ichthyologists and Herpetologists (ASIH). doi:10.1643/ch2020043.

Ferreira, R.B., Lourenço-de-Moraes, R., Zocca, C., Duca, C., Beard, K.H., and Brodie, E.D. 2019. Antipredator mechanisms of post-metamorphic anurans: a global database and classification system. Behav Ecol Sociobiol **73**(5): 69. doi:10.1007/s00265-019-2680-1.

Fick, S.E., and Hijmans, R.J. 2017. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. Intl Journal of Climatology **37**(12): 4302–4315. Wiley. doi:10.1002/joc.5086.

Frost, D.R. 2025. Rhinella Fitzinger, 1826 | Amphibian Species of the World. Available from https://amphibiansoftheworld.amnh.org/Amphibia/Anura/Bufonidae/Rhinella [accessed 24 July 2025].

Harvell, C.D., Mitchell, C.E., Ward, J.R., Altizer, S., Dobson, A.P., Ostfeld, R.S., and Samuel, M.D. 2002. Climate Warming and Disease Risks for Terrestrial and Marine Biota. Science **296**(5576): 2158–2162. American Association for the Advancement of Science (AAAS). doi:10.1126/science.1063699.

Ibraheem, N.A., Hasan, M.M., Khan, R.Z., and Mishra, P.K. 2012. Understanding Color Models: A Review. **2**(3).

IUCN. 2025. Available from https://www.iucnredlist.org/en [accessed 24 July 2025].

Koneru, M., and Caro, T. 2022. Animal Coloration in the Anthropocene. Front. Ecol. Evol. **10**: 857317. doi:10.3389/fevo.2022.857317.

Kwet, A., R, L., and M, D.-B. 2010. Pró-Mata: Anfíbios da Serra Gaúcha, sul do Brasil/Amphibien der Serra Gaúcha, Südbrasilien/Amphibiansofthe Serra Gaúcha, South of Brazil. EDIPUCRS, Porto Alegre.

Laumeier, R., Brändle, M., Rödel, M.-O., Brunzel, S., Brandl, R., and Pinkert, S. 2023. The global importance and interplay of colour-based protective and thermoregulatory functions in frogs. Nat Commun **14**(1): 8117. doi:10.1038/s41467-023-43729-7.

Lermen, L., Furtado, R., and Hartz, S.M. 2025. To be or not be seen: the influence of substrate on anuran community coloration. Journal of Natural History **59**(17–20): 1249–1265. doi:10.1080/00222933.2025.2480733.

Lima, A.P., Menin, M., and De Araújo, M.C. 2007. A new species of Rhinella (Anura: Bufonidae) from Brazilian Amazon. Zootaxa **1663**(1). Magnolia Press. doi:10.11646/zootaxa.1663.1.1.

Lopes-Rodrigues, E.N., De S. Mendonça, Jr, M., L. O. Rosado, J., and Loeck, A.E. 2010. Soil spiders in differing environments: Eucalyptus plantations and grasslands in the Pampa biome, southern Brazil. Rev. Colomb. Entomol. **36**(2): 277–284. doi:10.25100/socolen.v36i2.9159.

Mack, M., and Beaty, L. 2021. The influence of environmental and physiological factors on variation in American toad (Anaxyrus americanus) dorsal coloration. Journal of Herpetology **55**(2): 119–126. doi:10.1670/20-093.

Maia, R., Gruson, H., Endler, J.A., and White, T.E. 2019. pavo 2: New tools for the spectral and spatial analysis of colour in r. Methods in Ecology and Evolution **10**(7): 1–11. doi:10.1111/2041-210X.13174.

Maragno, F.P., and Souza, F.L. 2011. Diet of Rhinella scitula (Anura, Bufonidae) in the Cerrado, Brazil: the importance of seasons and body size. Rev.Mex.Biodiv. **82**(3). Universidad Nacional Autonoma de Mexico. doi:10.22201/ib.20078706e.2011.3.693.

McElroy, M.T. 2016. Teasing apart crypsis and aposematism - evidence that disruptive coloration reduces predation on a noxious toad. Biol. J. Linn. Soc. **117**(2): 285–294. Oxford University Press (OUP). doi:10.1111/bij.12669.

Menéndez-Guerrero, P.A., David M. Green, and Davies, T.J. 2020. Climate change and the future restructuring of Neotropical anuran biodiversity. Ecography **43**: 222–235. doi:10.1111/ecog.04510.

Merilaita, S., and Jormalainen, V. 1999. Optimization of cryptic coloration in heterogeneous habitats. Biological Journal of the Linnean Society **67**: 151–161.

Merilaita, S., Scott-Samuel, N.E., and Cuthill, I.C. 2017. How camouflage works. Philosophical Transactions of the Royal Society B: Biological Sciences **372**(1724). doi:10.1098/rstb.2016.0341.

Moreno-Rueda, G., and Comas, M. 2023. Evolutionary Ecology of Amphibians. *In* 1st edition. CRC Press, Boca Raton. doi:10.1201/9781003093312.

Morton, D.C., Rubio, J., Cook, B.D., Gastellu-Etchegorry, J.-P., Longo, M., Choi, H., Hunter, M., and Keller, M. 2016. Amazon forest structure generates diurnal and seasonal variability in light utilization. Biogeosciences **13**(7): 2195–2206. Copernicus GmbH. doi:10.5194/bg-13-2195-2016.

Norris, K.S., and Lowe, C.H. 1964. An Analysis of Background Color-Matching in Amphibians and Reptiles. Ecology **45**(3): 565–580. Wiley. doi:10.2307/1936109.

de Oliveira, M., de Avila, F.R., and Tozetti, A.M. 2017. Diet of Rhinella arenarum (Anura, Bufonidae) in a coastal habitat in southern Brazil. Herpetology Notes **10**: 507–510.

Park, C., No, S., Yoo, S., Oh, D., Hwang, Y., Kim, Y., and Kang, C. 2023. Testing multiple hypotheses on the colour change of treefrogs in response to various external conditions. Sci Rep **13**(1): 4203. doi:10.1038/s41598-023-31262-y.

Pereyra, M.O., Blotto, B.L., Baldo, D., Chaparro, J.C., Ron, S.R., Elias-Costa, A.J., Iglesias, P.P., Venegas, P.J., C. Thomé, M.T., Ospina-Sarria, J.J., Maciel, N.M., Rada, M., Kolenc, F., Borteiro, C., Rivera-Correa, M., Rojas-Runjaic, F.J.M., Moravec, J., De La Riva, I., Wheeler, W.C., Castroviejo-Fisher, S., Grant, T., Haddad, C.F.B., and Faivovich, J. 2021. Evolution in the Genus Rhinella: A Total Evidence Phylogenetic Analysis of Neotropical True Toads (Anura: Bufonidae). Bulletin of the American Museum of Natural History **447**(1). American Museum of Natural History (BioOne sponsored). doi:10.1206/0003-0090.447.1.1.

Pinheiro, J., R Core Team, and Bates, D. 1999, November 23. nlme: Linear and Nonlinear Mixed Effects Models. The R Foundation. doi:10.32614/cran.package.nlme.

Portik, D.M., Streicher, J.W., and Wiens, J.J. 2023. Frog phylogeny: A time-calibrated, species-level tree based on hundreds of loci and 5,242 species. Molecular Phylogenetics and Evolution **188**: 107907. Elsevier BV. doi:10.1016/j.ympev.2023.107907.

Postema, E.G., Lippey, M.K., and Armstrong-Ingram, T. 2023. Color under pressure: how multiple factors shape defensive coloration. Behavioral Ecology **34**(1): 1–13. doi:10.1093/beheco/arac056.

Prasetya, A.M., Peters, A., and Delhey, K. 2020. Carotenoid-based plumage colour saturation increases with temperature in Australian passerines. Journal of Biogeography **47**(12): 2671–2683. Wiley. doi:10.1111/jbi.13968.

R Core Team. 2024. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available from https://www.r-project.org/.

Roberts, S.M., Stuart-Fox, D., and Medina, I. 2022. The evolution of conspicuousness in frogs: When to signal toxicity? J of Evolutionary Biology **35**(11): 1455–1464. Oxford University Press (OUP). doi:10.1111/jeb.14092.

Robinson, C.D., and Gifford, M.E. 2018. Covariation between Thermally Mediated Color and Performance Traits in a Lizard. Physiological and Biochemical Zoology **91**(5): 1013–1025. doi:10.1086/699616.

Rojas, B. 2017. Behavioural, ecological, and evolutionary aspects of diversity in frog colour patterns. Biological Reviews **92**(2): 1059–1080. Blackwell Publishing Ltd. doi:10.1111/brv.12269.

Rojas, B., Lawrence, J.P., and Márquez, R. 2023. Amphibian Coloration: Proximate Mechanisms, Function, and Evolution. *In* Evolutionary Ecology of Amphibians, 1st edition. CRC Press, Boca Raton. pp. 219–258. doi:10.1201/9781003093312-12.

Rudh, A., and Qvarnström, A. 2013. Adaptive colouration in amphibians. Seminars in Cell and Developmental Biology **24**(6–7): 553–561. Elsevier Ltd. doi:10.1016/j.semcdb.2013.05.004.

Sabagh, L.T., Carvalho-e-Silva, A.M.P.T., and Rocha, C.F.D. 2012. Diet of the toad Rhinella icterica (Anura: Bufonidae) from Atlantic Forest Highlands of southeastern Brazil.

Biota Neotrop. **12**(4): 258–262. FapUNIFESP (SciELO). doi:10.1590/s1676-06032012000400027.

San-Jose, L.M., and Roulin, A. 2017. Genomics of coloration in natural animal populations. Philosophical Transactions of the Royal Society B: Biological Sciences **372**(1724). doi:10.1098/rstb.2016.0337.

Santos, A.D.C., Pereira, M.G., Anjos, L.H.C.D., Bernini, T.A., and Cooper, M. 2016. Genesis of Soils Formed from Mafic Igneous Rock in the Atlantic Forest Environment. Rev. Bras. Ciênc. Solo **40**(0). FapUNIFESP (SciELO). doi:10.1590/18069657rbcs20150056.

Sirisathitkul, Y., and Sirisathitkul, C. 2025. Decoding Soil Color: Origins, Influences, and Methods of Analysis. AgriEngineering **7**(3): 58. MDPI AG. doi:10.3390/agriengineering7030058.

Sousa, T.R. de, Benício, R.A., and Fonseca, M.G. 2022. POLIMORFISMO EM Rhinella diptycha (ANURA: BUFONIDAE) EM UMA ÁREA DE CAATINGA, ESTADO DO PIAUÍ, NORDESTE DO BRASIL. Biosphere Comunicações Científicas **1**(2): 16–22.

Stegen, J.C., Gienger, C.M., and Sun, L. 2004. The control of color change in the Pacific tree frog, *Hyla regilla*. Can. J. Zool. **82**(6): 889–896. doi:10.1139/z04-068.

Stevens, M., Párraga, C.A., Cuthill, I.C., Partridge, J.C., and Troscianko, T.S. 2007. Using digital photography to study animal coloration. Biological Journal of the Linnean Society **90**: 211–237.

Szuroczki, D., Koprivnikar, J., and Baker, R.L. 2019. Effects of dietary antioxidants and environmental stressors on immune function and condition in Lithobates (Rana) sylvaticus. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology **229**: 25–32. Elsevier BV. doi:10.1016/j.cbpa.2018.11.017.

Tattersall, G.J., Eterovick, P.C., and De Andrade, D.V. 2006. Tribute to R. G. Boutilier: Skin colour and body temperature changes in basking *Bokermannohyla alvarengai* (Bokermann 1956). Journal of Experimental Biology **209**(7): 1185–1196. doi:10.1242/jeb.02038.

Toldi, M., Bizarro, G.L., Da-Costa, T., Da Silva, V.L., Jantsch Ferla, J., Johann, L., De Freitas, E.M., Da Silva, G.L., and Ferla, N.J. 2021. Mite fauna associated with different environments in the Southern Pampa, Brazil. International Journal of Acarology **47**(5): 387–395. doi:10.1080/01647954.2021.1915378.

Toledo, L.F., and Haddad, C.F.B. 2009. Colors and Some Morphological Traits as Defensive Mechanisms in Anurans. International Journal of Zoology **2009**: 1–12. Hindawi Limited. doi:10.1155/2009/910892.

Umbers, K.D.L., Silla, A.J., Bailey, J.A., Shaw, A.K., and Byrne, P.G. 2016. Dietary carotenoids change the colour of Southern corroboree frogs. Biol. J. Linn. Soc. **119**(2): 436–444. doi:10.1111/bij.12818.

Vaz-Silva, W., Maciel, N.M., Bastos, R.P., and Pombal, J.P. 2015. Revealing Two New Species of the*Rhinella margaritifera*Species Group (Anura, Bufonidae): An Enigmatic Taxonomic Group of Neotropical Toads. Herpetologica **71**(3): 212–222. Herpetologists League. doi:10.1655/herpetologica-d-14-00039.

Vences, M., Galán, P., Vieites, D.R., Puente, M., Oetter, K., and Wanke, S. 2002. Field body temperatures and heating rates in a montane frog population: the importance of black dorsal pattern for thermoregulation. **39**: 209–220.

CONSIDERAÇÕES FINAIS E PERSPECTIVAS

Nesta Tese, investiguei os efeitos de variáveis ambientais sobre os padrões de coloração dorsal em anuros em diferentes escalas biológicas, abrangendo níveis populacionais, comunitários e macroevolutivos. Para isso, utilizei registros fotográficos padronizados dos animais (Stevens et al., 2007) e dados ambientais coletados em campo na região sul do Brasil, abrangendo habitats com distintas características ecológicas. Além dos dados obtidos em campo, utilizei também registros fotográficos de plataformas de imagens online (CalPhotos, iNaturalist) e informações ambientais provenientes de bancos de dados (IUCN, WorldClim) complementados por dados filogenéticos. Essas informações foram analisadas com diferentes abordagens estatísticas para compreender como fatores como tipo de habitat, temperatura e precipitação modulam a variação na coloração dorsal dos anuros e de que forma esses fatores ambientais contribuem para a diversificação e manutenção dos padrões de coloração no grupo.

Em escala populacional, os resultados demonstram que a coloração dorsal responde a gradientes ambientais locais de forma complexa e multifuncional. No primeiro capítulo, populações de *Dendropsophus minutus* que habitam áreas campestres apresentaram maior saturação de cor em comparação às populações florestais, sugerindo que ambientes abertos podem favorecer maior investimento reprodutivo ou estratégias visuais associadas ao aumento do estresse ambiental, conforme observado em *Epidalea calamita (Laurenti, 1768)* (Zamora-Camacho; Comas, 2019). Alternativamente, a menor saturação em florestas pode refletir uma resposta às condições de luminosidade impostas pelo dossel, favorecendo a camuflagem em ambientes de luz filtrada (Endler, 1993; Rojas; Lawrence; Márquez, 2023). No segundo capítulo, verificou-se que a luminosidade aumentou significativamente com a temperatura, reforçando seu papel termorregulatório (Laumeier et al., 2023), embora a ausência de diferenças entre habitats indique que pressões seletivas opostas, como a proteção contra radiação ultravioleta em áreas abertas, possam atuar de forma antagonista, anulando seus efeitos (Endler, 1993; Rudh; Qvarnström, 2013; Burraco; Orizaola, 2022). Além disso, a saturação apresentou um padrão dependente da interação entre habitat e precipitação, com maior saturação em campos sob condições úmidas e redução geral com o aumento da temperatura, sugerindo sensibilidade térmica desse componente da cor (Stegen; Gienger; Sun, 2004). Em conjunto, esses resultados indicam que diferentes componentes da coloração dorsal respondem de maneira distinta aos gradientes ambientais locais. Esses achados reforçam a relevância ecológica da coloração e evidenciam a vulnerabilidade dos fenótipos às alterações de habitat e às mudanças climáticas, uma vez que modificações na cobertura do dossel, nos regimes de precipitação e nas condições térmicas podem comprometer a manutenção da funcionalidade da coloração (Barnett et al., 2021a; Koneru; Caro, 2022).

Em escala comunitária, os resultados indicam que o tipo de habitat exerce papel central na estruturação da diversidade interespecífica da coloração. Contrariamente às expectativas de maior variação na coloração das comunidades em florestas com estrutura vertical complexa (Delhey et al., 2023; Spaniol et al., 2020), as comunidades campestres exibiram maior diversidade de cores interespecífica. Esse padrão é consistente com a maior pressão de predação registrado em ambientes abertos (Seymoure et al., 2018; Goßmann et al., 2023), que pode favorecer uma maior correspondência entre coloração e micro-habitats disponíveis, enquanto florestas, sob menor risco de predação e com luz espectralmente filtrada, tendem a favorecer colorações intermediárias que funcionam de forma satisfatória em diferentes contextos do substrato (Merilaita; Lyytinen; Mappes, 2001; Cuthill; Matchette; Scott-Samuel, 2019; Endler, 1993). Esses achados ressaltam a importância dos campos naturais como ambientes capazes de sustentar elevada variação fenotípica, frequentemente subestimados em estratégias de conservação.

Já em uma escala macroevolutiva, a análise do gênero Rhinella, caracterizado por ampla amplitude ecológica, revelou que a coloração dorsal resulta da interação entre fatores ambientais contemporâneos e a história evolutiva do grupo. Matiz e saturação aumentaram com a temperatura, e espécies florestais apresentaram valores mais elevados de matiz, associados a tons avermelhados, possivelmente relacionados à camuflagem na serapilheira e ao mimetismo com o substrato florestal (Ferreira et al., 2019; Toledo; Haddad, 2009; Santos et al., 2016; Sirisathitkul; Sirisathitkul, 2025). A associação entre climas mais quentes e colorações mais saturadas pode estar relacionada à maior disponibilidade dietética de carotenoides, adquiridos por meio do consumo de artrópodes, bem como a possíveis funções associadas à resistência a parasitas em regiões próximas ao equador, onde a incidência desses organismos tende a ser mais elevada (Prasetya; Peters; Delhey, 2020; Koneru; Caro, 2022; Harvell et al., 2002; Szuroczki; Koprivnikar; Baker, 2019). Ademais, a diversidade de cores intraespecífica respondeu à interação entre temperatura e habitat, aumentando em ambientes abertos mais quentes, mas sendo restringida em florestas quentes. Esse padrão pode ocorrer porque florestas densas, como a Amazônica, favorecem estratégias de camuflagem mais especializadas em *Rhinella*, o que tende a limitar a variação de coloração (Barnett et al., 2021b; Merilaita; Scott-Samuel; Cuthill, 2017), enquanto habitats abertos permitem maior variabilidade cromática em função da maior exposição à luz e da maior heterogeneidade ambiental (Amézquita; Hödl, 2006; Endler, 1993). Esses achados enfatizam a complexa interação entre pressões ecológicas, condições climáticas e história evolutiva na determinação da coloração dos anfíbios, ao mesmo tempo em que reforçam a vulnerabilidade dessas características às mudanças ambientais globais em curso (Koneru; Caro, 2022).

De forma geral, os resultados dos quatro capítulos demonstram que os diferentes componentes da coloração dorsal não respondem de maneira uniforme às variáveis ambientais, refletindo funções adaptativas distintas associadas não apenas à termorregulação, mas também à reprodução, à sobrevivência e à camuflagem. A temperatura, em particular, emerge como um fator central ao influenciar simultaneamente a luminosidade, a saturação e a diversidade de cores, indicando que seus efeitos vão além do balanço térmico. Em contraste, ambientes florestais parecem impor pressões seletivas mais restritivas sobre a coloração, limitando a variabilidade intra e interespecífica, possivelmente em função da luz filtrada pelo dossel e da necessidade de camuflagem eficiente. Em todas as escalas analisadas, a coloração mostrou-se vulnerável às mudanças ambientais, especialmente à modificação da cobertura vegetal e às alterações nos regimes térmicos e de precipitação, com potencial prejuízo às funções adaptativas essenciais desse traço (Koneru; Caro, 2022).

Como perspectivas futuras, destaca-se a necessidade de experimentos controlados em laboratório para avaliar diretamente os efeitos da temperatura, da precipitação e da presença do dossel sobre a coloração, bem como investigar a sensibilidade térmica da saturação. A incorporação de modelos visuais permitirá avaliar se as variações observadas são perceptíveis a predadores e coespecíficos, contribuindo para a compreensão funcional da coloração em diferentes contextos ecológicos. Além disso, investigar a disponibilidade de carotenoides na dieta e sua relação com a coloração em ambientes florestais e campestres, assim como aprofundar a relação entre diversidade de predadores, diversidade de cores e estratégias defensivas, constitui um avanço importante. Por fim, a ampliação das escalas espacial, temporal e taxonômica será fundamental para avaliar a generalidade dos padrões observados e compreender de forma mais abrangente a evolução da coloração em anfíbios anuros diante das mudanças ambientais globais.

REFERÊNCIAS

AMÉZQUITA, Adolfo; HÖDL, Walter. HOW, WHEN, AND WHERE TO PERFORM VISUAL DISPLAYS: THE CASE OF THE AMAZONIAN FROG HYLA PARVICEPS. **Herpetologica**, v. 60, n. 4, p. 420–429, 14 jul. 2006.

BARNETT, James B. *et al.* Habitat disturbance alters color contrast and the detectability of cryptic and aposematic frogs. **Behavioral Ecology**, v. 32, n. 5, p. 814–825, 2021a.

BARNETT, James B. *et al.* Colour pattern variation forms local background matching camouflage in a leaf-mimicking toad. **Journal of Evolutionary Biology**, v. 34, n. 10, p. 1531–1540, out. 2021b.

BURRACO, Pablo; ORIZAOLA, Germán. Ionizing radiation and melanism in Chornobyl tree frogs. **Evolutionary Applications**, v. 15, n. 9, p. 1469–1479, set. 2022.

CUTHILL, Innes C.; MATCHETTE, Samuel R.; SCOTT-SAMUEL, Nicholas E. Camouflage in a dynamic world. **COBEHA**, v. 30, p. 109–115, 2019.

DELHEY, Kaspar *et al.* Evolutionary predictors of the specific colors of birds. **Proceedings of the National Academy of Sciences**, v. 120, n. 34, p. e2217692120, 22 ago. 2023.

ENDLER, John A. The Color of Light in Forests and It's Implications. **Ecological Monographs**, v. 63, n. 1, p. 1–27, 1993.

FERREIRA, Rodrigo B. *et al.* Antipredator mechanisms of post-metamorphic anurans: a global database and classification system. **Behavioral Ecology and Sociobiology**, v. 73, n. 5, p. 69, maio 2019.

GOSSMANN, Anika *et al.* Habitat openness and predator abundance determine predation risk of warningly colored longhorn beetles (Cerambycidae) in temperate forest. **Journal of Insect Science**, v. 23, n. 2, 1 mar. 2023.

HARVELL, C. Drew *et al.* Climate Warming and Disease Risks for Terrestrial and Marine Biota. **Science**, v. 296, n. 5576, p. 2158–2162, 21 jun. 2002.

KONERU, Manisha; CARO, Tim. Animal Coloration in the Anthropocene. **Frontiers in Ecology and Evolution**, v. 10, p. 857317, 22 abr. 2022.

MERILAITA, S.; LYYTINEN, A.; MAPPES, J. Selection for cryptic coloration in a visually heterogeneous habitat. **Proceedings of the Royal Society B: Biological Sciences**, v. 268, n. 1479, p. 1925–1929, 2001.

MERILAITA, Sami; SCOTT-SAMUEL, Nicholas E.; CUTHILL, Innes C. How camouflage works. **Philosophical Transactions of the Royal Society B: Biological Sciences**, v. 372, n. 1724, 2017.

PRASETYA, Audrey Miranda; PETERS, Anne; DELHEY, Kaspar. Carotenoid-based plumage colour saturation increases with temperature in Australian passerines. **Journal of Biogeography**, v. 47, n. 12, p. 2671–2683, dez. 2020.

ROJAS, Bibiana; LAWRENCE, J. P.; MÁRQUEZ, Roberto. Amphibian Coloration: Proximate Mechanisms, Function, and Evolution. *In*: MORENO-RUEDA, Gregorio;

COMAS, Mar (Eds.). **Evolutionary Ecology of Amphibians**. 1. ed. Boca Raton: CRC Press, 2023. p. 219–258.

RUDH, Andreas; QVARNSTRÖM, Anna. Adaptive colouration in amphibians. **Seminars in Cell and Developmental Biology**, v. 24, n. 6–7, p. 553–561, 2013.

SANTOS, Adailde Do Carmo *et al.* Genesis of Soils Formed from Mafic Igneous Rock in the Atlantic Forest Environment. **Revista Brasileira de Ciência do Solo**, v. 40, n. 0, 2016.

SEYMOURE, Brett M. *et al.* Environment-dependent attack rates of cryptic and aposematic butterflies. **Current Zoology**, v. 64, n. 5, p. 663–669, 2018.

SIRISATHITKUL, Yaowarat; SIRISATHITKUL, Chitnarong. Decoding Soil Color: Origins, Influences, and Methods of Analysis. **AgriEngineering**, v. 7, n. 3, p. 58, 25 fev. 2025.

SPANIOL, Ricardo Luís *et al.* Discolouring the Amazon Rainforest: how deforestation is affecting butterfly coloration. **Biodiversity and Conservation**, p. 1–18, 3 jun. 2020.

STEGEN, James C.; GIENGER, C. M.; SUN, Lixing. The control of color change in the Pacific tree frog, *Hyla regilla*. **Canadian Journal of Zoology**, v. 82, n. 6, p. 889–896, 1 jun. 2004.

STEVENS, Martin *et al.* Using digital photography to study animal coloration. **Biological Journal of the Linnean Society**, v. 90, p. 211–237, 2007.

SZUROCZKI, D.; KOPRIVNIKAR, J.; BAKER, R. L. Effects of dietary antioxidants and environmental stressors on immune function and condition in Lithobates (Rana) sylvaticus. **Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology**, v. 229, p. 25–32, mar. 2019.

TOLEDO, Luís Felipe; HADDAD, Célio F. B. Colors and Some Morphological Traits as Defensive Mechanisms in Anurans. **International Journal of Zoology**, v. 2009, p. 1–12, 12 mar. 2009.

ZAMORA-CAMACHO, Francisco Javier; COMAS, Mar. Beyond Sexual Dimorphism and Habitat Boundaries: Coloration Correlates with Morphology, Age, and Locomotor Performance in a Toad. **Evolutionary Biology**, v. 46, n. 1, p. 60–70, 2019.

ANEXOS

Artigos que compõem a Tese

1. **LERMEN, Luísa;** LOEBMANN, Daniel. The influence of environmental factors on dorsal coloration patterns in a neotropical tree frog. **Biological Journal Of The Linnean Society, 2025.**

Artigos publicados durante o processo de doutoramento que não compõem a tese (Agosto/2021-dezembro/2025)

2. **LERMEN, Luísa Nunes**; FURTADO, Raíssa; HARTZ, Sandra Maria. Color polymorphism and variation in microhabitat selection: a defensive strategy in males of Boana pulchella (Anura: Hylidae)? **Acta Scientiarum. Biological Sciences (Online)**, v. 44, p. e58763-8, 2022.

3. **LERMEN, Luísa**; FURTADO, Raíssa; HARTZ, Sandra Maria. To be or not be seen: the influence of substrate on anuran community coloration. **Journal Of Natural History**, v. 59, p. 1249-1265, 2025.

COMISSÃO DE ÉTICA EM USO ANIMAL
Universidade Federal do Rio Grande
Pró-Reitoria de Pesquisa e Pós-Graduação - PROPESP
ceua@furg.br http://www.propesp.furg.br

CEUA

CERTIFICADO Nº P034/2022

Certificamos que o projeto intitulado "Influência do ambiente na evolução dos padrões de coloração corporal de anfíbios anuros", protocolo n^0 23116.001718/2022-35, sob a responsabilidade de Daniel Loebmann - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao Filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa – encontra-se de acordo com os preceitos da Lei n° 11.794, de 8 de outubro de 2008, do Decreto n° 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi APROVADO pela COMISSÃO DE ÉTICA EM USO ANIMAL DA UNIVERSIDADE FEDERAL DO RIO GRANDE (CEUA-FURG), em reunião de 31 de agosto de 2022 (Ata 008/2022).
A CEUA lembra aos pesquisadores que qualquer alteração no protocolo experimental ou na equipe deve ser encaminhada à comissão para avaliação e aprovação. Um relatório final deve ser enviado à CEUA no término da vigência do seu projeto.

CEUA Nº	Pq008/2022
COLABORADORES AUTORIZADOS A MANIPULAR OS ANIMAIS	
VIGÊNCIA DO PROJETO	31/07/2025
ESPÉCIE / GRUPOS TAXONÔMICOS	Anuros
NÚMERO DE ANIMAIS	Informar nos relatórios
N° SOLICITAÇÃO / AUTORIZAÇÃO SISBIO	
ATIVIDADE(S)	(X) CAPTURA ( ) COLETA DE ESPÉCIMES ( ) MARCAÇÃO ( ) OUTRAS:
LOCAL(is) REALIZAÇÃO ATIVIDADES	Rio Grande do Sul
ENVIO DE RELATÓRIO PARCIAL	Janeiro de 2023; Janeiro de 2024; Janeiro de 2025
ENVIO DO RELATÓRIO FINAL	Agosto de 2025

Rio Grande, 01 setembro de 2022.

gov.br Documento assinado digitalmente
MARCIO DE AZEVEDO FIGUEIREDO
Data: 01/09/2022 09:13:04-0300
Verifique em https://verificador.iti.br

Med. Vet. Márcio de Azevedo Figueiredo
Coordenador da CEUA-FURG

Controle da CEUA - Parecer(es) prévio(s) relacionado(s) ao processo: P031/2022. Página 1/1